BLOCK A

Display: Monday 17 – Tuesday 18 April

Presentation session: Monday 17 April (16.30-17.40)

Session Topic: Secondary adaptations: the fuel of diversification in parasites and protists

P001

Evolution and adaptation of a broken mitotic entry switch in *Schizosaccharomyces pombe*

Chantelle Endeley ORCID iD[1,2], Maria Rosa Domingo-Sananes ORCID iD[1]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

The mitotic entry switch is a regulated biological process functioning as a bistable switch which enables robust and abrupt entry into mitosis. While this system has been studied from a theoretical and experimental perspective, little is known about the origin of the system and how it continues to evolve and adapt. In this study, we use experimental evolution to determine how the system adapts when the switch is defective. Wild type (PN1) and mutant (Wee1Δ and Cdc25-ts (temperature sensitive)) strains of the fission yeast *Schizosaccharomyces pombe* were evolved for over 200 generations. Evolved populations and isolated single colonies were then phenotypically characterised (cell size, generation time, and temperature sensitivity). Minimal changes in cell size at division and generation time were seen in the evolved wild type and Wee1Δ populations, but notable changes in cell size at division were seen in the majority (>70%) of the single clones. Significant phenotypic differences were observed in the evolved Cdc25-ts populations compared to the ancestral strain, with cell size at division, generation time and temperature sensitivity all decreasing. Variations in phenotypes were apparent in the evolved Cdc25-ts single clones, in terms of cell size, temperature sensitivity and generation times, suggesting the existence of multiple adaptation pathways to defects in mitotic entry. While a reversion of the mutation causing temperature sensitivity has not been observed in the evolved Cdc25-ts strains, work is ongoing to determine the genetic basis of adaptation through genetic crosses and whole genome sequencing.

P002

Intestinal Parasitic Infections among Sudanese psychiatric patients; a preliminary study.

Abdelhakam G. Tamomh ORCID iD, Hafiz Y. Mohammed, Israa A. Bashir, Ola O. Ali

University of El Imam El Mahdi, Kosti, Sudan

Abstract

Background: Intestinal parasitic infections are considered a major health problem, particularly in tropical countries such as Sudan. Due to poor hygiene practices, psychiatric patients may acquire parasitic infections. Until now, no published data or available information regarding the prevalence rate of intestinal parasitic infections among Sudanese psychiatric patients, therefore, our present study aimed to determine the prevalence of intestinal parasitic infections and the potential associated risk factors among Sudanese psychiatric patients.

Methods: A hospital-based cross-sectional study was conducted and a total of 422 stool samples were randomly collected from psychiatric patients attending the psychiatric section at Kosti teaching hospital in the White Nile State of Sudan during the period from September 2021 to March 2022. Socio-demographic data were gathered using a structured questionnaire. All stool samples were examined using different parasitological techniques.

Results: The overall prevalence rate of intestinal parasitic infection among psychiatric patients was 120/211(56.8%) and non-psychiatric 66/211(31.3%). The prevalence rate of intestinal parasites (IPs) among psychiatric patients were (Entamoeba histolytica (29.9%), Gardia lamblia (19.4%), Entamoeba coli (5.2%%), A. lumbricoides(0.9%) , Hymenolepis nana (0.9%), and Enterobius vermicularis (0.5%). There was no relationship between intestinal parasitic infection and age, sociodemographic features, sources of drinking water, contact with domestic animals, washing of hands, eating of raw vegetables/meats, and having psychiatric disorders (P>0.05).

Conclusions: Studying the prevalence rate of intestinal parasites among psychiatric patients may help to improve their health status, leading to better health services, diagnosis, and treatment.

Session Topic: Plant-microbe interactions

P003

Phyllospheric Bacterial Diversity of *Helianthus annuus*

SHAHIDA HASNAIN, SEHAR MAHMOOD

Institute of Microbiology and Molecular Genetics, Quaid e Azam Campus, University of the Punjab, Lahore, Pakistan

Abstract

Background: Phyllosphere embodies the largest global interface of the aerial plant parts and exibit diverse microbial communities. The microbial ecology of Phyllosphere is of great concern regarding nature sustainability, agricultural worth, and economic importance.

Methodology: In the present work the bacterial diversity, population density and community structure in terms of spatial and temporal variations of two varieties of sunflower were explored. During the growth season, bacterial isolates were harvested from the abaxial and adaxial surface of dicotyledonous leaf, young mature and old leaves of vegetative, reproductive and flowering stages of Hysun-33 and Hysn-39 varieties of sunflower. Plant growth-promoting attributes of the individual bacteria and consortium phyllosphere bacteria were evaluated in natural field conditions by applying inoculum on seed. Using GFP-labelled Pseudomonas sp MeA-P42 images localization of epiphytic phyllospheric bacteria on root, stem and leaves of both hybrids was examined using CLSM.

Results: Overall culturable bacterial carrying capacity of leaves remain same. Leaf associated bacteria showed a paradigm shift in community composition and structure across the developmental stages. Phyllospheric bacteria pronouncedly improves *Helainthus annuus* plant growth. Phyllosheric bacteria colonized the aerial parts and roots of plant. Co-culturing of phyllospheric isolates Pseudomonas sp. MehA-P41 and Bacillus sp. MehA-P43 with fungal strain Trichoderma hamatum showed significant antagonistic activity against pathogens. Biocontrol activity against Sclerotinia sclerotium was taken as the main function of chitinase enzyme produced by the phyllospheric bacteria.

Conclusion: Phyllosheric bacterial community exhibited variation with development stages of sunflower plant. Bacterial inoculation improved plant growth significantly and revealed biocontrol activity.

P004

Elucidation of *Bacillus* sp. strain MHSD28 spore structure and development of *Bacillus*-based formulation.

Sephokoane Cindy Makuwa ORCID iD, Prof Mahloro Hope Serepa-Dlamini ORCID iD

University of Johannesburg, Johannesburg, South Africa

Abstract

Endophytes are an endosymbiotic group of microorganisms that exist inside plant tissues without causing any negative impact. It has several properties such as biocontrol through production of antifungal metabolites and induction of disease resistance as well as plant growth promotion through fixing biological nitrogen and increasing mineral uptake in plants. Furthermore, the application of endophytes offers an alternate to the use of chemical fungicides in the management of plant diseases. *Bacillus* species can produce spores, which gives them the ability to sustain harsh environmental conditions. As a result, their capacity to produce endospores ensures that they can withstand unfavourable environmental conditions, making them effective biocontrol agents. The objective of this study was characterizing the spore structure and to develop a carrier-based formulation of a novel bacterial endophyte *Bacillus* sp. strain MHSD28 as a biocontrol agent. Powder formulation of three different carrier material which includes talcum powder, activated charcoal and sodium alginate were evaluated and a talc-based formulation was optimized for longer shelf life in terms of storage temperature and microbial concentration.

P005

Antibiotic resistance profile of *E. coli* isolates from lettuce, poultry manure, irrigation water and soil in Kumasi, Ghana

Abigail Abena Anokyewaa Appau Appau, Linda Aurelia Ofori

Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract

Background: Inputs such as irrigation water and poultry manure used in lettuce cultivation have been found to be associated with antibiotic resistant pathogens. The study assessed the antibiotic resistance profile of *Escherichia coli* found in these inputs.

Methods: A total of 156 lettuce, irrigation water, soil and manure samples were collected from 3 farms over a period of 7 weeks. *E. coli* were enumerated using Most Probable Number technique. Using the Kirby Bauer method, antibiotic sensitivity testing was performed against 225 biochemically confirmed *E. coli* with twelve antibiotics. *E. coli* were screened for Extended Spectrum Beta Lactamase (ESBL) production using Cefotaxime (CTX) and Cefotaxime/Clavulanic Acid (CTX/CLA) disc. ESBL positives were confirmed with Polymerase Chain Reaction (PCR) and amplified using gel electrophoresis

Results: 98% of the samples were positive for *E. coli*. Microbial loads differed significantly among the treatments. Manure recorded the lowest load while soil recorded the highest load. Relatively high resistances were recorded for some members of the beta-lactam class; Meropenem – 94.2%, Ampicillin – 91.9%, Cefuroxime – 95.1%, Ceftriaxone – 94,7% and Cefotaxime – 94.2%. PCR confirmed presence of ESBL gene bla_{CTX-M} one each from poultry manure, irrigation water.

Conclusion: Occurrence of antibiotic resistant *E. coli* on vegetable farm inputs is alarming and poses serious health threats to the public. Presence of ESBL bla_{CTX-M} genes on *E. coli* from farm inputs recorded for the first time in Ghana requires enforcement by regulatory bodies on the inappropriate use of antibiotics in the country.

P006

Getting to the root of the co-evolution of plants and their endophytic *Streptomyces*

Louisa Perry ORCID iD, Damien Gayrard ORCID iD, Jake Newitt, Samuel Prudence, Matt Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

The bacterial genus *Streptomyces* has been well studied for its medicinally beneficial specialised metabolites. Most commonly known as soil dwelling bacteria, their specialised metabolites play an essential role in their ability to defend their environmental niches from microbial competition. Understanding *Streptomyces*' ecological role may provide new insights into why and how these specialised metabolites are produced.

Previous studies have shown that some *Streptomyces* bacteria are wheat root endophytes (Prudence et al., 2021) with some strains showing activity against the wheat pathogen, Wheat Take-all (Worsley et al., 2020). Through Illumina and CCS PacBio sequencing we show that the abundance and composition of the endophytic *Streptomyces* taxa vary between plant species of different phyla (liverwort (*Marchantia polymorpha*), C-Fern (*Ceratopteris richardii*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*)), grown in the same agricultural soil, suggesting that some plants have evolved to specifically recruit beneficial microbes. *Streptomyces scabies*-like species were most abundant in the endosphere of wheat and barley, relative to the surrounding rhizosphere. We continue to explore how co-evolution might have occurred between *Streptomyces* scab-causing relatives and these domesticated crop plants.

Prudence, S. et al., 2021. Soil, senescence and exudate utilisation: characterisation of the Paragon var. spring bread wheat root microbiome. Environmental Microbiome.

Worsley, S. et al. 2020. *Streptomyces* Endophytes Promote Host Health and Enhance Growth across Plant Species. Applied and Environmental Microbiology

P007

Aromatic amino acids influence virulence gene expression in *Pseudomonas syringae*

Chloe Harper[1,2], Nattapong Sanguankiattichai[1], Gail Preston[1]

[1]University of Oxford, Oxford, United Kingdom. [2]University of Warwick, Coventry, United Kingdom

Abstract

The plant pathogen *Pseudomonas syringae* utilises a type III secretion system (T3SS) to suppress plant immunity. T3SS expression is regulated by signals present in the plant environment. As precursors for plant defence compounds, aromatic amino acids (AAAs) accumulate in the plant environment upon infection and are rapidly taken up by *P. syringae*. However, they are a poor source of nitrogen or carbon and at high concentrations can be inhibitory to *P. syringae* growth. We investigated the role of AAAs in regulating virulence gene expression. Bioinformatic analyses showed that the aromatic amino acid permease *aroP*, and the regulators *tyrR* and *phhR* are highly conserved across *P. syringae* pathovars, whilst downstream metabolic genes have a varied distribution. Knockout mutants were generated for *aroP*, *tyrR* and *phhR* in *P. syringae* pv. *tomato* DC3000, and the effect of AAAs on virulence gene expression was assessed by measuring luminescent signals from *lux* reporter constructs for the T3SS regulator *hrpL* and the T3SS-secreted effector *avrPtoB*. The concentration of AAAs and the choice of media (mannitol-glutamate (MG) media pH 7 vs. M9 fructose pH 5.5) significantly influenced the growth and T3SS expression of WT and mutant *P. syringae* strains. Results obtained using *ΔaroP* strains further suggested that AroP has a role in regulating virulence gene expression, which is further mediated by the environment. Overall, our results show that AAAs can influence T3SS expression and that the environment plays an instrumental role in regulating gene expression in *P. syringae*.

P008

Bacterial microbiomes in irrigation water and leafy green vegetables associated with small-scale farms in South Africa

Degracious Kgoale, Jarishma Gokul, Erika Du Plessis, Lise Korsten

University of Pretoria, Hatfield, South Africa

Abstract

Irrigation with poor-quality water can result in contamination of fresh produce with water- and food-borne pathogens, especially in small-scale farming communities, where proper hygiene measures are often neglected. To identify potential pathogens in the production environment and the shifts in community structure because of their presence, we characterized the bacterial communities in irrigation water and leafy greens from five small-scale farms in South Africa. Illumina MiSeq high-throughput sequencing targeting the V4-V5 variable region of the 16S rRNA gene region revealed high relative abundance of Proteobacteria, Firmicutes and Actinobacteria, including prominent families such as Burkholderiaceae (48%), Enterobacteriaceae (34%), Bacillales family XII (8%), Rhodobacteraceae (3%), Micrococcaceae (1.98%) and Pseudomonadaceae (1.79%). Diversity analysis showed that each farm exhibited a unique bacterial profile with core communities specific to plant or irrigation water source evident. Potential pathogenic Enterobacteriaceae such as *Enterobacter*, *Salmonella*, *Shigella*, *E. coli*, *Citrobacter* and *Klebsiella* were the dominant genera identified overall. These findings highlight the presence of specific bacterial communities in irrigation water and leafy green vegetables in different areas and the existence of human pathogens within the irrigation water-fresh produce interface. This research suggests that improved water quality in the small-scale production systems is necessary and can aid in the prevention of potential foodborne outbreaks.

P009

Exploring the role of oxanthromicin, an antifungal metabolite produced by endophytic *Streptomyces*, in the reduction of wheat take-all disease symptoms.

Jake Newitt[1], Sibyl Batey[2], Pedro De-Resende[1], Louisa Perry[1], Edward Hems[1], Barrie Wilkinson[1], Matt Hutchings[1]

[1]John Innes Centre, Norwich, United Kingdom. [2]BactoBio, London, United Kingdom

Abstract

Streptomyces is a genus of soil dwelling actinomycetes that plays an important role in plant health through association with plant roots. They benefit their plant host by increasing nutrient availability, enhancing plant growth, and preventing infectious disease. Specialised antimicrobial metabolites are thought to underpin disease protection, although this is not clear. In this study, we explore the biological and chemical interactions between *Streptomyces* isolated from wheat roots and wheat root pathogen *Gaeumannomyces tritici.* (Wheat take-all) both *in vitro* and *in planta*, and the role of antifungal secondary metabolites produced by *Streptomyces* in the wheat root endosphere.

The roots of field grown Kerin, Soisson and Paragon variety wheat plants were sampled at maturity and 54 *Streptomyces* spp. were isolated from both the rhizosphere and endosphere microbiome. A total of 17 strains were found to inhibit wheat take-all fungus *in vitro* and 3 of these showed a clear reduction in take all disease symptoms *in planta*. Antifungal metabolites were successfully extracted for 15 strains. The extract from *Streptomyces* spp. SES1, which showed the clearest reduction in disease was purified using preparative HPLC, and several antimicrobial compounds were identified and structurally characterized using NMR. The genomes for these strains were sequenced using a hybrid short/long read approach. AntiSMASH analysis identified a candidate for the cryptic antifungal metabolites produced by SES1.

This study explores the role of antifungal compounds produced by streptomycetes in the wheat root microbiome and provides a platform for further probing the chemical ecology of wheat/*Streptomyces* interactions.

P010

Are we ready to harness effective but genetically modified *Burkholderia* and *Paraburkholderia* biopesticides?

Gordon Webster ORCID iD, Alex Mullins ORCID iD, Yoana Petrova ORCID iD, Eshwar Mahenthiralingam ORCID iD

Cardiff University, Cardiff, United Kingdom

Abstract

Fungal and oomycete damping-off diseases cause global crop losses. Chemical pesticides are used to control disease, but with their withdrawal due to safety concerns, farmers have few protective options. Until 1999, *Burkholderia* bacteria were used commercially as biopesticides in the US, but concerns relating to their opportunistic pathogenicity led to a moratorium on their exploitation unless they could be proven safe. *Burkholderia* produce multiple specialised metabolites with plant-protective properties, including antimicrobial polyynes such as cepacin A. This metabolite is a key mediator in the ability of *Burkholderia* ambifaria biopesticides to protect germinating peas against *Globisporangium* (formerly *Pythium*) *ultimum* damping-off disease. We systematically investigated the biological control ability of other polyyne-producing *Burkholderia, Collimonas, Trinickia*, and *Pseudomonas* bacteria. When applied as a seed coat, *B. ambifaria* (cepacin A polyyne), and *Burkholderia gladioli* and *Burkholderia plantarii* (caryoynencin pollyne), were the only species to effectively protect peas against *G. ultimum* damping-off. Two genetic modification strategies to improve the safety profile of these opportunistic pathogen species were investigated. Firstly, a virulence-attenuated third replicon mutant of B*. ambifaria* in which gene-editing had removed this large virulence plasmid (>1000 genes), proved to be highly protective against damping-off disease in greenhouse-scale pea experiments. Secondly, transfer of the *B. gladioli* caryoynencin gene pathway to inherently safe *Paraburkholderia* species also facilitated effective biocontrol of *G. ultimum* disease. In summary, genetically modified *Burkholderia* and *Paraburkholderia* are effective as biopesticides. The next challenge will be to address the regulatory and societal understanding needed to enable their wider testing and potential use.

P011

Understanding resistance to *Botrytis cinerea* in strawberries

Finlay Bourquin[1,2], Charlotte Nellist[1], Helen Cockerton[3], Matthew Dickinson[2], Tim Robbins[2]

[1]National Institute of Agricultural Botany, Cambridge, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom. [3]University of Kent, Kent, United Kingdom

Abstract

Botrytis cinerea is a virulent necrotrophic pathogen responsible for causing soft fruit rot both pre- and post-harvest in strawberries, consequently damaging crops throughout the production, transport and retail stages. Strawberries are a high value crop, hence harvest losses at all stages of production have an economic impact and resistance to fungicides is increasing. The aims of this project are to identify novel strawberry susceptibility or resistance factors to *B. cinerea* and novel *B. cinerea* virulence factors. Pathogenicity assays have been developed for *Fragaria vesca* leaves, *F. vesca* flowers, *Fragaria x ananassa* fruits and for *Nicotiana benthamiana* leaves, and differences in *B. cinerea* virulence has been found between isolates ($p < 2.2e-16$) and across strawberry organs and hosts. To investigate *B. cinerea* virulence factors, isolates will undergo UV mutagenesis and pathogenicity testing. Should virulence be altered, genome sequencing and analysis will be carried out, leading to an increased understanding of the *B. cinerea*-strawberry pathosystem. To investigate strawberry susceptibility factors, *F. vesca* seeds have been mutagenized, grown to maturity and the M2 generation will be screened for differences in susceptibly or resistance to *B. cinerea* using the pathogenicity assays. Any plants exhibiting increased susceptibility or resistance will undergo genome sequencing to determine which genomic loci are responsible for the change in phenotype. As *F. vesca* is the dominant ancestor of cultivated strawberries, this work could lead to the development of selective markers for breeding or targets for genetic modification, which could result in the production of *B. cinerea* resistant strawberry cultivars.

P012

Ficus thonningii Phyllosphere: Potential Source of Novel Antimicrobials

Anderson O Oaikhena ORCID iD[1,2], Morenike E Coker[1], Wisnu Adi Wicaksono[2], Iruka N Okeke[1], Gabriele Berg[2]

[1]University of Ibadan, Ibadan, Nigeria. [2]Technical University of Graz, Graz, Austria

Abstract

Medicinal plants have been explored for antimicrobial potentials but their microbiomes have been relatively understudied. Phyllosphere bacteria contribute to plant health, regulation and production of antimicrobial compounds. We characterized the culturable leaf-microbiome of *Ficus thonningii,* a tropical medicinal plant, and investigated their antimicrobial potential.

Fresh leaves of *F. thonningii* were collected. Bacteria was isolated from the leaves on 0.1X tryptone soy agar containing cycloheximide and identified by 16S rRNA sequencing. Antimicrobial challenge tests on selected control strains was conducted with agar well diffusion and the genomes of antagonistic isolates and probable new species were Illumina sequenced. Genome mining was conducted using antiSMASH and PRISM.

A total of 395 isolates were identified and belonged to four phyla- Proteobacteria (253), Firmicutes (94), Actinobacteria (40) and Bacteroidetes (8). Bacillaceae (70) was the most predominant family detected, and species belonging to Bacillaceae, Enterobacteriaceae and Xanthomonadaceae previously unknown to science were recovered. Cell-free supernatant of 19 of 38 tested bacteria produced zones of inhibition on challenge organisms including *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. A total of 311 biosynthetic gene clusters, including 284 previously undescribed were detected from 35 sequenced isolates. Biosynthetic gene clusters were predicted to encode aryl polyenes, non-ribosomal peptides, polyketides, lanthipeptides and terpenes, all groups of compounds with potent antimicrobial activity.

Antimicrobial activity of phyllosphere bacteria from *F. thonningii* suggests that the plant's microbiome may contribute directly to observed medicinal effects. *F. thonningii* microbiome may be a rich source of secondary metabolites that should be explored for antimicrobial drug discovery.

P013

Future Oak: a microbiome-wide association study of acute oak decline severity in Quercus robur

Jim Downie ORCID iD[1], Alejandra Ordonez ORCID iD[1], Usman Hussain[1], Marine Cambon ORCID iD[1], Jasen Finch ORCID iD[2], Nathan Brown ORCID iD[3], Sandra Denman ORCID iD[3], James McDonald ORCID iD[1,4]

[1]Bangor University, Bangor, United Kingdom. [2]Aberystwyth University, Aberystwyth, United Kingdom. [3]Forest Research, Farnham, United Kingdom. [4]Birmingham University, Birmingham, United Kingdom

Abstract

Acute Oak Decline (AOD) is a complex decline disease of oak in which weeping stem lesions are caused by a multi-species bacterial "pathobiome", leading to tissue necrosis and often tree death. AOD is spreading across the UK, causing concern about the future of oak trees, but to date there are no effective means of management. One target may be the oak microbiome, which might contain microbial taxa that facilitate protection against the activity of these pathogenic species, either through their direct inhibition or competitive exclusion. To locate such microbial taxa, we are conducting a microbiome-wide association study, characterising the leaf, stem and rhizosphere microbiomes of 300 *Quercus robur* trees from 30 sites across the UK using shotgun metagenomic sequencing, as well as 16S rRNA and ITS amplicon sequencing. These trees represent a wide range of AOD severity, from healthy trees to severely affected. To date, we have constructed 424 bacterial and 965 fungal MAGs associated with the oak phyllosphere. These MAGs provide key insight into the composition and function of the microbiome with changing disease severity, as well as the physiological changes occurring in the tree following disease onset. Future work will use a Bayesian mixed modelling approach to identify which, if any, of these taxa are positively associated with oak health for further testing as part of a microbial treatment.

P014

Implementing a DNA-based diagnostic assay on a Lateral Flow Device (LFD) – Development of in field tools for early detection of fungal storage rots of strawberry

Olaide Ogunsanya[1,2,3], Richard Colgan ORCID iD[1], Andrew Armitage ORCID iD[1]

[1]Natural Resources Institute, Chatham Maritime, United Kingdom. [2]International Institute of Tropical Agriculture, Ibadan, Nigeria. [3]Federal University of Agriculture, Abeokuta, Nigeria

Abstract

Botrytis cinerea is a devastating pre- and postharvest pathogen of over 1,000 plant species and represents the primary disease of harvested strawberries. In strawberry, the fungus colonises open flowers (primary infections) or fruit (secondary infections) and switches to a necrotrophic life stage upon fruit ripening. Monitoring and control of the pathogen is critical, with risk of >80% losses in the absence of treatment. Lab-based molecular diagnostics, designed directly from the pathogen's genome, provide high-specificity to the target organism. However, PCR and qPCR assays require access to lab-equipment, molecular biology training and as such, cannot typically be deployed on-site.

We have developed an isothermal Recombinase Polymerase Amplification (RPA) assay, with results detectable on a Lateral Flow Device. Comparative genomic analysis of four sequenced *Botrytis cinerea* isolates, alongside 35 additional *Botrytis* spp. genomes, allowed identification of lineage-specific effector complements to *Botrytis cinerea*. Design of primers and probes to these regions allowed specific detection of *B. cinerea* amongst common fungal and oomycete pathogens of strawberry. With increasing public familiarity of LFDs, such assays offer an opportunity to put genome-informed molecular diagnostics in the hands of growers, agronomists and fruit processors.

P015

Providing human and machine readable FAIR data resources to explore and intercompare diverse plant infecting pathogens

Martin Urban ORCID iD[1], Alayne Cuzick[1], James Seager[1], Kim Rutherford[2], Valerie Wood[2], Dan Smith[1], Keywan Hassani-Pak[1], Kim Hammond-Kosack[1]

[1]Rothamsted Research, Harpenden, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Complex phenotypes, such as virulence, pathogenicity and resistance, are important observable traits controlled by the genomes of interacting organisms and individual virulence/avirulence genes. PHI-base (www.phi-base.org) is a gold-standard manually curated phenotype database that stores molecular information on genes implicated in microbial disease. It aims to help researchers to uncover new fundamental insights, share phenotype data in a machine readable format, and discover novel intervention targets for disease control.
Our November 2022 release provides information on 283 pathogens tested on 234 hosts, covering 19,881 interactions and 8,993 genes curated from > 4,847 peer reviewed articles. PHI-base provides information on target sites of anti-infective chemistries and the first host targets of pathogen effectors. It is a primary information source for researchers studying plant, animal, and/or human pathogens. Species neutral high-level phenotypes are implemented to allow comparative plant-pathogen analysis using the Pathogen-Host Interaction Phenotype Ontology. PHI-base phenotypes are exported to Ensembl, NCBI, UniProtKB, FungiDB and KnetMiner knowledge graphs. These resources link phenotypes to genomes and evolutionary trees, and enable computational analysis that utilises comparative genomics, gene network analysis, and mappings to biochemical pathways. We also demonstrate PHI-Canto, a new web-based community annotation tool (canto.phi-base.org) that permits authors to capture wild-type and mutant phenotype data at the point of publication. Knowledge graphs for the microbial fungus *Fusarium culmorum* were created using KnetMiner and used to identify a number of candidate virulence genes associated with secondary metabolite pathways.

P016

Multi-phasic identification of causative pathogen responsible for foliar and pod diseases of African yam bean (Sphenosylis stenocarpa) in Nigeria

Olaide Ogunsanya[1,2,3,4], Clement Afolabi[3], Andrew Armitage[1], Richard Colgan[1], Elinor Thompson[4], Alejandro Ortega-Beltran[2], Micheal Abberton[2]

[1]Natural resources centre, Kent, United Kingdom. [2]International Institute of Tropical Agriculture, Ibadan, Nigeria. [3]Federal University of Agriculture Abeokuta, Abeokuta, Nigeria. [4]University of Greenwich, Kent, United Kingdom

Abstract

African yam bean (AYB; Sphenostylis stenocarpa, Fabaceae) serves as a security crop, which is used for food, and feed, and it possesses medicinal properties. Despite the nutritional value of AYB, low grain yield as a result of fungal diseases deters farmers from large-scale cultivation. The causal agents of the pod and leaf blight diseases on AYB are largely uncharacterized. To investigate the prevalence of AYB fungal diseases, a disease survey was conducted in 2018 in major AYB-growing areas in Nigeria. Morphological and molecular assays were conducted to identify the causal agents and diseases responsible for low grain yield in this crop. The 143 fungal isolates collected from diseased AYB leaves and pods were identified by morphology and sequencing of the Internal Transcribed Spacer (ITS4 and 5). Colletotrichum gleosporiodes complex, Neopestalotiopsis spp, Curvularia akaii, and Fusarium oxysporum were recovered from leaf blight samples, whereas C. gleosporiodes complex and C. truncatum were collected from pod blight diseased samples. Koch's postulates were satisfied for C. truncatum, and C. gleosporiodes complex, leading to progressive dark lesions on healthy detached AYB leaves, pods, and flower buds. Other fungal pathogens did not produce any symptoms in healthy AYB tissues. This investigation confirms that Colletotrichum species are the predominant agents of these diseases, with evidence for host-preference of Colletotrichum spp. to different tissues. Given the importance of AYB in Western- and Eastern Nigerian diets, accurate pathogen species identification is vital to understand disease epidemiology and to deploy cultivars with appropriate resistance.

P017

Balancing the scales: Impact of irrigation and pathogen burden on potato blackleg disease and soil microbial communities

Ciara Keating ORCID iD[1], Elizabeth Kilbride[1], Joel Milner[2], Mark Stalham[3,4], Charlotte Nellist[4], Simon Smart[4], Sonia Humphris[5], Ian Toth[5,1], Barbara Mable[1], Umer Zeeshan Ijaz[6]

[1]School of Biodiversity, One Health, and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom. [2]Plant Science Group, College of Molecular, Veterinary & Life Sciences, University of Glasgow, Glasgow, United Kingdom. [3]Mark Stalham Potato Consultancy, Cambridge, United Kingdom. [4]The National Institute of Agricultural Botany, Cambridge, United Kingdom. [5]Cell & Molecular Sciences, James Hutton Institute, Dundee, United Kingdom. [6]School of Engineering, University of Glasgow, Glasgow, United Kingdom

Abstract

Potatoes, a critical food crop, are highly susceptible to pathogens and management practices to limit disease are a balancing act. Dry conditions favour common scab (*Streptomyces* spp.), while wet conditions favour blackleg (*Pectobacterium* spp.) disease. Watering regimes thus alter disease susceptibility, but it is not known if this perturbs the soil microbiome and whether this affects crop yields. Using field trials, we aimed to determine how the pathogen load of potato seed stocks (at 'Zero', 'Low', and 'High' levels of *Pectobacterium* spp) and irrigation management (unirrigated and three irrigation regimes) impacted: i) crop yields; ii) disease development (blackleg or common scab) and iii) microbial communities. Using sophisticated statistical tools, we quantified differences in microbial communities between stocks, irrigation treatments, and sampling time. We found that irrigation increased crop yields and increased blackleg symptoms in the Low and High stocks (22-34%) but not in the Zero stock (2-6%). Blackleg disease developed at a similar rate and incidence in plots planted with the Low stock as compared to the High stock. Not irrigating led to increased common scab symptoms (2-5%) and reduced crop yields by ~100 tubers per hectare. Irrigation alone did not impact the composition of the rhizosphere microbiome, but planting the seed tubers with a high blackleg pathogen burden resulted in increased abundance of *Planctomycetota*, *Chloroflexi* and *Acidobacteria* species compared to the Low and Zero stocks. We conclude that the initial pathogen burden on seed tubers substantially affects soil community dynamics, while irrigation regime had little influence on the soil microbiome.

P018

Differences in effector gene complement and expression between *Fusarium oxysporum* f. sp. *lactucae* race 1 and race 4 show novel effector candidates and evidence of horizontal gene transfer

Helen Bates ORCID iD[1], Sascha Jenkins[2], Jamie Pike[2], John Connell[1], Andrew Legg[2], Richard Harrison ORCID iD[1,3], John Clarkson ORCID iD[2]

[1]NIAB, Cambridge, United Kingdom. [2]University of Warwick, Warwick, United Kingdom. [3]Wageningen University and Research, Wageningen, Netherlands

Abstract

The *Fusarium oxysporum* species complex (FOSC) is a soil borne ascomycete with over 100 different pathogenic formae speciales (f.spp.) adapted to different host plants including many crops of agronomic importance. *Fusarium oxysporum* f. sp. *lactucae* (FOL) causes Fusarium wilt disease on lettuce and is further subdivided into races showing differential pathogenicity on different host varieties. FOL race 1 (FOL1) is the most widespread and a particular problem in Southern Europe and USA. Recently, race 4 (FOL4) has emerged in Northern Europe predominantly affecting protected lettuce and causing extensive losses of up to 50-80%. The genomes of the FOSC consist of a set of core chromosomes showing high identity between members of the FOSC and a set of Lineage Specific (LS) chromosomes rich in transposons and effectors that are unique to each f. sp. FOL1 and FOL4 have identical TEF1alpha sequences suggesting a common origin. We assembled a range of FOL1 and FOL4 isolates from Europe and compared them through long-read Nanopore genome sequencing and *in planta* transcriptome analysis. Key differences in putative effector gene complement between and within FOL races as well as comparison to other *F. oxysporum* f. spp. will be presented alongside differential expression data of these effectors in lettuce.

P019

Using newly isolated bacteriocins to treat blackleg disease of potatoes

Andrea Rico Montaña[1], Ray Chai[2], Janet Laird[1], Daniel Walker[2], Joel Milner[1], Ciara Keating ORCID iD[1,3]

[1]Plant Science Group, College of Molecular, Veterinary & Life Sciences, University of Glasgow, Glasgow, United Kingdom. [2]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom. [3]School of Biodiversity, One Health, and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom

Abstract

Blackleg disease is a softrot disease of potatoes, which loses the UK production industry £50M every year. In the UK, disease is principally caused by enterobacterial soft rot phytopathogen *Pectobacterium atrosepticum*, but can also be caused by *Pectobacterium parmentieri* and *Pectobacterium brasiliense*. Bacteriocins are proteinaceous, highly selective antibacterial proteins weaponised by bacteria to kill related species and establish dominance in a niche. If used against pathogens, they could comprise a safer alternative to chemical pesticides as they allow for selectivity. PX1 is a bacteriocin produced by specific species of *Pectobacterium* and could potentially be used as a sustainable treatment against blackleg disease. *Pectobacterium spp.* growth and pectolytic activity vary with temperature. Therefore, bacteriocins must be effective for multiple *Pectobacterium spp.* and across a range of temperatures. Using the spot test methodology, we examined the efficacy of PX1 against different species of *Pectobacterium* (*P. atrosepticum, P. parmentieri* and *P. brassiliense*) at different temperatures (15°C and 27°C). We determined that nearly all of the 25 UK *P. atrosepticum* isolates tested were highly susceptible to PX1, even when the bacteriocin was diluted to 6 nM concentrations. Mutants that developed resistance after treatment were isolated for future characterization of the PX1 mechanism of action. In contrast, two out of three *P. brasiliense* isolates tested and all 7 strains of *P. parmentieri* tested were resistant to PX1. Overall, we established that PX1 is highly effective at killing *P. atrosepticum* at temperatures between 15°C & 27°C and shows potential as a biocontrol agent.

P020

Genetic diversity of Barley yellow dwarf virus (BYDV) across the UK

Lawrence Bramham ORCID iD, Izayana Sandoval-Carvajal, Kim Hammond-Kosack

Rothamsted Research, Harpenden, United Kingdom

Abstract

Sap-sucking aphids detrimentally impact plants through direct feeding and the secretion of sucrose rich honeydew, promoting saprophytic fungi and reducing the photosynthetic ability of a colonised host. The capacity to also readily transmit devastative plant viruses renders aphids a notable threat to important crop species. Common wheat, *Triticum aestivum*, is one such species affected by aphids and the viruses they transmit, most prominently Barley yellow dwarf virus (BYDV). In terms of both worldwide distribution and economic significance, BYDV is one of the most important viral diseases affecting cereal crops, reducing yield by up to 80%. Genetic sources of resistance to aphids and/or BYDV may provide an attractive control solution; this forms the basis for much of our work.

The presence and variation of BYDV strains across the UK has not been thoroughly explored, with purely strains 'MAV' and 'PAV' reported. This is despite knowledge of BYDV diversity being crucial for developing improved diagnostics, robust monitoring, effective disease management and the identification of resistance-breaking viral variants. Here, work has explored UK BYDV diversity via viral coat protein sequencing and phylogenetic analyses using viruliferous aphid samples collected from suctions traps across the nationwide Rothamsted Insect Survey .

Besides BYDV-MAV and PAV already known to be prevalent across the UK, our results suggest the widespread occurrence of an unreported BYDV strain, PAS; this has potentially significant ramifications and may be a key finding for improved disease management. Identified UK BYDV substrain variation has also assisted with the development of UK-specific diagnostic assays.

Session Topic: Microbiology meets machine learning

P021

Identifying genetic events that potentiate multi-drug resistance in *Escherichia coli*

Elizabeth Cummins ORCID iD, Alan McNally ORCID iD, Nicole Wheeler ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Multi-drug resistant (MDR) *Escherichia coli* poses an alarming threat to global public health. MDR clones of *E. coli* account for a significant proportion of bloodstream and urinary tract infections, which are becoming increasingly difficult to treat with antibiotics. New methods to understand how these pathogens emerge by identifying key evolutionary events throughout their formation will be vital for future treatment strategies. The ever-increasing generation of genomic data provides a rich pool of information that microbiologists can now explore with complex computational techniques to answer important research questions. What are the common genetic signatures that underpin the evolution of MDR clones? Do specific mutations or gene gain and loss patterns potentiate MDR clone formation? We have curated a dataset of over 20,000 genomes representing the full phylogenetic diversity of *E. coli* to allow us to address these questions. We quantified the burden of atypical mutations in protein coding genes using the delta bitscore metric to then apply a machine learning approach to probe this unique dataset. We assess the ability of a random forest classifier to identify atypical mutations in protein coding genes that are precursory to MDR *E. coli* clone formation and evaluate how well signatures identified by the random forest generalise across distinct *E. coli* lineages.

P022

AURORA: a new machine learning tool for analyzing bacterial habitat adaptation

Dalimil Bujdoš ORCID iD[1,2], Jens Walter ORCID iD[1,2,3], Paul O'Toole ORCID iD[1,2]

[1]APC Microbiome, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland.
[3]Department of Medicine, University College Cork, Cork, Ireland

Abstract

Understanding which genes contribute to the fitness of microbial strains in a habitat is one of the main goals of functional genomics. However, there is a lack of computational approaches that facilitate such a task. This type of analysis falls under a group of methods called Genome-Wide Association Studies (GWAS). There are already several bioinformatic platforms for microbial GWAS, but available tools have limitations as they use statistical hypotheses that are not appropriate for the analysis of genes responsible for habitat adaptation. We developed a new tool called AURORA (AUtochthonous, Random fOrest, Random wAlk) that can deal with confounders of typical GWAS (population structure and recombination). Additionally, AURORA has two functionalities: machine learning algorithms, able to recognize strains that differ significantly based on gene content from other strains from the same habitat. These strains are most likely allochthonous and their presence in the dataset reduces statistical power to detect significant associations. However, AURORA can also identify cases where the analyzed strains are “nomadic” (i.e. are not specialized to a habitat). We demonstrate the utility of AURORA on *Limisilactobacillus reuteri* whose adaptations to the rodent gastrointestinal system have already been intensively studied, and essential genes experimentally identified. We show that AURORA can unambiguously identify essential genes while other tools struggle with false negatives. Moreover, we demonstrate that AURORA correctly classified *Lactiplantibacillus plantarum* as a nomadic species. AURORA is implemented in the R programming language as an open-source package.

P023

Resolving the prokaryote-to-eukaryote transition using new phylogenomic models of endosymbiosis

Anna Nenarokova ORCID iD[1], Nina Dombrowski ORCID iD[2], Lénárd Szánthó ORCID iD[3], Philip C. J. Donoghue ORCID iD[1], Davide Pisani ORCID iD[1], Gergely J. Szöllősi ORCID iD[3], Anja Spang ORCID iD[2], Tom A. Williams ORCID iD[1]

[1]University of Bristol, Bristol, United Kingdom. [2]Royal Netherlands Institute for Sea Research, Den Burg, Netherlands. [3]Eötvös Loránd University, Budapest, Hungary

Abstract

The origin of eukaryotes is a crucial event in the history of life on Earth. The development of complex eukaryotic cells allowed for the emergence of more advanced organisms, paving the way for the incredible diversity of life we see today.

While the details of this transition are still the subject of ongoing research, the scientific consensus is that the last common ancestor of eukaryotes was a chimeric organism, formed from the merger of at least two different prokaryotic cells - an Asgard archaeon and a bacterium, close to modern alpha-proteobacteria. However, the specific order of events and the sequence in which the different features of the eukaryotic cell emerged is not well understood. There is also controversy about the number of symbiotic partners that contributed to eukaryogenesis.

In this study, we are using new phylogenomics methods that take into account the endosymbiotic nature of eukaryotic organisms, recognizing that eukaryotic genes can have multiple evolutionary histories. For instance, eukaryotic genes can come from the archaeon, the mitochondrial symbiont, or the other symbionts that may have been engulfed along the way, or could be acquired as a result of horizontal gene transfers.

With the help of these methods, we are planning to investigate the prokaryotic origins of individual eukaryotic families and the timing of individual events during eukaryogenesis.

P024

Feed Your Model More Protein: Novel features based on viral protein composition and predicted interaction improve and slimline models of viral zoonoses

Matthew Arnold ORCID iD[1,2], Simon Crouzet ORCID iD[3], Nardus Mollentze ORCID iD[2,1], Daniel Streicker ORCID iD[1,2], Simon Babayan ORCID iD[1]

[1]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom. [2]MRC Centre for Virus Research, University of Glasgow, Glasgow, United Kingdom. [3]Sorbonne University, Paris, France

Abstract

Of known viruses, over half infect multiple species and spillover events between populations can lead to outbreaks of disease, both from novel viruses and novel strains of known viruses. Tools to inform viral surveillance are therefore essential to controlling the risk of emerging viral disease, so the maintenance host populations for these viruses can be rapidly identified and effective control measures designed. The "need for speed" in epidemic response recommends the use of models based on viral genome sequence.

Using machine learning models based on gradient boosted tree algorithms, we have leveraged viral genome data to make predictions of the host taxon of a virus based on features calculated as biologically-informed summary metrics of its genomic information. Our previous approaches centred on models based on viral phylogeny and summarised genome composition. We present here improved models incorporating novel amino acid- and protein motif-based features, integrating functional understanding of viral proteins and their host interactions into our genome-derived models, which achieve state-of-the-art accuracy.

Using these novel renderings of viral sequences we propose a lightweight model with similar predictive capabilities using a smaller set of features designed to incorporate viral protein post-translational modification and protein-protein interaction. Additionally, we find that these sequence representations can be mapped to phylogeny, providing insights into virus-host coevolution.

Crucial to the development of these models is engagement with real-world application; our future work will aim to deliver our models to stakeholders via a user-friendly interface and gain feedback on avenues to improve model performance and usefulness.

P025

A Machine Learning Approach to Understanding Pangenomes

JAMES MCINERNEY, Alan Beavan

University of Nottingham, Nottingham, United Kingdom

Abstract

We estimate the causal dependence on genetic background for the establishment of an incoming gene or set of genes. We do this by development of a machine learning approach where we evaluate the "importance" of the contribution of genetic background to the presence or absence of a focal gene. We use a diverse set of complete genomes from *Escherichia coli*. These genomes have evolved divergent gene content over time – so much so, that a gene that is horizontally transferred from one sequence type (ST) to another will find itself in a considerably different ensemble genetic background. In many ways, we are testing a natural equivalent of a "historical difference experiment". To understand the roles of contingency, repetition, and determinism on pangenome evolution, we developed a random forest approach, where decision trees are constructed using nodes to represent a gene family within the pangenome. At each node, the genomes are divided into two groups, according to whether a member of the gene family is present or absent. In this way, the decision tree can predict the presence or absence of a gene based on the path taken through the tree. We find that evolutionary history has structured the *E. coli* pangenome in a way that results in a significant amount of gene content predictability in any given genome, and that evolution has repeated itself in this pangenome many times. This is the first incarnation of a machine learning system of modelling pangenomes. Future versions will include promoters and other metadata.

P026

Investigating the antimicrobial effects of metal-doped bioactive glass fibres on chronic wound biofilms - an experimental and computational approach

Sandeep Shirgill ORCID iD[1], Sarah Kuehne[1], Gowsihan Poologasundarampillai[1], Sara Jabbari[1], John Ward[2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Loughborough University, Loughborough, United Kingdom

Abstract

Chronic wounds (principally pressure sores, venous leg ulcers and diabetic foot ulcers) are a drain on global health services and remain a major area of unmet clinical need. Chronic wounds are characterised by a bacterial biofilm which hinders innate immune response and can prevent wound healing. Recently, research has delved into "biofilm-based wound care" as an effective strategy for healing wounds, where the first step of treatment is to disrupt the biofilm. Bioactive glass fibres, which have a "cotton-wool" appearance, offer a promising biofilm-based wound care treatment for chronic wounds. Fibres can be doped with antimicrobial metal ions, for example, silver and copper, which will be released when fibres are packed into the wound. Here, an in vitro chronic wound biofilm model has been developed. This model is used to test the antimicrobial activity of fibres doped with silver and copper on single-species and dual-species biofilms containing common bacterial species found in chronic wound biofilms *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Ag-doped fibres possessed stronger antimicrobial effects against single-species *P. aeruginosa* biofilms and dual-species biofilms than Cu-doped fibres, which were more antimicrobial against single-species *S. aureus* biofilms. However, there was a larger reduction in viability in biofilms treated with copper-doped fibres followed by silver-doped fibres rather than 2 sequential treatments of silver-doped fibres. Experimental results are compared to results from a partial differential equation mathematical model of the system, furthermore, the mathematical model is used to make predictions about the likelihood of biofilm eradication based on fibre and biofilm properties.

P027

Bioinformatic analysis of metagenomic and RNA expression data from 57 colorectal cancer samples and controls allow identification of certain bacteria in the tumour microenvironment

Onalenna Neo[1,2], Steven Van Laere[3], Sarah A. Kuehne[1], Fedor Berditchevski[2]

[1]Oral Microbiology Group, School of Dentistry and Institute of Microbiology and Infection, College of Medical and Dental Sciences, The University of Birmingham, Birmingham, United Kingdom. [2]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom. [3]Faculty of Medicine and Health Sciences, Campus Drie Eiken, University of Antwerp, Universiteitsplein 1 2610 Wilrijk, Antwerp, Belgium

Abstract

An association between certain bacteria and colorectal cancer (CRC) has recently been established. We analysed metagenomic and gene expression data from 57 CRC tumour samples and their normal paired controls. Using the R-tsne algorithm the samples were grouped based on their metagenomic profile. Five clusters were identified and analysed with Deseq2 to reveal significant differences in the metagenomic profiles of the groups. Bacteria such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were significantly associated with tumour-sample rich clusters and a depletion of bacteria such as *Bifidobacterium breve* - a potential probiotic was observed. Differences in gene expression between clusters with high abundance of these bacteria and those with low abundance were observed. Significant differences in the expression of genes such as those involved in apoptosis and in cellular proliferation were noted. R-tsne clusters were also generated based on the expression profile of the samples. Consensus TME was used to estimate immune cell abundance allowing for the determination of differences in immune cell activity between clusters. Furthermore, pathway analyses were performed to determine the activity of hallmark signalling pathways between the clusters. Several tetraspanin proteins are known to regulate attachment of various bacteria to epithelial cells. Therefore, pathway activities in function of Tetraspanins were investigated and a correlation analysis performed. Differences in the activities of hallmarks such as myc targets and WNT beta signalling were positively correlated with high expression of TSPAN6. Understanding the metagenomic and expression profile of CRC tumours can further the understanding of the role of bacteria in CRC.

P028

Detecting higher-order interactions in pangenomes with random forests

Nathan Heslop[1], James McInerney[2], Conor Meehan ORCID iD[1], Maria Rosa Domingo-Sananes ORCID iD[1]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Gene-gene interactions between accessory genes in pan-genomes have been an area of focus in recent years, with programmes dedicated to detecting possible links between genes being developed. However, most of these programmes are only able to detect pairwise interactions between genes and many of the interactions can be explained by the phylogenetic signal of the pan-genome. To identify more complex relationships between genes, we ask if it is possible to predict the presence of a focal gene based on the gene content of the rest of the genome. If prediction is possible, this implies the presence of interactions between the focal gene and other accessory genes. To test whether the prediction of gene presence could be used to identify interactions we downloaded 788 publicly available Rhizobium leguminosarum genomes from the genome taxonomy database. We then filtered based on multiple metrics to select only high-quality genomes and constructed a pangenome. After only retaining accessory genes with low phylogenetic signal, we trained random forest classifiers to predict the presence of each gene based on the rest of the accessory genome. We then extracted feature importance to construct networks that indicate how genes contribute to explain the presence of each other. Groups of tightly connected genes could signify biologically relevant relationships between genes and/or environments. We expect that this approach can be easily applied to multiple species and other types of data (e.g., single-nucleotide polymorphisms and phenotypes) and can help direct further research.

P029

A critical analysis of tandem mass spectra in the context of *de novo* peptide identification

Kevin McDonnell ORCID iD, Enda Howley ORCID iD, Florence Abram ORCID iD

University of Galway, Galway, Ireland

Abstract

Proteomics involves the characterisation and analysis of protein expression in a sample. Typically tandem mass spectrometry and peptide fragmentation are followed by a database search to identify peptides (and hence proteins) from the mass spectra. However, using this approach, 75% of spectra remain without a significant peptide match. While historically database searching provided greater sensitivity, advancements in *de novo* peptide identification directly from mass spectra makes it a viable alternative. A deep knowledge of mass spectra data is key to the optimal design of *de novo* algorithms, which ideally could be tested, refined and evaluated using artificial data. To this end, we characterised tandem mass spectra with regards to noise and missing fragmentation cleavages. Noise peaks were found to account for more than 80% of the total peaks, while more than 70% of the spectra had at least one missing fragmentation cleavage. By modelling noise and introducing it into artificial spectra, we were able to substantially increase the performance of a state-of-the-art algorithm (PointNovo) trained on augmented artificial data and tested on real data. This work highlights the potential improvements that can be made to the utility of artificial spectra. Customisable test data in the form of realistic artificial spectra would allow for the evaluation of *de novo* algorithms under all possible conditions of both missing fragmentation cleavages and noise. Comprehensive algorithm evaluation and a greater understanding of the underlying data are essential to the design and development of the next generation of *de novo* peptide identification algorithms.

P030

The key roles of non-AMR annotated genes can be identified by interpretable machine learning techniques

Lucy Dillon, Christopher Creevey, Nicholas Dimonaco

Queen's University Belfast, Belfast, United Kingdom

Abstract

Background:

Antimicrobial resistance (AMR) genes are found to be ubiquitous within microbiomes, even when antimicrobial usage is absent. To identify AMR phenotype, the most common method is to use a laboratory-based assay. Yet, when dealing with microbiome samples, many species are difficult to culture within the laboratory. To avoid this, a computational approach may be more favourable. AMR gene finder tools are efficient at determining the AMR genotype, but they have a lower accuracy when predicting AMR phenotype and they do not investigate the action of non-AMR annotated genes. To address this, we generated 23 machine-learning (ML) models specific to the major drug classes of therapeutic antibiotics to allow accurate prediction of AMR phenotypes from genotypic data.

Methods:

To evaluate the relationship between AMR phenotype and the organism's genotype, 16,950 genomes from BV-BRC which had corresponding MIC values were used to build 23 decision tree (DT) models. Two different types of DT models were developed based on the presence and absence of AMR genes and all functional genes.

Results:

The DT models could predict AMR phenotype accurately using known AMR genes and from all functional genes, the average accuracy was 91.7% and 92.2% respectively. The results found that gene co-occurrence, presence and absence of AMR and non-AMR genes are key factors to analyse when identifying AMR phenotype from genomic data. Our results highlight the downfalls of commonly used AMR gene-finder tools. This emphasizes why we should continue to research the relationship between AMR phenotype and genomic data.

Session Topic: Clinical virology

P031

Induction of autophagy by Nucleocapsid protein of Peste des petits ruminants virus mediated by phosphatidylinositol-3-kinase complex

Yash Chaudhary, Rajeev Kaul

Department of Microbiology, University of Delhi South Campus, New Delhi, India

Abstract

Autophagy is a crucial and highly conserved catabolic process essential in the battle against intracellular pathogens. Viruses have evolved several ways to alter the host defense mechanism. Likewise, PPRV also interacts with components of defense system of the recipient host causing enhanced autophagy. In this study, we demonstrated that PPRV N protein interacts with core components of the class III phosphatidylinositol-3-kinase (PI3K) complex that induces autophagy in the host cell. The LC-MS analysis indicated the interaction of PPRV N protein with core components of PI3K-complex which were further validated by co-immunoprecipitation and GST-pulldown assay. The immunofluorescence studies were performed using confocal microscopy. The functional significance of these interactions was analyzed by monitoring the autophagic activity in cultured cells. The proteomics data suggested the interaction between PPRV N protein and cellular autophagy proteins. Co-immunoprecipitation data clearly showed that viral N protein and PI3K-complex proteins interact with each other stably in whole cell lysates. However, GST-pulldown assay showed that VPS34 protein of PI3K-complex interacts directly with PPRV N. Furthermore, co-localization studies showed that viral protein nicely co-localizes with the host proteins but the interaction does not affect the sub-cellular localization of the host protein. Formation of autophagosomes was identified morphologically by transmission electron microscopy (TEM). Immunoblot analysis of LC3B and p62/SQSTM1 indicated that PPRV N protein causes increase in autophagic activity inferring to induction of autophagy. Together, our findings provided the evidence that PPRV N protein has a critical role in the induction of autophagy mediated by PI3K complex of the host.

P032

Understanding the mechanism of action of novel antivirals using high content imaging

Charlotte Cumper, Rebecca Roberts, Sandy Kimber, Claire Richards, Louise Brackenbury

Charles River Laboratories, Portishead, United Kingdom

Abstract

Background The impact of infectious diseases, particularly of viral origins, on human health is enormous, exemplified by the COVID-19 pandemic. Viral infection is a complex process involving the interaction of both viral and host components, and many viruses have developed strategies to hijack host cellular pathways to enable propagation. One of the major reasons for a lack of effective interventions is limited understanding of viral entry and replication and how these can be targeted to limit disease. Here we use H1N1 influenza to demonstrate how high content imaging, alongside supportive assays and *in vivo* models, can be used to screen for efficacy testing, and to determine the mechanism of action of antivirals.

Methods Both primary and continuous cells were infected with H1N1, with or without antivirals over a time-course and co-stained for viral structural elements or replication markers, together with host sub-cellular localization markers. Images were taken using a Cell Insight CX7 HCS Platform. A range of antivirals were screened for their ability to prevent H1N1-induced cell death in an MTT assay. A murine infection model, testing antivirals, was also employed.

Results Antiviral screening showed inhibition of H1N1 infection *in vitro* and *in vivo* over-time. Specific antivirals blocked H1N1 entry, replication or egress as shown by quantitative high throughput imaging-based analysis of intracellular trafficking of the virus, supported by in vivo model data.

Conclusions Taken together, this screening platform combines *in vitro* and *in vivo* assays to offer a powerful approach to identify novel antivirals or nAb.

P033

The Pathogenesis of Human Papillomavirus (HPV) in Cancer of the Oropharynx

Rebecca Hanwell[1,2], Thomas Illingworth[1], Laurice Fretwell[1], Bindy Sahota[3], Elizabeth Marsh[1]

[1]University of Derby, Derby, United Kingdom. [2]HistologiX, Nottingham, United Kingdom. [3]Royal Derby Hospital, Derby, United Kingdom

Abstract

Background: Human papillomavirus (HPV)-mediated Oropharyngeal Cancer (OPC) incidence is increasing, with high-risk HPV16 and 18 causing ~86-90% of cases. OPC aetiology is not well established but believed to be comparable with Cervical Cancer, where HPV infects traumatised epithelium such as the tonsillar reticulated crypts. We will investigate the natural history of HPV within the oropharynx.

Methods: Biobank-sourced OPC sections (n=5) underwent p16 immunohistochemistry and HPV in situ hybridisation (HPV ISH) for HPV confirmation as per current NHS guidelines. Multiplex immunofluorescence was performed using CK7, EGFR, PD-1, PD-L1, ER and CD8 biomarkers, together with HPV16 E2/E6/E7 and HPV18 E6 antibodies.

Our clinical study (Derby, Burton, and Leicester hospitals) recruits adults over 18 years undergoing routine tonsillectomy. Participants donate non-cancerous tonsils and answer a short lifestyle questionnaire on demographics, sexual behaviours and drinking/smoking habits. HPV-positive tonsils identified by qPCR undergo histological characterisation and ancillary testing as above.

Results: Three OPCs were p16/HPV ISH-negative, with two OPCs p16/HPV ISH-positive. Both p16/HPV ISH-positive samples demonstrated non-keratinising and keratinising (hybrid) morphologies. Two p16/HPV ISH-negative samples demonstrated non-keratinising morphology, which is rare, as this is synonymous with HPV infection. Unfortunately, HPV antibodies demonstrated non-specific staining, with positive staining in negative controls and alternate tissue types.

Conclusions: Not all non-keratinising OPCs are p16/HPV ISH-positive, therefore it is not synonymous with HPV infection. Commercially available HPV antibodies tested to date show non-specific staining. The ongoing work will further understand OPC aetiology, and aims to establish alternative marker(s) for HPV detection in clinical samples.

P034

Expanding the capacity of PrimalScheme algorithms to handle pan-species variation

Dr. Josh Quick, Christopher Kent ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

During the last 3 years, amplicon sequencing has emerged as the dominant method for viral whole genome sequencing, due to its low cost and high sensitivity. Due to the widescale adoption and experience in Covid sequencing, this is an ideal and impactful time to further the ability of amplicon sequencing.

One of the main issues with the current generation of PrimerScheme design is variation between the primer and genome sequences cause reduced primer binding affinity, leading to amplicon dropout and loss of coverage. The old method of handling this is to avoid regions with variation. However, once variation reaches a certain point this "subtractive" approach leaves little to no available primers to use in the scheme.

In this project, we have generated new methods which can handle variation, and Tm normalisation via dynamic primer length, which vastly increases the number of available amplicons to use. These new methods have enabled the generation of pan-virus schemes such as pan-measles and pan-RSV, which have been designed to accommodate all variability currently found within the given TAXID.

A typing mode has also been generated, in which amplicons which the greatest Shannon entropy (variance) are picked, to provide the most information with the least number of amplicons. This enables, both differentiating between different virus species and the generation of typing information for the virus strain. As intra-virus variance is often due to selection pressure, the regions covered are biased towards regions of public health interest such as RBD and highly-antigenic sites.

P035

Developing Oncolytic Gene Therapies that Target Cancer Cells Expressing Defined Cell-Surface Molecules

Elif Aktar[1], Callum Ditondo[1], Graham P. Cook[2], Eric G Blair[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Leeds Institute for Medical Research, Leeds, United Kingdom

Abstract

Cancer is a major global disease because of its high morbidity and mortality. Although established treatments such as surgery, chemotherapy and radiotherapy have improved patient survival, new therapeutic approaches are needed. One approach involves oncolytic viruses which can be developed using conditionally-replicating adenovirus (CRAD) vectors. These vectors are designed to replicate selectively in cancer cells and kill them. We aim to develop CRADs as a potential therapy for pancreatic and other cancers of the digestive tract using cancer-specific promoters. Initial studies have been focussed on the identification of suitable cell lines that show either high or low levels of expression of the CEACAM5 protein that is often highly expressed in pancreatic cancers compared to normal pancreatic epithelial cells. The activity of the CEACAM5 proximal promoter (cloned in a reporter gene plasmid) in such cell lines was tested using luciferase assays. This confirmed that the CEACAM5 promoter was specifically active in cells that expressed high levels of CEACAM5 protein. The CEACAM5 promoter was then incorporated into an Adenovirus 5 genome in place of the immediate early E1A promoter by recombineering, for eventual intra-tumoral delivery of the virus. The Adenovirus 5 fibre was also replaced with that of Adenovirus 35 to expand the cell tropism of the virus. The resulting recombinant Adenovirus (pCEA-Ad5/F35) and the parental Ad5/F35viruses have been analysed by transduction of the identified cell lines by cell viability (MTS) and viral replication (intracellular hexon staining) assays. Results of these analyses will be presented.

Session Topic: Infection Forum

P036

Rosa indica mediated synthesis of titanium oxide nanoparticles and its effects on bacterial biofilms

MAHRUKH MAQBOOL[1], Saira Riaz ORCID iD[2], Anjum Nasim Sabri ORCID iD[1]

[1]Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid e Azam Campus, Lahore, Pakistan. [2]Center for solid state physics, University of the Punjab, Quaid e Azam campus, Lahore, Pakistan

Abstract

Nanoparticles synthesized by the use of medicinal plants have been proven more beneficial in the field of medicine. They can be used as an antimicrobial, antibiofilm, antitumor, and drug delivery agent.

Titanium dioxide nanoparticles were synthesized by using crude extract of *Rosa indica* , Titanium butoxide [Ti(OBu)4] (precursor), and Ethylene Glycol. The change in color from orange to dark brown confirmed the nanoparticle synthesis. The TiO2-NPs nanoparticles gave a characteristic peak at 294nm in the UV-Visible absorption spectrum. FTIR spectrum showed peaks at 467cm-1, 554cm-1, 1070cm-1, 1390cm-1 and 1638cm-1. The range of 2θ for the X-ray diffraction analysis set between 30°–80°, revealed the Brookite structure of Titania nanoparticles. Agar well diffusion assay and MIC determination showed growth inhibitory effects of TiO2-NPs and extract of *R.indica* on *Bacillus*, *E.coli & Pseudomonas.* 2,3,5- Triphenyl-Tetrazoliumchloride reduction showed that non respiring cells appeared creamy white, and viable cells appeared red in color on the plate around clear zones. Cell proliferation MTT ((3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay showed the change in color in the presence of metabolically active cells.

With the increasing concentration of TiO2- NPs and rose extract slime production, bacterial cell adherence, cell aggregation, planktonic cell density, tightly bound cells decreased significantly, which is a clear indication of antibiofilm activity. Hence the synthesized nanoparticles are a good candidates for biofilm dispersion/ detachment and have a killing effect on bacteria.

P037

Antimicrobial susceptibility of Escherichia coli isolated from diabetic patients in Mogadishu, Somalia

Shafie Hassan ORCID iD[1], Yousif Alobaid[2], Bashiru Garba ORCID iD[3]

[1]Jamhurya univesity of science and technology, Mogadishu, Somalia. [2]Univresty of El imam E Mahdi, Kosti, Sudan. [3]Department of Veterinary Public Health & Preventive Medicine, Usmanu Danfodiyo University Sokoto, Sokoto, Nigeria

Abstract

Background:

The most common organism that causing urinary tract infections in diabetic patients is Escherichia coli, which is also one of the most common resistant bacteria in humans. Diabetic patients are more likely to develop urinary tract infections due to frequent urination and high blood sugar levels. The aim of this study was to determine the antimicrobial susceptibility of Escherichia coli isolated from diabetic patients in Mogadishu, Somalia

Methods: This is a descriptive cross-sectional study conducted at Ummah Hospital, Mogadishu, Somalia, from November 2020 to April 2021. Urine samples were taken from diabetic patients attending or admitted at the hospital with target sample size of 350 participants. All samples were cultured in CLED media (Himedia, India). Biochemical tests were performed to identify the isolated organisms. Kirby-Bauer disc diffusion was used to determine antibiotic susceptibility.

Results: Out of the total 350 urine, E. coli was isolated in 220(63%). All isolates were found to be resistant to cefpodoxime (100%). In addition, high resistance rates were observed with ofloxacin (90.8%), ciprofloxacin (77.5%), amikacin (60.8%), ceftriaxone (58.3%) and cefepime (51.7%). The most sensitive antibiotics were colistin and imipenem (99.2% and 88.3% respectively) followed by gentamycin (70%).

Conclusions: The results of this study show high rates of antimicrobial resistance to ofloxacin, ciprofloxacin, amikacin, ceftriaxone and cefepime. colistin, imipenem and gentamycin are considered suitable for empirical treatment of E. coli in the study area. Continuous antimicrobial susceptibility monitoring in both community and hospital settings is recommended.

Keywords: UTI, diabetes mellitus, E. coli, Antibiotic resistance, Somalia

P038

A modified flow model for growing multi-species chronic wound-like biofilms.

Ammara Khalid[1], Alan R Cookson[2], David Whitworth[2], Mike Beeton[1], Lori Robins[3], Sarah E Maddocks[1]

[1]Cardiff Metropolitan University, Cardiff, United Kingdom. [2]Aberystwyth University, Aberystwyth, United Kingdom. [3]University of Washington, Washington, USA

Abstract

BACKGROUND

Understanding chronic wound infection is limited by a paucity of relevant laboratory models. A model with clinical relevance has value for wound care, diagnostics, antimicrobial stewardship, and advancing knowledge of microbial interactions in complex biofilm infections.

METHODS

A biofilm flow device was modified to mimic the chronic wound environment. This included introducing simulated wound fluid, a collagen-based 3D biofilm matrix, and a five-species mixture of clinically relevant bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Citrobacter freundii). Total viable counts determined population composition over 14 days, and reduction of resazurin for metabolic activity. Transmission electron microscopy and confocal microscopy established biofilm architecture, and the efficacy of topical antimicrobial treatments was determined by total viable count over 72h.

RESULTS

Mixed biofilms were cultured for 14 days with consistent numbers of bacteria that exhibited reduced metabolic activity, which increased with a high dose of glucose. S. aureus was recovered from biofilms as a small colony variant, but as a normal colony variant if P. aeruginosa was excluded from the system. Bacteria within the biofilm did not co-aggregate but formed discrete species-specific clusters. Biofilms demonstrated differential tolerance to the topical antimicrobials Neosporin and HOCl, consistent with protection due to the biofilm lifestyle.

CONCLUSION

The modified a polymicrobial biofilm flow system simulated a chronic infected wound where the bacteria therein exhibited characteristics matching those of real-world wound biofilms. This yields a powerful in vitro tool that is versatile, inexpensive, and pivotal for understanding chronic wound infection.

P039

Knowledge and practices regarding toxoplasmosis among High School and University female students, and pregnant women: A cross sectional study in Kuwait

Jamshaid Iqbal, Najat Khadadah

College of Medicine, Kuwait, Kuwait

Abstract

This study retrospectively analyzed the seroprevalence of anti-T. gondii antibodies among a total of 2262 subjects during a nine-year period (January 2010 to December 2018) in Kuwait. We documented a low level (540 of 2262; 23.9%) of seropositivity for T. gondii among all subjects. Although there was no significant difference in seropositivity rate between male and female subjects, the number of seropositive non-Kuwaiti females (294; 71.4%) was significantly higher than Kuwaiti females (118; 28.6%) (P<0.001%). The annual number of seropositive cases varied between 28-103 cases during the study period. The age of subjects ranged from <1 to 90 years with a mean of 30.2 years (95% CI: 29.6, 30.8). The mean age of seropositive female subjects (34.4 years) was significantly higher than that of males (28.7 years) (P=0.001). Furthermore, the seropositivity was significantly higher in subjects >30 years of age in comparison with those <30 years of age (P<0.001). An overwhelming majority of seropositive subjects had anti-Toxoplasma IgG antibodies (n=508; 94.1%) while both IgG and IgM antibodies were found in 29 (5.4%) subjects. Only 3 (0.5%) patients were positive for IgM antibodies alone. The seroprevalence rates recorded in this study were significantly lower than the incidence of 53.1% for IgG and 13.8% for IgM anti-T. gondii antibodies reported earlier in a retrospective cohort study conducted >10 years ago. The decreasing trend in toxoplasmosis seroprevalence in Kuwait is attributed to improvement in economic and literacy status, and well-organized awareness campaigns by increasingly modern health facilities throughout the country.

P040

Factors impacting movement of antimicrobial resistance genes within biofilms

Ho Yu Liu ORCID iD[1,2], Mark Webber ORCID iD[1,2]

[1]Quadram Institute Biosciences, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Most infections involve a biofilm component, and these infections are associated with poor patient outcomes due to the intrinsically antimicrobial resistant (AMR) nature of cells when growing as a biofilm. As well as this inherent resistance to antibiotics, it has been shown that horizontal gene transfer (HGT) can occur within biofilms allowing evolution of high-level resistance. However, it is not clear what factors influence the rate of HGT within biofilms.

To study this, we established a model multispecies biofilm community. A panel of *Escherichia coli* food isolates were grown in competition with *Salmonella enterica* serovar Typhimurium, and viable numbers of each species were measured over time. The *E. coli* and *S.* Typhimurium mixed species biofilms were grown on different substrates and at different temperatures to test their stability under different conditions. Marked versions of both species were created to monitor plasmid movement between species within communities.

Three *E. coli* food isolates were found to co-exist and form stable communities with *S.* Typhimurium (with no significant differences ($p \geq 0.05$) found between numbers of the two species on both glass and steel beads) at both 37°C and 22°C. A range of common epidemic plasmids carrying different resistance genes have also been characterised and assessed for their mobility in both species. We have used this model to assess factors that impact the movement of an IncFII plasmid carrying $bla_{\text{CTX-M-14}}$ between the species in different conditions. We describe here some key conditions that impact the development of AMR within biofilms.

P041

Characterising the Role of Kupffer Cells During Infection in an Ex vivo Liver Segment Perfusion Model

Shiying Tang[1], Neama Alnabati[1], Trisha Kanani[1,2], Wen Chung[2], Ashley Dennison[2], Marco Oggioni[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University Hospitals of Leicester, Leicester, United Kingdom

Abstract

Liver resident macrophages, Kupffer cells (KCs), are essential in maintaining homeostasis of the liver and the development of immunotolerance. Recent data in a murine sepsis model suggested Streptococcus pneumoniae evades uptake by KCs through a serotype dependent manner. However, differences in microanatomy between species can lead to translatability issues therefore, we aim to investigate if serotype dependent uptake by KCs also occurs in a human liver segment perfusion model.

Authorisation was approved for the perfusion and infection of human liver segments and spleens (ClinicalTrial.gov Identifier: NCT05255042, REC 21/PR/0287, and ClinicalTrial.gov Identifier: NCT04620824, REC 18/EM/0057 respectively). Each organ was perfused with supplemented Oxyglobin and infected with a cocktail of S. pneumoniae and Streptococcus mitis strains containing a mixture of invasive and carriage serotypes for six hours. Samples were taken for bacterial counts and microscopy analysis.

Serotype-dependent uptake by KCs was not observed in both 106 and 107 CFU doses. In comparison to the spleen, majority of the bacteria were removed within less than an hour of infection at 107 CFU. At a higher infective dose of 108 CFU, the spleen again was able to demonstrate effective killing independent of serotype expression. Tissue resident macrophages in both organs was found to be associated with bacteria.

The results in this study suggested that the role of tissue resident macrophages differs from each organ and although KCs may not play a huge role in differential killing of pneumococcus, it does partake in bacterial killing potentially at a lower efficiency compared to the spleen.

P042

The role of sub-species variation in *Fusobacterium nucleatum* in virulence of human disease

Kitty Guo, David Edwards

University of Dundee, Dundee, United Kingdom

Abstract

Historically, *Fusobacterium nucleatum* has been described as a commensal organism, but discovery of its role in the development of two of the most abundant non communicable diseases worldwide: caries and periodontitis, along with the well-defined mechanisms in the development of colorectal cancer has led to its emergence as an important human pathogen.

This Gram negative anaerobe has a complex genome that includes genes displaying a diverse evolutionary origin, extending to those found in Gram positive and archaeal genomes. This complexity extends within the species itself, with four assigned sub-species which cannot be distinguished through traditional 16s rRNA identification.

We present an evaluation of sub-species variation in virulence and antimicrobial sensitivity through virulence factor screening by PCR and in vitro testing against a panel of antimicrobial agents. These tests have been validated on four test strains and extended to clinical isolates obtained from the oral cavity of healthy volunteers.

Our approach seeks to establish how variation in the carriage and gene dosage associated with key virulence factors relates to the development of pathologies including cancer. In addition, we wish to understand how sensitivity to clinical and experimental antibiotics differs between sub-species and individual isolates, and consequently evaluate different therapeutic approaches for targeting F. nucleatum.

We hope that that our findings will provide a foundation for improved surveillance and treatment of this pathogen.

P043

Combined effects of acetic acid, commercial vinegars and medical-grade honey on biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Freya Harrison, Anisa Blower, Erin Connelly

University of Warwick, Coventry, United Kingdom

Abstract

Vinegar and honey are common ingredients in historical and traditional medical remedies across time and across cultures, often being combined to treat skin, wound and other soft-tissue infections. The individual potential of these complex natural products to kill pathogenic microbes is reflected in current clinical use of acetic acid and medical-grade honeys to treat wounds colonised with biofilm-forming bacteria. We characterised a range of commercial vinegars and found that some vinegars had lower MICs against *Staphylococcus aureus* and/or *Pseudomonas aeruginosa* than would be predicted from their acetic acid content alone. In one case, this translated into vinegar having a much lower EC_{50} against mature biofilms than predicted from its acetic acid content, suggesting the presence of compounds that potentiate or synergise with acetic acid to enhance biofilm-killing efficacy. Further, we assessed the ability of selected vinegars, and acetic acid, alone and in combination with medical-grade honey wound gels, to eradicate mature biofilms. Our results show potential for combined acetic acid + honey treatments to yield synergistic activity against *S. aureus* biofilms, and for certain vinegar+honey combinations to yield synergistic activity against both *S. aureus* and *P. aeruginosa* biofilms. There has been very little research into the potential for vinegars to contain compounds which potentiate or synergise with the antibacterial activity of acetic acid, and almost no published research into combination acetic acid + honey dressings. Our results suggest that both of these research avenues could produce useful suggestions for further development of effective bioactive dressings to combat biofilm infection.

P044

A Review of the Inter-laboratory Variability of Caspofungin MICs for *Nakaseomyces glabrata* isolates

Saied Ali ORCID iD, Meadhbh Collison, Sinead McNicholas, Sinead McDermott

St. Vincent's University Hospital, Dublin, Ireland

Abstract

Background
The use of automated broth-microdilution systems for caspofungin antifungal susceptibility testing (AST) for *Nakaseomyces glabrata* is prone to high inter-laboratory variation. We aim to review caspofungin minimum inhibitory concentrations (MICs) of clinically significant *N.glabrata* isolates, as reported locally and compare with reference laboratory results for the same isolate.

Methods
All clinically significant *N.glabrata* isolates from 2019-2021 inclusive were reviewed. Caspofungin MICs were obtained locally using the VITEK2 system and strains were referred to the Mycology Reference Laboratory for confirmatory testing via E-Tests. MICs were compared using categorical and essential agreement.

Results
Of the forty-one isolates reviewed, marked discrepancies were noted in interpretative breakpoints between assays, producing 16 Minor and Major Category Errors. Categorical agreement was found to be 22%, with the VITEK2 over-estimating resistance. Doubling dilution differences revealed an essential agreement of 61% within ±1 log2 dilution.

Conclusion
Performing caspofungin AST using broth-microdilution methods is prone to high inter-laboratory variation, and potentially results in the mis-classification of susceptible isolates as highlighted in our study. The use of E-tests has been shown to be highly reliable and not prone to inter-laboratory variation. It may be worthwhile looking into adopting a similar testing approach locally from an economic and patient-centred view.

P045

Using CRISPR-CAS9 screens to uncover genes of interest in avian viral oncogenic diseases

Toby Look[1], Yongxiu Yao[1], Venu Nair[1], Finn Grey[2]

[1]The Pirbright Institute, woking, United Kingdom. [2]The Roslin Insituite, Edinburgh, United Kingdom

Abstract

Avian oncogenic viruses cause a significant burden both financially and to global food security within the commercial poultry farming industry. Marek's Disease Virus (MDV) and Avian Leukosis virus (ALV) are two of the most notable. MDV is a highly contagious viral neoplastic disease in chickens resulting in T-cell lymphomas. Avian leukosis virus is a retrovirus that can lead to B-cell lymphomas. Both viruses currently cause sporadic outbreaks and are endemic globally. In MDV T-cell malignancies arise from MDV latent infection but the processes contributing to the transformed phenotype remains to be thoroughly investigated. To identify essential genes of the transformed phenotype of host (HP8) cells we performed a genome-wide loss-of-function screen in HP8 cells. We mutagenized an MDV-transformed cell line with 75,000 sgRNAs. Cells lost during selection from the pool of sgRNA-transduced cells most quickly could be identified by targeted PCR and sequencing. The HP8 cell line screen identified 281 host genes uniquely essential to HP8 cells. ALV have a defined set of specific receptors for viral entry to host cells, beyond these receptors less is known on genes required for ALV infection and replication. To identify these genes, we used a similar methodology but subsequently infected with ALV-GFP virus in combination with fluorescent associated cell sorting we identified 675 genes involved in viral infection and replication. Using this unbiased systematic approach to analyse genes has the potential to guide and increase our understanding of viral transformation in MDV and the requirements for ALV infection and replication.

P046

Pseudomonas aeruginosa isolates recovered from catheter-associated urinary tract infections in an Egyptian hospital show phenotypic and genotypic heterogeneity

Mohamed Eladawy ORCID iD[1,2], Jonathan Thomas ORCID iD[2], Lesley Hoyles ORCID iD[2]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. [2]Department of Biosciences, School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom

Abstract

Catheter-associated urinary tract infections (CAUTIs) represent one of the major healthcare-associated infections in Egypt. *Pseudomonas aeruginosa* is a common bacterium linked to CAUTIs, yet little is known about *P. aeruginosa* isolates contributing to CAUTIs in Egyptian clinical settings. Between October and November 2021, 31 isolates were recovered from the Urology and Nephrology Center, Mansoura, Egypt. These were characterized phenotypically [antimicrobial resistance (AMR), biofilm formation] and by whole-genome sequencing. They were confirmed to be *P. aeruginosa* based on average nucleotide identity analysis with the genome of *P. aeruginosa* DSM 50071^T. The genomes represented eight different multilocus sequence types – 244, 357, 381, 621, 773, 1430, 1667, 3765. AMR testing (EUCAST) showed the isolates were highly resistant to quinolones, and to a lesser extent tobramycin and cephalosporins. *In silico* analyses revealed all isolates encoded an arsenal of AMR genes predicted to confer resistance to aminoglycosides, β-lactamases and fluoroquinolones, with many of these (*OXA-395, OXA-494, OXA-520, OXA-846, OXA-847, OXA-903, OXA-914, PDC-5, PDC-11, PDC-14, and PDC-16*) not previously reported in Egypt. One isolate carried a pBT2436-like megaplasmid, representing the first report of such a genetic element in *P. aeruginosa* from the Middle East and North Africa region. Isolates formed strong (24/31), moderate (6/31) or weak (1/31) biofilms. Characterization of their biofilm-associated genes is ongoing. The present study represents a detailed analysis of *P. aeruginosa* isolates contributing to CAUTIs in Egypt. Results will inform development of improved monitoring and treatment regimens for CAUTIs in the source hospital and potentially throughout Egypt.

P047

Streptococcus gallolyticus and its Associations: A Quality Assurance Study

Saied Ali ORCID iD[1], Marcela Merc[2]

[1]St Vincent's University Hospital, Dublin, Ireland. [2]University Hospital Waterford, Waterford, Ireland

Abstract

Background

Studies suggest that S*treptococcus gallolyticus* bacteraemia is associated with colonic tumours (premalignant and malignant) and full bowel examination is recommended. Furthermore, these lesions may serve as portals of entry for invasive infections. The aim of this study is to assess whether these associations are noted through evaluation of treatment and referral pathways for *S.gallolyticus* bacteraemic patients.

Methods

A retrospective chart review was performed for all inpatient presentations resulting in a positive blood culture for *S.gallolyticus* from January 2015 to January 2020 inclusive. Demographic data, antimicrobial therapy and investigative information including echocardiography, radiology and endoscopy was noted.

Results

A total of 11 patients were identified, with an average age of 79 years. All patients suffered significant co-morbidity. Each individual was treated with targeted antibiotic therapy, validated by follow-up negative blood cultures. Echocardiography outruled infective endocarditis in all cases. Simple contrast computerized tomography was performed for all persons, with a colorectal tumour identified in 1 person. 8/11 cases proceeded to undergo colonoscopy. 2 cases revealed adenocarcinomas, 3 cases identified non-dysplastic adenomas, and the remainder was clear. There was no endoscopic follow-up. The remaining 3 patients were neither referred nor had any evidence of endoscopic evaluation.

Conclusion

Majority of cases were in line with recommendations understanding the relationship between *S.gallolyticus* and colorectal cancer. However, notable deficits lie in the absence of scheduled follow-up. Whether this was due to patient preference, endoscopist decision or reassurance of a single negative endoscopy report or clear radiographic imaging is unclear and further study is recommended.

P048

Non-Ionic Surfactant Vesicles as Drug Delivery Systems for Antimicrobials

Holly Van Dessel[1], Stuart Woods[2], Riccardo D'Elia[3], Gary Burgess[3], Craig Roberts[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of the West of Scotland, Paisley, United Kingdom. [3]Defence Science and Technology Laboratory, Salisbury, United Kingdom

Abstract

Biothreat agents and pandemic capable pathogens can reside in a variety of different tissue types and organs. Once they have set up in the host cells, the pathogens have evolved mechanisms making them highly skilled at evading innate or adaptive immune responses. Thus, these pathogens can be highly virulent and difficult to clear if left untreated. Treatment using antibiotics and antivirals have been found to be very effective in managing infections, however total clearance of the pathogens is difficult as the tissues they reside in can be inaccessible to therapies. Therefore, there is a demand to develop a delivery system to target these hard-to-reach areas. Non-ionic surfactant vesicles (NISVs) have long been used as a delivery system for drugs and vaccine adjuvants. These vesicles are well characterised and can entrap both lipophilic and hydrophilic molecules. NISVs are very similar to an organic counterpart, liposomes. However, NISVs can deliver the same results with reduced toxicity due to the absence of phospholipids. NISVs also are readily modifiable, with the addition of bile salts to enhance stability for oral drug delivery, and the addition of ligands to their membrane for use in cell receptor targeting. Bile salt NISVs (bilosomes) can be used for the delivery of antibiotics (such as doxycycline, levofloxacin, ciprofloxacin), while the ligand NISVs have been used to deliver antibodies across the blood brain barrier (BBB). This PhD will explore the development novel formulations of the NISV for specific tissue targeting and the oral delivery of antibiotics restricted to IV use.

P049

Bile upregulates extracellular polysaccharide production and protects *Staphylococcus aureus* biofilms from the human immune system and neutrophils.

Mary Turley[1], Karolina Klosinska[1], Gonzalo Yebra[2], Aisling Towell[1], Kirsten Schaffer[3], J. Ross Fitzgerald[2], Joan A. Geoghegan[1,4]

[1]Trinity College Dublin, Dublin, Ireland. [2]The Roslin Institute, Edinburgh, United Kingdom. [3]St Vincent's University Hospital, Dublin, Ireland. [4]Institute of Microbiology and Infection, Birmingham, United Kingdom

Abstract

Staphylococcus aureus is a dominant pathogen of cystic fibrosis (CF) airways. Gastroesophageal reflux affects 80% adults and 40% children with CF resulting in bile aspiration into the lungs. We investigated the impact of bile on biofilm formation by CF isolates of *S. aureus*. Using genetically related strains, ED83 and ED86, isolated from the same patient we found that both formed biofilm in media containing bile but, for ED86, biofilm formation was completely dependent on the presence of bile. Dispersal by treatment with sodium metaperiodate but not proteinase K demonstrated biofilms were comprised of polysaccharide. Exopolysaccharide poly-β(1-6)-N-acetylglucosamine (PNAG) mediates staphylococcal biofilm accumulation with biosynthesis relying on the icaADBC operon. Quantitative RT-PCR established *icaA* gene expression was upregulated upon addition of bile to the medium. Confocal microscopy and western immunoblotting consolidated this by showing the quantity of extracellular PNAG produced by bacteria grown in the presence of bile was greater than without bile. *S. aureus* biofilms grown in media containing bile were significantly more resistant to dispersal by oxacillin. Similarly, biofilms formed in the presence of bile better resisted disruption by human neutrophils than those without. In summary, our data indicates that bile induces expression of the *icaADBC* operon, increasing production of exopolysaccharide and that this protects the bacteria from the human immune system and antibiotics. This suggests that bile is an important host signal that can alter the behaviour of *S. aureus*. Current studies are focused on elucidating the molecular relationship between bile and sensorial networks in *S. aureus*.

P050

Unravelling bile resistance in zoonotic and invasive *Salmonella* serovars

Annis Newman, Prerna Vohra

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Salmonella enterica is a zoonotic pathogen of global importance, responsible for an estimated 93 million cases of human non-typhoidal salmonellosis annually, with infections commonly acquired via the food chain from farmed animals. *Salmonella* infection can range from self-limiting gastroenteritis, where the bacteria are largely gut restricted, to invasive typhoid-like disease where *Salmonella* spreads systemically to organs like the gallbladder, liver and lymph nodes. Colonisation of both the gastrointestinal tract and gallbladder requires tolerance to bile, albeit at different concentrations, making bile an important environmental cue. While the mechanisms of *Salmonella* bile resistance have been well-studied in *S*. Typhimurium and, more recently, in *S*. Typhi, little is known about other serovars. The aim of this study is to investigate bile resistance in invasive host-restricted serovars in livestock including *S*. Dublin (cattle) and *S*. Choleraesuis (pigs), and gastroenteritis-causing *S*. Typhimurium to understand serovar-specific differences in the type of disease caused. This will be done by characterising the global transcriptomic responses of all three serovars using RNA-Seq following exposure to host-specific bile at intestinal (3%) and gallbladder concentrations (10%) of ox-bile and porcine bile. The involvement of two component systems (TCS) in bile resistance in *S*. Dublin will also be investigated by screening of a library of 31 TCS sensor kinase (SK) transposon insertion mutants in 3% ox-bile and identifying those with enhanced or reduced growth compared to the wild-type. Taken together, these studies will inform how diverse *Salmonella* serovars survive in reservoir hosts.

P051

Modelling the gastrointestinal carriage by *Klebsiella pneumoniae* infections

Ricardo Calderón González ORCID iD, Alix Lee, Guillermo López Campos ORCID iD, Steven J. Hancock ORCID iD, Joana Sá Pessoa ORCID iD, Amy Dumigan ORCID iD, Ronan McMullan, Eric L. Campbell ORCID iD, Jose A. Bengoechea ORCID iD

Wellcome-Wolfson Institute for Experimental Medicine (School of Medicine, Dentistry and Biomedical Sciences / Queen's University Belfast), Belfast, United Kingdom

Abstract

Klebsiella pneumoniae is a leading cause of nosocomial and community acquired infections, making *K. pneumoniae* the second pathogen associated with the most deaths attributed to any antibiotic resistant infection. *K. pneumoniae* colonises the nasopharynx and the gastrointestinal tract in an asymptomatic manner without dissemination to other tissues; importantly gastrointestinal colonisation is a requisite for infection. Our understanding of *K. pneumoniae* colonisation is still based on interrogating mouse models in which animals are pre-treated with antibiotics to disturb the colonisation resistance imposed by the gut microbiome. In these models, infection disseminates to other tissues. Here, we report a murine model to allow for the study of the gastrointestinal colonisation of *K. pneumoniae* without tissue dissemination. Hypervirulent and antibiotic resistant strains stably colonise the gastrointestinal tract of an inbred mouse population without antibiotic treatment. The small intestine is the primary site of colonisation followed by a transition to the colon over time without dissemination to other tissues. Our model recapitulates the disease dynamics of metastatic *K. pneumoniae* strains able to disseminate from the gastrointestinal tract to other sterile sites. Colonisation is associated with mild to moderate histopathology, no significant inflammation, and no effect on the richness of the microbiome. Our model sums up the clinical scenario in which antibiotic treatment disturbs the colonisation of *K. pneumoniae* resulting in dissemination to other tissues. Finally, we establish that the capsule polysaccharide is necessary for the colonisation of the large intestine whereas the type VI secretion system contributes to colonisation across the gastrointestinal tract.

P052

Characterizing the Proteome of *Salmonella enterica* Outer Membrane Vesicles Under Infection-Relevant Conditions

Alastair Scott ORCID iD, Prerna Vohra ORCID iD

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Salmonella enterica is a zoonotic pathogen responsible for 93.8 million annual cases of gastroenteritis globally. Livestock are a major reservoir of *Salmonella* and human infections are commonly a consequence of handling or ingestion of contaminated food products. *Salmonella* serovars can broadly be divided into generalists like *S.* Typhimurium that typically cause acute gastroenteritis in humans and animals, and host-restricted serovars such as *S.* Dublin and *S.* Choleraesuis that cause systemic disease in cattle and pigs, respectively, and occasionally in humans. Like other Gram-negative organisms, *Salmonella* produces outer membrane vesicles (OMVs) that bleb from the bacterial surface during growth and can play a role in pathogenesis by fusing to host cells to deliver virulence factors and manipulate the host immune system. The aim of this research is to characterise the OMV proteomes of *S.* Typhimurium, *S.* Dublin and *S.* Choleraesuis when exposed to intestinal levels of bile to identify serovar-specific protein packaging that may relate to differences in the type of disease these serovars cause in livestock. Strains were cultured in Luria-Bertani (LB) broth and in the presence of 1% ox and porcine bile until late logarithmic phase of growth. Culture supernatants were collected, concentrated and subjected to density gradient ultracentrifugation to purify OMVs, which were quantified using nanoparticle tracking analysis and a bicinchoninic acid assay. Visualisation of OMV preparations using SDS-PAGE indicates differences in OMV protein content between the serovars that are more pronounced in LB than in bile. Mass spectrometry will be used to confirm these observations.

P053

Comparing the use of fungicides and *Bacillus* biocontrol for the treatment of cobweb disease on mushroom (*Agaricus bisporus*) crops

Joy Clarke ORCID iD[1,2], Helen Grogan ORCID iD[2], David Fitzpatrick ORCID iD[1], Kevin Kavanagh[1]

[1]Department of Biology, Maynooth University, Maynooth, Ireland. [2]Teagasc, Horticulture Development Department, Ashtown Research Centre, Dublin 15, Ireland

Abstract

Cobweb disease, caused by members of the genus *Cladobotryum* is a major concern for growers of the white button mushroom (*Agaricus bisporus*). Fungicides are currently employed to manage this disease, however the number of approved fungicides is decreasing rapidly. Biocontrol agents are being investigated as alternative, more environmentally friendly treatment options. In this work a large-scale growth trial which investigated the treatment of cobweb disease during *A. bisporus* cultivation was carried out. The resistance of two *C. mycophilum* strains (1546 & 618) was investigated against two fungicides (Prochloraz-Mn and Metrafenone). Prochloraz-Mn could control both strains of *C. mycophilum* with efficacy values of 73% against 1546 and 70% against 618 (p-value >0.05). Metrafenone could only control strain 618 with efficacy of 96% (p-value >0.05) but was not effective against strain 1546. Two biocontrol treatments were also included in this trial. *B. velezensis* QST 713 is the active agent in the commercially available biocontrol product Serenade ® while *B. velezensis* Kos is a novel strain which has been shown to be inhibitory to *C. mycophilum* in vitro. The commercially available strain QST 713 was not effective against *C. mycophilum* 1546 in vivo. The novel Kos strain did show some efficacy against the *C. mycophilum* in terms of reducing disease incidence (efficacy=30%) but did not perform as well as the fungicide treatments. Fungicides will continue to play a role in disease treatment on mushroom crops, however their role will be limited in the future necessitating the development of novel, environmentally sustainable control strategies.

P054

Acetic acid exerts bactericidal activity without generating resistance

Sara Henderson ORCID iD[1], Thomas Robinson[2], Liam Grover[2], Joan Geoghegan[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom.
[2]Healthcare Technologies Institute, University of Birmingham, Birmingham, United Kingdom

Abstract

Burn wounds are frequently colonised with pathogenic bacteria resulting in infections, delayed healing times, and increased cost of treatment. Acetic acid has antimicrobial, bactericidal, fungicidal, and antibiofilm activity against a range of clinically relevant bacteria including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This study aims to increase our understanding about the mechanism of killing of bacteria by acetic acid and to establish if acetic acid-resistant mutants can arise. We found that at concentrations above 1% (v/v) acetic acid exerts bactericidal activity against *S. aureus* by inducing lysis while we found the MIC values to be in the range of 0.04-0.16% (v/v) acetic acid. To determine if acetic acid resistance can emerge spontaneously, we exposed *P. aeruginosa*, *A. baumannii* and *S. aureus* to acetic acid concentrations close to the minimum inhibitory concentration (MIC) [0.1-0.5%(v/v)]. No resistant mutants were recovered indicating that resistance is unlikely to emerge in a single step following exposure to acetic acid. To determine if bacteria become more tolerant to acetic acid following prolonged exposure to sub-MIC concentrations, we serially passaged *S. aureus* and *P. aeruginosa* in medium containing ¼ of the MIC. Following 15 serial passages we did not detect any increase in tolerance to acetic acid. Our results highlight the promise that acetic acid holds as an antibacterial agent that can decolonise sites such as infected burn wounds.

P055

What's LPS got to do with it? The role of lipopolysaccharide structure in biocide susceptibility and virulence in *Proteus mirabilis*

Ocean Clarke, Harriet Pelling, Vicky Bennett, Jonathan Nzakizwanayo, Anthony J. Slate, Brian V. Jones

University of Bath, Bath, United Kingdom

Abstract

Chlorhexidine (CHD) is a cationic biocide used ubiquitously in healthcare settings. *Proteus mirabilis*, an important pathogen of the catheterised urinary tract, is often described as intrinsically "resistant" to CHD containing products used for catheter infection control. To identify the mechanisms underlying reduced CHD susceptibility in *P. mirabilis*, we subjected the CHD tolerant clinical isolate RS47 to random transposon mutagenesis and screened for mutants with reduced CHD MICs. One mutant recovered from these screens (designated RS47-2) exhibited ~ 8-fold reduction in CHD MIC. Complete genome sequencing of RS47-2 showed a single mini-Tn5 insert in the *waaC* gene involved in LPS inner core biosynthesis. Phenotypic screening of RS47-2 revealed a significant increase in cell surface hydrophobicity compared to the wildtype and confirmed defects in LPS production congruent with *waaC* inactivation. Disruption of *waaC* was also associated with increased susceptibility to a range of other cationic biocides, and human serum but did not affect susceptibility to antibiotics tested. Complementation studies showed that repression of *smvA* efflux activity in RS47-2 further increased susceptibility to CHD and other cationic biocides, reducing CHD MICs to values comparable with the most CHD susceptible isolates characterised. *In vitro* bladder models showed that formation of crystalline biofilms and blockage of urethral catheters was also significantly attenuated in RS47-2. Taken together, these data show that aspects of LPS structure and upregulation of the *smvA* efflux system function in synergy to modulate susceptibility to CHD and other cationic biocides, and that LPS structure is also an important factor in *P. mirabilis* crystalline biofilm formation.

P056

Microscopy-based phenotypic profiling of infection by *Staphylococcus aureus* clinical isolates reveals intracellular lifestyle as a prevalent feature

Ines Rodrigues Lopes[1], Laura Maria Alcantara[1], Ricardo Jorge Silva[1], Jerome Josse[2], Elena Pedrero Vega[3], Ana Marina Cabrerizo[3], Melanie Bonhomme[2], Daniel Lopez[3], Frederic Laurent[2,4], Francois Vandenesch[2,4], Miguel Mano[1,5], Ana Eulalio[1,6]

[1]Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal. [2]Centre International de Recherche en Infectiologie (CIRI), Université de Lyon, Lyon, France. [3]National Centre for Biotechnology, Spanish National Research Council (CNB-CSIC), Madrid, Spain. [4]Centre National de Référence des Staphylocoques, Lyon, France. [5]King's College London, London, United Kingdom. [6]Imperial College London, London, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of life-threatening nosocomial and community-acquired infections. *S. aureus* was initially considered an extracellular pathogen, though cumulating evidence demonstrates that it can invade a range of host cells. Nevertheless, the significance and pervasiveness of the *S. aureus* intracellular lifestyle remain controversial. Here, we evaluated the ability of *S. aureus* to invade, replicate and persist inside host cells, employing 191 *S. aureus* clinical isolates collected from patients with bone/joint infections, bacteremia, and infective endocarditis. By applying fluorescence microscopy-based infection assays and automated image analysis, we characterized in detail the interaction of these clinical isolates with four host target cells, specifically epithelial and endothelial cells, osteoblasts, and macrophages. This multiparametric analysis revealed that 187 out of 191 *S. aureus* isolates (98%) invade host cells and that a large fraction of those replicates within infected cells. Furthermore, most isolates persisted within host cells at 48h post-infection, particularly in macrophages and endothelial cells. Nonetheless, the isolates presented distinct intracellular lifestyles in non-professional or professional phagocytes. Phenotypic clustering of the isolates highlighted interesting intracellular behaviours. Among these, a group of isolates exhibited a striking phenotype characterized by high and prolonged intracellular replication, albeit inducing delayed host death both in vitro and in vivo. We discovered that these isolates are deficient for the staphylococcal cysteine protease staphopain A. Overall, this study demonstrates that the intracellular lifestyle is a prevalent feature of *S. aureus* clinical isolates, highlighting the importance of eliminating intracellular bacteria for the effective treatment of staphylococcal infections.

P057

Detection of Respiratory Pathogens and Antimicrobial Resistance Genes by High-Throughput Nanofluidic PCR in Bronchoalveolar Lavage (BAL) Samples from ICU Patients with Suspected Pneumonia

Leanne Kermack[1], Ellen Higginson[1], Andrew Conway-Morris[2], Stephen Baker[1], Vilas Navapurkar[2]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Addenbrookes Hospital, Cambridge, United Kingdom

Abstract

The burden of respiratory infections is a significant global concern, causing 4 million deaths annually. Diagnosing these infections is challenging as patient symptoms are not always pathogen-specific and culture as the 'gold standard' has limited sensitivity. Therefore, empirical treatment over directed therapy if often necessary, hindering attainment of antimicrobial stewardship goals. Molecular approaches such as the TaqMan Array Card (TAC) represent a faster and more sensitive alternative. However, these are higher cost and cover a limited breadth of organisms. To address these shortcomings, this study developed a HT-qPCR respiratory panel (R-Panel) able to detect up to 93 respiratory organisms (viruses, bacteria and fungi) and antimicrobial resistance genes in 96 samples simultaneously.

Samples originated from 157 Intensive Care Unit (ICU) suspected pneumonia patients, with documented results from microbial culture and TaqMan Array Card (TAC). We retrospectively analysed the BAL extracts using our R-Panel, before comparing levels of pathogen detection across the three methods.

Only 79 (50%) samples yielded detection of at least one organism by culture. The R-Panel and TAC demonstrated superiority for the detection of multiple organisms, and showed correlation with detections for the majority of targets. 76.5% of targets were detected by both culture and the R-Panel. In terms of concordance with culture, the difference between TAC and R-Panel was not statistically significant ($p=0.28$).

The R-Panel developed in this study could be customised to detect both existing and emerging respiratory pathogens and AMR genes. This would support timely, directed antimicrobial therapy to improve treatment effectiveness and reduce antimicrobial resistance.

P058

PROMOTION OF SAFE-FOOD TO CONSUMERS OF MILK AND DAIRY PRODUCTS AT KIBAHA TOWN COUNCIL (KTC) – TANZANIA

Damas Theobald Msaki[1], Sigrid Agenas[2]

[1]Kibaha Education Centre, Kibaha, Tanzania, United Republic of. [2]Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Abstract

A cross-sectional baseline survey was conducted at Kibaha Town Council (KTC), Pwani Region (Tanzania), to assess awareness and practices of smallholder dairy producers towards antimicrobial use (AMU). The aim was to improve consumers' confidence towards consumption of milk and other dairy products. Quantitative data were collected from a total of 30 randomly selected respondents that keep dairy cattle in 3 administrative wards of Tumbi, Picha ya Ndege and Maili-Moja. A structured questionnaire was administered through face to face conversation between the interviewer and the interviewees. Six other respondents participated in the Focus Group Discussions for qualitative data collection. Data were analysed using the statistical package for social science (SPSS, Version 16) and Microsoft Office Excel 2010. There were more male respondents (63.3%) than female whereas the level of education was primary (46.7%), secondary (33.3%) and tertiary level for remaining 20%. About 76.6% respondents (Males 53.3% and females 23.3%) were using antimicrobial agents to treat lactating dairy cows, mainly Oxytetracycline (OTC 20%), Penicillin and Peni-Strep, but respondents with tertiary education rarely administered antimicrobials themselves. The common diseases being treated were diarrhea (46.7%), mastitis (43.3%) and respiratory diseases (36.7%). A proportion of this milk with antimicrobial residues was fed to calves (33.3%), sold to milk collection centres (26.7%), fed to other animals (26.7%) and little was consumed by the family at home (6.7%). In conclusion, dairy producers administer antimicrobials to lactating animals by themselves and thereby, posing health challenges among the milk consumers. We advise them to avoid the unnecessary AMU.

P059

Methods to increase Escherichia coli electroporation efficiency

Feiyang Yu

Trinity College Dublin, Dublin, Ireland

Abstract

Electrotransformation of genomic material into bacteria, including Gram-negatives such as Escherichia coli, is a key tool for cloning and mutagenesis. However, the transformation efficiency (the relative uptake of the aforementioned genetic material) remains limited in some bacterial strains, especially with electroporation of large, foreign DNA (>10Kb). The aim of this research is to optimise transformation efficiency to subsequently permit the utilisation of electroporation to transform bacteria with bacteriophage genomes. The use of different transformation media and temperature was explored, in addition to altering cell permeability through the use of a polymyxin antibiotic derivative, polymyxin B nonapeptide. In this study, the electroporation efficiency of E. coli K-12 strain MG1655 and urosepsis isolate CFT073 transformed with plasmid vectors was significantly improved, using a combination of the methods mentioned above. Subsequently, a large plasmid vector was transformed by the novel methods. Moreover, it was then shown that using the novel method for transformation, the genome of a bacteriophage virus was transformed into strain MG1655 at an efficiency 480-folds greater than that of the standard transformation protocol. This bacteriophage targets MG1655 and prototypic urosepsis isolate CFT073. The transformation of MG1655 with bacteriophage DNA will permit phage genome manipulation in a non-pathogenic background, to allow for eventual therapeutic use in the treatment of urinary tract infections and sepsis resulting from infection with uropathogenic E. coli, such as strain CFT073.

P060

Mutations caused by adaptation to chlorhexidine are highly prevalent in clinical *Proteus mirabilis* isolates and are significantly associated with increased tolerance to other biocides and antimicrobials

Vicky Bennett[1], Ocean Clarke[1], John Nzakizwanayo[1], Pelling Harriet[1], Anthony Slate[1], Matthew Wand[2], Emma Denham[1], Mark Sutton[2], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]UK Health Security Agency, Salisbury, United Kingdom

Abstract

Biocides such as chlorhexidine (CHD) are widely used in infection control measures. The Gram-negative pathogen *Proteus mirabilis* (*P. mirabilis*) frequently causes catheter associated urinary tract infections, however clinical isolates are often tolerant to higher CHD concentrations than found in many clinical products. Our previous work has identified highly tolerant clinical isolates which contain mutations that modulate both efflux activity and cell surface charge.

We employed directed evolution approaches coupled with genomic and transcriptomic analyses to determine the prevalence and impact of these mutations in *P. mirabilis* clinical isolates. We surveyed a panel of ~550 genomes and mapped genomic to phenotypic changes in ~80 clinical strains. Expression changes in significant genes, and cross-resistance to antibiotics and other biocides was assessed. Virulence profiles were determined using serum-based assays.

Mutations which upregulate SmvA efflux pump expression were highly prevalent (60% of genomes) and significantly associated with high tolerance to CHD and other biocides. Many of the more susceptible clinical isolates contained mutations in lipopolysaccharide and surface charge modulation associated genes. However, adaptation to CHD can reverse these mutations and significantly increase tolerance to other biocides and membrane acting antibiotics. Increased virulence was also common in these isolates.

We have here demonstrated the high prevalence of mutations associated with CHD and biocide tolerance within the wider *P. mirabilis* clinical population, as well as the potential for less tolerant strains to gain these mutations through CHD adaptation. This leads not only to higher biocide tolerance, but also increased virulence and cross-resistance to certain antimicrobials.

P061

Do metabolically inactive bacteria in biofilms contribute to antimicrobial tolerance?

Steve Trendell, Ammara Khalid, Sarah Maddocks

Cardiff Metropolitan University, Cardiff, United Kingdom

Abstract

The aim of the project was to establish if the presence of glucose to kick-start microbial metabolism in a complex biofilm, made the bacteria more susceptible to antimicrobial treatment.

Five of the most common species of bacteria that contribute to chronic wound infections were chosen to create the biofilm (Staphylococcus aureus, Pseudomonas aeruginosa, Citrobacter freundii, Enterococcus faecalis and Escherichia coli). These five species were cultured in an agarose-collagen matrix and placed into a Duckworth Biofilm Device. Four assays were conducted and varied by being supplemented with simulated wound fluid, simulated wound fluid and D-glucose, and with or without a topical antimicrobial treatment (Neosporin). The bacteria were recovered at 24-, 48- and 72-hour timepoints and community compositions were determined by total viable count using selective agars.

Data analysis showed no significance in the antimicrobial susceptibility of the bacteria in the presence of glucose and the original hypothesis that glucose would raise the tolerance of biofilm bacteria to antimicrobial treatment was rejected. Greater antimicrobial susceptibility and a reduction in bacterial numbers over time was observed in the Gram-negative species between 48 and 72 hours, whereas a slight increase in bacterial numbers was seen in the Gram-positive species in the same time frame under the same conditions.

Although there was no significance in the data, the Gram-positive species within the biofilm showed a slight decrease in susceptibility to antimicrobial treatment when metabolically active and reflects the difficulties in the treatment of chronic wound infections for people with underlying health conditions such as diabetes.

P062

Characterisation of two biofilm-forming Non-Typeable *Haemophilus influenzae* strains isolated from the COPD lung using an air-liquid-interface small airways epithelium co-culture model system.

Jake Weeks[1], C. Mirella Spalluto[1,2], V. Maria Humbert[1,2], Kristoffer Ostridge[3,1], Tom Wilkinson[1,2], Karl Staples[1,2]

[1]Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Southampton, United Kingdom. [2]National Institute for Health Research (NIHR) Southampton Biomedical Research Centre, Southampton, United Kingdom. [3]Translational Science and Experimental Medicine, Research and Early Development, Respiratory & Immunology, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden

Abstract

Non-typeable *Haemophilus influenzae* is a commensal-turned pathogen with emerging biofilm lifestyle properties. In Chronic Obstructive Pulmonary Disease (COPD), a progressive inflammatory syndrome of the lungs, NTHi proliferations account for approximately 25% of periodic acute exacerbations of COPD that lead to hospitalisation. This research aims to characterise host-pathogen interactions using two COPD lung-derived, biofilm-forming NTHi strains by co-culturing NTHi on a small airways epithelium (SAE) grown at air-liquid interface (ALI) model system with improved pathophysiological relevance to the COPD small airways.

The relative expression of 18 biofilm-associated genes were quantified by RT-qPCR and advanced imaging techniques including fluorescence in-situ hybridisation (FISH) and biofilm matrix stains SYPRO Ruby, Calcofluor White, Concanavalin A and TOTO-1 were used to assess differences in biofilm strategies and structure between the NTHi strains. The integrity of the model system was also evaluated for de-differentiation, cytotoxicity, tight junction barrier degradation and increased permeability using TEER, LDH and FITC-Dextran assays, respectively.

Differences were detected in NTHi gene expression between NTHi strains, highlighting the particular importance of genes conferring adhesin expression, hypoxia and oxidative stress tolerance and cell surface modifications – all hallmarks of the biofilm lifestyle. Florescence imaging revealed extracellular NTHi aggregates consistent with biofilm formation, and analysis of pro-inflammatory cytokine release has demonstrated time- and strain-dependent host innate immune responses to NTHi biofilms confirming that relevant host-pathogen interactions are occurring in the model. Together these findings may help explain the persistence of NTHi in the COPD lung and may expose novel therapeutic targets against NTHi biofilm strategies.

P063

Beauveria bassiana as a novel source of antifungals against *Aspergillus fumigatus*

Ho Ting Lucio Fung, Kotta Loizou Ioly

Imperial College London, London, United Kingdom

Abstract

Aspergillosis is a lung infection caused by *Aspergillus fumigatus* affecting more than 3 million immunosuppressed individuals annually. Novel drugs for aspergillosis are needed due to the prevalence of azole-resistant *A. fumigatus*. The entomopathogen *Beauveria bassiana* and *Aspergillus* coexist in insects, raising the potential of *B. bassiana* as a source of antifungals against *A. fumigatus*. To this end, dual plating of *B. bassiana* and *A. fumigatus* was performed to determine candidate strains with inhibitory effects. The inhibition was quantified by measuring radial growth of *A. fumigatus* in the presence of *B. bassiana* on six commonly used fungal media. Finally, pre-inoculation of B. bassiana 2, 1, and 0 days before *A. fumigatus* provided insights on potential time-dependent mechanisms. *B. bassiana* strain ATCC 74040 has a statistically significant inhibitory effect (Student t-test, P-value<0.001) on *A. fumigatus* as shown by an observable inhibition halo in dual plating. Among the 6 tested media, the highest inhibition of *A. fumigatus* is seen in yeast extract sucrose (YES; 67.3%), followed by potato dextrose agar (PDA; 52%) and Sabouraud dextrose agar (SDA; 40%). A statistically significant difference (Student t-test) in inhibition of *A. fumigatus* is observed on PDA (P-value<0.001), YES (P-value<0.01) and SDA (P-value<0.005) when *B. bassiana* is pre-inoculated 1 day as compared to simultaneous inoculation, despite no significant differences in *B. bassiana* radial growth, suggesting the inhibition may be due to a lag phase component. This finding provides a potential candidate for novel drug development against *A. fumigatus* which is urgently needed to tackle azole-resistant strains.

P064

Characterisation of Novel Bacterial Factors Affecting Systemic Dissemination of *Burkholderia pseudomallei*

Rose Doyle, Joanne Stevens, Madeleine G. Moule

The University of Edinburgh, Edinburgh, United Kingdom

Abstract

Melioidosis, caused by the tropical, soil-dwelling bacterium, *Burkholderia pseudomallei*, is an infectious disease which is often nicknamed "the Great Mimicker" due to its non-specific and varied clinical presentations. Multiple organ involvement and sepsis are severe manifestations of the disease, associated with the ability of *B. pseudomallei* to escape the initial site of infection and disseminate systemically. However, the mechanisms and pathways involved in *B. pseudomallei* dissemination are poorly understood. We hypothesise that by identifying bacterial factors involved in this process, we can shed insight into this aspect of *B. pseudomallei* pathogenesis. Previously, Transposon-directed Insertion-site Sequencing (TraDIS) was performed to identify novel *B. pseudomallei* virulence factors. We have recently reanalysed this dataset to identify genes important for dissemination by comparing the population of mutants that were capable of colonising the initial infection site of the lungs with the population of extrapulmonary mutants found in the spleen. Candidate genes have been selected by bioinformatic analyses for further characterisation, including genes encoding a chemotaxis protein and an esterase. Unmarked deletion mutants for each gene have been constructed in the model organism *Burkholderia thailandensis*, and characterised using a range of *in vitro* assays including infections in lung epithelial cell cultures. Elucidating the role of these genes may provide insight into dissemination mechanisms employed by *B. pseudomallei*, thereby increasing our understanding of *B. pseudomallei* pathogenesis for the development of new therapies and diagnostics.

P065

Understanding the ecology of the cystic fibrosis (CF) airway microbiome using probabilistic modelling

Pok-Man Ho, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

I am interested in understanding better the interactions between microbes in human airway infection scenarios, and in particular, whether these interactions change if the system is perturbed e.g., through the introduction of new species or following antibiotic challenge. In order to tease apart the ecological interactions between species, we have developed a statistical-mathematical pipeline for analysing microbial population dynamics data in the cystic fibrosis (CF) airways. The airways of people with CF are frequently infected by a mixture of species, including well known pathogens such as *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA). To investigate this further, we obtained time-series data (containing information about species presence/absence for the period 2008-2020) from the UK Cystic Fibrosis Registry. Based on these data, we have been able to establish the long-term ecological relationship(s) between species e.g., whether pairs of species display commensalism, amensalism, parasitism etc, along a rolling time-frame. Our data indicate that ecological relationships are shaped not only by antibiotic treatment, but also by other forms of medical intervention. We have also been using the pipeline to model inter-species interactions in an experimentally-perturbable in vitro polymicrobial model of the CF airways. This has allowed us to quantify the ecological impact of mutating key genes, and to study, in defined conditions, the influence of antibiotics on inter-species interactions. In summary, we have developed a pipeline which accurately captures the dynamic nature of inter-species interactions in complex, polymicrobial infection scenarios.

P066

Organoid models to study the role of *Helicobacter pylori* VacA in gastric adenocarcinoma development

Harriet Giddings ORCID iD[1], Claire Shannon-Lowe[2], Karen Robinson[3], Amanda Rossiter[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom.
[2]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom.
[3]Nottingham Digestive Diseases Biomedical Research Centre & Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

Abstract

Infection with the stomach-colonising oncobacterium *Helicobacter pylori* is usually asymptomatic but can lead to development of gastric adenocarcinoma. *H. pylori* secretes vacuolating cytotoxin A (VacA), which induces vacuole formation and disruption of intercellular junctions in gastric epithelial cell lines and contributes to pathogenesis. Effects of VacA on cell lines are clear, and although reproducible, cell lines do not accurately represent the physiological epithelia or architecture of the stomach. Recently, alternative approaches to model infection of the stomach have emerged, such as the use of primary human gastric organoids. Using these models of infection may increase understanding of how VacA contributes to gastric carcinogenesis. In this project a recently established healthy gastric organoid mucosoid monolayer was used to determine whether different allelic forms of *H. pylori* VacA target specific cells within the stomach and interrupt intercellular junctions. Cellular phenotypes were characterised using immunofluorescence to confirm the presence of all relevant components of the model, such as E-cadherin, MUC5AC, stem, parietal and chief cells. Organoid monolayers and AGS cell lines were then exposed to extracts from *H. pylori* strains expressing the active (s1/i1) or inactive (s2/i2) forms of VacA (s1/i1 or s2/i2). As expected, vacuolation was more prominent in cell lines exposed to the s1/i1 form of VacA. No vacuolation was found in organoid monolayers, however. Live *H. pylori* cells will be used in further work to determine whether this difference is also seen in a coculture system and whether VacA targets specific cell types or disrupts intercellular junctions within the monolayer.

P067

The role of non-typeable *Haemophilus influenzae* in cystic fibrosis lung infection

Phoebe Do Carmo Silva[1], Freya Harrison[1], Darryl Hill[2]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Non-typeable *Haemophilus influenzae* (NTHi) colonises the lungs of people with cystic fibrosis (CF) in infancy. An understanding of early lung colonisers may become increasingly important as modulator therapy progresses, which could stop patients progressing to "classic" chronic infection with later stage pathogens, like *Pseudomonas aeruginosa*. There is currently little research on NTHi lung infection in CF, compared with later stage pathogens.

I am using an *ex-vivo* pig lung model as a high validity model for respiratory infections, combined with synthetic CF sputum media (SCFM) to mimic the nutritional composition of CF sputum. This is the first time this model has been used for NTHi. I aim to answer key questions about how NTHi infection affects CF lung disease progression including:

- What is the biofilm structure of NTHi on host lung tissue?
- Does the growth of NTHi as a biofilm in the lungs affects susceptibility to standard antibiotic treatment?
- Does NTHi alter the lung environment to assist other CF pathogens to colonise the lungs?

I have optimised the formulation of SCFM for use with NTHi, determining that supplementation with additional molecules found in CF lung mucus is required for biofilm growth *in vitro*. This was assessed through growth curves and static biofilm assays. Using this supplemented SCFM, NTHi biofilm forming ability, biofilm structure and antibiotic susceptibility can be assessed in the lung model. I will use this optimised model to better understand the physiology and ecology of early-stage pathogen infection in CF, and elucidate the role of NTHi in CF disease progression.

P068

Dissecting immune evasion mechanisms in *Staphylococcus aureus*

Toska Wonfor, Maisem Laabei

University of Bath, Bath, United Kingdom

Abstract

Staphylococcus aureus is a multi-drug resistant human pathogen responsible for a variety of diseases, from skin infections to bacteraemia. Complement is a tightly regulated proteolytic cascade which simultaneously labels microorganisms and recruits immune cells to facilitate phagocytosis. *S. aureus* has the largest number of complement disarming proteins, collectively termed complement evasins (CE), identified among pathogens. We are interested in understanding the individual contribution of CEs to complement inhibition which could direct future therapies.

To measure *S. aureus*-mediated complement inactivation, we modified a serum bactericidal assay employing complement susceptible *E. coli* incubated with normal human serum in the presence or absence of *S. aureus* supernatant. We screened a panel of 14 genetically diverse *S. aureus* strains, which indicated CE as a variable virulence trait. Next, we created mutants of all secreted CE in two *S. aureus* genetic backgrounds and identified 3 key secreted proteins SCIN, SSL7 and aureolysin that cause significant reduction in complement inactivation when disrupted.

Our assay was optimised to include computational colony counting permitting high-throughput screening of 134 closely related genome-sequenced clinical isolates. Our results indicate that this phenotype varies substantially even within the same clonal group. Genome-wide association studies identified several hypothetical proteins associated with complement evasion. Lastly, we identified a moderate correlation between CE and cytotoxicity, both secreted virulence factor phenotypes. We highlighted the role of several two-components regulators important in CE but not cytotoxicity. Future work will dissect the specific regulatory pathways of individual CEs, facilitating a greater understanding of staphylococcal immune evasion mechanisms.

P069

Antibiotic-mediated remodelling of species trajectories in a polymicrobial airway infection model

Eva Bernadett Benyei, Martin Welch ORCID iD

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa (PA) is designated by the WHO to be a critical priority pathogen. In many airway infection scenarios, PA shares the niche with a veritable "zoo" of other microbes, including Gram-negative, Gram-positive and fungal species – yet little is currently known about how these co-habiting species affect the response of PA to antibiotic challenge, or about how targeting of one species affects the trajectories of others. These are the topics that I will be addressing in my presentation.

PA is a very aggressive pathogen, and has proven difficult to stably co-culture with other species *in vitro*. However, this problem has been recently overcome with the introduction of a robust experimental platform that allows us to stably co-culture PA in artificial sputum medium with other airway-associated microbes, including *Staphylococcus aureus* (SA) and *Candida albicans* (CA), for long periods of time. All three species frequently co-colonise the airways of people with cystic fibrosis.

We found that the presence of other species greatly enhances the survival of PA when challenged with antibiotics, compared with survival of the species in monoculture. Some elements of this enhanced resistance were heritable, whereas others are associated with phenotypic adaptation. The relative efficacy of anti-pseudomonal antibiotics could be improved when they were applied in combination. However, this combination therapy, which is a common tactic deployed in the clinic, inadvertently led to a "blooming" of the fungal pathogen. The possible biochemical basis for this antibiotic-mediated population remodelling (e.g. quorum sensing, virulence factors, Type VI Secretion *etc*.) will be discussed.

P070

Survival of *Streptococcus dysgalactiae* on soil and farm bedding substrates, and the efficacy of calcium oxide as a disinfectant

Louise Jackson, Jennifer Duncan, Dorina Timofte

University of Liverpool, Liverpool, United Kingdom

Abstract

Background: *Streptococcus dysgalactiae* subspecies *dysgalactiae* (SDSD) is the leading cause of infectious arthritis (joint ill) in young lambs. This study aims to: (i) use laboratory-modelling of the farm environment to determine SDSD survival on soil and bedding, in damp and dry states, and (ii) provide farmers with practical guidelines for disinfection.

Methods: Sterilised unused straw, wood shavings, and soil, were added to universals either dry or dampened with sterile water. A 0.5 McFarland suspension of a clinical SDSD isolate was added and substrates were stored aerobically in a humidity-controlled incubator across 7 time points (1-21 days), following an initial day 0 reading. Average temperature and humidity was deduced using real-time farm data and were cycled in two-hour intervals over a 24-hour period. The study was repeated with the addition of calcium oxide at laboratory conditions, for 10-60 minutes. Samples were cultured on Streptococcal-selective media at 37^{o}C.

Results: At day 14, there was a decrease in SDSD of 2.1 and 2.0 log average cfu/ml in straw and damp straw, respectively; SDSD was unculturable by day 21. Soil and damp soil maintained SDSD survival across all time points; by day 21, there was 0.5 and 1.3 log decrease in cfu/ml respectively. In wood shavings, SDSD was unculturable at all time points. Calcium oxide addition rendered SDSD unculturable after 10 minutes across all substrates.

Conclusion: The importance of a dry, clean lambing environment is highlighted, further supporting current advice on bedding/pen management. Calcium oxide offers a potential disinfectant for SDSD.

P071

Understanding the key readouts to test the efficacy of novel immunomodulators to combat respiratory viral infection

Sandy Kimber, Charlotte Cumper, Hon Shing Lam, Matt Miller, Kate James, Lorena Sueiro Ballesteros, Chris Kirkham, Patrick Walters, Yenenesh Tekletsion, Claire Richards, Louise Brackenbury

Charles River Discovery, Portishead, United Kingdom

Abstract

Background In addition to reducing the burden of pathogens within the lung during an infection, it is also essential to consider limiting aberrant immunopathology. To understand how to best screen novel immunomodulators within the context of influenza infection, we have assessed a variety of *in vivo* and *ex vivo* readouts, in both BALB/c and C57BL/6J mice.

Methods Animals were divided into six groups for influenza A/PR/8/34 infection (standard v high dose) including controls. Bodyweight and clinical scores were monitored. At termination on day 6, plasma and bronchoalveolar lavage fluid (BALF) were taken for cytokine detection. Lung immune cell infiltrate was examined by flow cytometry. Lung tissue was taken for histopathology and viral titres. Additional readouts such as BALF protein quantification and lung consolidation were used to assess gross lung damage.

Results Following infection at day 6, cellular lung infiltrate shows a clear depletion of alveolar macrophages in both strains indicating infection. While C57BL/6J mice presented higher survival, BALB/c mice presented with higher clinical scores, tissue damage and levels of key inflammatory mediators such as IL-6.

Conclusion Data from these key readouts differ between mouse strains, which likely supports the differential skewing of the helper T cell responses of BALB/c and C57BL/6J mice. This data demonstrates that mouse strain and influenza dose are critical when designing experiments to test the efficacy and mode of action of immunomodulators. While both models are useful, influenza infection in BALB/c mice provides a larger window for therapeutic intervention with novel immunomodulators.

P072

Cysteine protease 5 and Phospholipase B genes in the genomic DNA of *Entamoeba histolytica*

Banaz Kareem ORCID iD[1], Hawri Bakr[2], Rozha Othman[3], Fares Bahrami[4], Shler Faqe[3], Heshu Rahman[1]

[1]Sulaimani University-College of Medicine, Sulaymaniyah, Iraq. [2]Hawler Medical University- College of Medicine, Erbil, Iraq. [3]Sulaimani University- College of Medicine, Sulaymaniyah, Iraq. [4]Kurdistan University of Medical Sciences- Zoonoses Research Center, Research Institute for Health Development,, Sanandaj, Iran, Islamic Republic of

Abstract

Introduction: *Entamoeba histolytica* infections range from asymptomatic, intestinal amoebiasis to extra-intestinal abscesses formation. The difference in the clinical outcome of the *Entamoeba histolytica* infections could be attributed to genetic diversity and parasite intrinsic factors such as virulence, the current study conducted to investigate *Entamoeba histolytica* strain diversity through the presence of the main virulence gene (cysteine protease 5) in the asymptomatic samples and compared to the symptomatic specimens. The study also examined the presence of another gene (phospholipase B) in symptomatic and asymptomatic samples.

Materials and Methods: nested PCR was utilized to detect the cysteine protease 5 gene among 40 symptomatic and 57 asymptomatic samples of *E. histolytica* (all samples were identified as *E. histolytica* by PCR prior to analysis). The phospholipase B gene was also targeted in the symptomatic and asymptomatic samples using a nested PCR technique.

Results: for the cysteine protease 5 gene, 100% of the symptomatic samples were positive, yet only 75.43% of the asymptomatic samples were positive, a statistically significant difference. The phospholipase B gene was also present in a significantly greater proportion of the symptomatic (80%) than asymptomatic (59.65%) samples.

Conclusions: the significant differences in the presence of both genes, indicating variation in the genetic makeup between symptomatic and asymptomatic samples of *E. histolytica*, results in differences in the clinical outcome of the disease.

P073

Mycobacterium avium paratuberculosis – a pain in the gut!

Charlotte Winspear

Moredun Research Institute, Penicuik, United Kingdom. The James Hutton Institute, Aberdeen, United Kingdom. The University of Edinburgh, Edinburgh, United Kingdom

Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a widespread bacterial pathogen and is the causative agent of Johne's Disease. It appears worldwide, with large economic losses from the livestock industry and it has been shown to be associated with Crohn's Disease in humans – although, the argument of MAP being zoonotic is still largely debated. Johne's Disease affects the intestine of several ruminant species including cattle, goats, and sheep; it has no cure, despite repeated efforts of developing a number of diagnostics to combat the disease, producing an unclear picture of how we move forward. However, emerging evidence within gut microbiome research and the environmental preferences of MAP may unlock the answers needed to understand the transmission of this pathogen. This project aims to take what we know and delve further into gut microbiome composition research, alongside environmental assessments of selected farmland including soil and water sources, to understand the effect of treatments and vaccines in parasitic and MAP co-infected animals - and to hopefully develop improved preventative/control measures. The first year of this project has focused on developing and optimising a qPCR assay, to improve the detection and quantification of MAP in environmental and faecal samples, with hopes of applying said optimised assay into current identification and diagnostics. Over time, we expect to apply our findings to the real-world, which will hopefully improve the outcome of livestock affected by Johne's Disease.

P074

Alternating rigid and flexible regions of the passenger enhance function of the inverse autotransporter intimin

Julia Weikum[1], Kristine Stub Rønø Clausen[2], Jack C. Leo ORCID iD[3], J. Preben Morth ORCID iD[2,1]

[1]Centre for Molecular Medicine Norway, Oslo, Norway. [2]Technical University of Denmark, Lyngby, Denmark. [3]Nottigham Trent University, Nottingham, United Kingdom

Abstract

Inverse autotransporters, or type 5e secretion systems, are adhesins of Gram-negative bacteria exemplified by intimin of enteropathogenic and enterohaemorrhagic *Escherichia coli*. The adhesive extracellular region of intimin (the passenger) is exported through the outer membrane-embedded β-barrel domain through a hairpin-like intermediate. The energy for folding is provided by the sequential folding of individual domains in the passenger. We have solved the crystal structures of the outer-membrane proximal domains D00 and D0, which are both immunoglobulin (Ig)-like domains. However, whereas D0 is structurally very similar to the D1 and D2 domains of intimin, D00 is more divergent. D00-like domains are predicted at the N-terminus of most inverse autotransporters, and recent crystal structures from other representatives of the family confirm this prediction. The connector between D00-D0 forms an S-shaped hydrophobic loop that appears to confer a rigid orientation to the D00-D0 tandem. By contrast, the connections between D0-D1 and D1-D2 are flexible. Making the D00-D0 connector flexible by replacing the residues with glycine and serine did not have an effect on the stability of the domains but had a drastic effect on protein levels in vivo. Simulations show that the rigid connector between D00-D0 increases the reach of the adhesin, whereas the flexibility between D0, D1 and D2 domains allows the distal adhesive D3 domain to adopt the optimal orientation for receptor binding. Thus, alternating rigid and flexible regions within the intimin passenger are important for its function.

P075

Exposure to Klebsiella pneumoniae culture filtrate inhibits Aspergillus fumigatus growth and stimulates gliotoxin production.

Aaron Curtis ORCID iD[1], Michelle Ryan[1], Kevin Kavanagh ORCID iD[2]

[1]Maynooth University, Kildare, Ireland. [2]Maynooth Univerisity, kildare, Ireland

Abstract

Background

Aspergillus fumigatus is an opportunistic pathogen and is rarely the sole isolate from an infected lung, it often interacts and competes with bacterial pathogens in the microenvironment. Previous work has demonstrated Pseudomonas aeruginosa has antagonistic physical and secretory affects on fungal development, similar affects are attributed to Klebsiella pneumoniae physical interactions although little is known about the secreted factors contributing to the observed affects.

Methodology

Cultures of A. fumigatus (48 hrs) were exposed to 25% (v/v) K. pneumoniae culture filtrate and the effect on wet weight and gliotoxin production was assessed after 24 hours. Qualitative proteomic analysis was employed to identify possible inhibitory proteins in K. pneumoniae culture filtrate. Label free quantitative proteomics was used to characterise the changes in the A. fumigatus proteome following exposure to K. pneumoniae culture filtrate

Results

Exposure of A. fumigatus to K. pneumoniae culture filtrate inhibited fungal growth and increased gliotoxin production. Qualitative proteomic analysis of the K. pneumoniae culture filtrate identified proteins associated with metal sequestering, enzymatic degradation and redox activity which may impact fungal development. Proteomic analysis of A. fumigatus revealed reduced abundance of proteins involved in fungal growth and development and increased abundance of proteins encoded by 5 of the 12 genes associated with the gliotoxin biosynthesis cluster

Conclusion

Exposure of A. fumigatus to K. pneumoniae culture filtrate results in a reduction in growth but an increase in gliotoxin production and in the abundance of associated proteins. Such an interaction in vivo could exacerbate infection, having adverse patient implications.

P076

Developing an in-vitro polymicrobial biofilm model for ventilator associated pneumonia

Dean Walsh[1], Trevor Lithgow[2], Ana Traven[2], Freya Harrison[1]

[1]University of Warwick, Warwick, United Kingdom. [2]Monash University, Melbourne, Australia

Abstract

Numerous pathogens are capable of causing ventilator associated pneumonia (VAP), including Pseudomonas aeruginosa, Klebsiella pneumoniae and Candida albicans. Here, we showcase a reproducible model that simulates the chemical, biological, and material environment of biofilms implicated in VAP. Defined as a pneumonia occurring after more than 48 hours of mechanical ventilation via an endotracheal tube (ETT) or tracheostomy tube, VAP results from biofilm forming on the ETT and seeding the lower airways with pathogenic microbes. The prevalence of VAP varies between 9-65% with mortality rates as high as 76%. Furthermore, VAP drives the use of broad-spectrum antibiotics, adding to already high selection pressures and further increasing antibiotic resistance. Currently there is a lack of accurate in vitro models of the VAP environment. This greatly limits our understanding of how the VAP environment alters pathogen physiology and the efficacy of existing and novel therapies. In our biofilm model each of the critical pathogens are grown on ETT segments in the presence of a novel synthetic airway surface liquid (ASL) growth medium and porcine trachea to simulate the VAP environment. Antibiotic susceptibility testing (AST) in this model reveals that these pathogens require far higher concentrations to eradicate, if eradicated at all, compared to when grown under the standard laboratory conditions usually employed for AST. Our model can be used to inform on how the VAP environment alters biofilm formation and antibiotic susceptibility. This model is a promising platform for the testing of new and existing therapies to tackle VAP.

P077

Stopping a killer superbug: unravelling the molecular mechanisms of *C. difficile* sporulation

Charlotte Roughton, Paula Salgado

Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Clostridioides difficile is an antibiotic resistant bacterial human pathogen that colonises the large intestine in the absence of a healthy competing gut microbiota. Current treatments for *C. difficile* infection (CDI), the most common cause of healthcare-associated gastrointestinal disease, entail use of antibiotics that perpetuate gut dysbiosis and enable disease recurrence. This highlights an urgent requirement to further understand the fundamental biology of this pathogen so novel therapies can be sought. A promising, albeit unexploited, target for new therapeutics is the process by which *C. difficile* forms spores, dormant cell forms resistant to environmental stressors and the primary CDI infectious agent. Sporulation begins with an asymmetrical cell division, and entails substantial peptidoglycan remodelling as the smaller forespore is engulfed by the larger mother cell prior to spore maturation. The molecular details of sporulation require further unravelling to find potential pharmaceutical targets to interrupt this process, thereby eliminating the CDI transmission route. SpoIIP is a dual amidase and endopeptidase involved in peptidoglycan remodelling during engulfment, but the localisation of SpoIIP in vivo and the identity of its endopeptidase catalytic residues remain unknown. Previous work has identified two cleavage sites targeted by the SpoIVB2 peptidase, proposed to be a mechanism of liberating enzymatically active SpoIIP isoforms. Our current work seeks to further investigate this, with each isoform having been generated using inverse PCR and tested for enzymatic activity using peptidoglycan digestion assays. By furthering understanding of the mechanisms of forespore engulfment, the crucial pursuit of novel CDI therapies will be aided.

P078

Incorporating the genetic diversity of *Klebsiella pneumoniae* into antibody screening using novel high content imaging

Peter Brian Gallagher ORCID iD, Caroline Zellmer ORCID iD, Pantelis Samartsidis ORCID iD, Gosh Sanmitra ORCID iD, Plamena Naydenova, Simon Clare, Deniela De Angelis ORCID iD, Stephen Baker ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

Klebsiella pneumoniae (Kp) presents a major threat to global health. Previous studies have described multiple methods to generate Kp vaccines, from live-attenuated, vesicle, to conjugated vaccines. Despite this, no vaccine currently exists that can prevent Kp infection or reduce virulence. One reason for this may be the vast genetic of Kp. To investigate how this diversity impacts antibody responses, a collection of 175 clinically relevant isolates was procured and characterised. From this collection, outer-membrane vesicles (OMVs) were harvested from 5 isolates and used to immunise mice. The resulting 5 groups of polyclonal sera were harvested, characterised, and screened against live isolates using high content imaging (HCI). We found that the OMV derived polyclonal antibodies were cable of both homotypic and heterotypic binding to extracted OMVs, proteins and polysaccharides. Screening all 175 isolates against the 5 serum groups and a mixed serum group using HCI revealed that the polyclonal antibodies were only cable of strongly binding (binding >70% of bacteria imaged) 3-25 of the isolates and binding 39-133 of the isolates binding weakly (binding <30% of bacteria imaged) depending on serum group. This study represents the first use of HCI to screen an antibody response against a large collection of Kp isolates. We have demonstrated that OMV derived antibodies are capable of binding a number of unrelated isolates, however this is likely to be a small section of global Kp population. Future work will focus on investigating isolate characteristics that contribute to antibody binding, in order to increase isolate coverage.

P079

Roles for haemolysin in extra-intestinal pathogenic *Escherichia coli* serum resistance

Naoise McGarry, Stephen Smith

Trinity College, Dublin, Ireland

Abstract

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) is a major cause of urinary tract infections, bacteraemia and sepsis. CFT073 is a prototypic, urosepsis isolate. This laboratory, among others, has previously shown that strain CFT073 is serum-resistant, with virulence factors such as the exopolysaccharide capsule and other extracellular polysaccharides imparting resistance to the complement system. In this study, the regulation and interplay of lipopolysaccharide (LPS) and capsule in CFT073 serum resistance was further characterised, with surface charge being implicated as a major determinant to cell integrity, association of exopolysaccharides with the cell and subsequently, serum resistance. Additionally, it was shown that culture supernatants were protective in standardised serum killing assays, when compared to fresh medium. Diluting cultures in fresh medium in place of conditioned medium significantly increased sensitivity of CFT073 to serum, indicating that a secreted factor may provide resistance to serum. Haemolysin, a pore-forming toxin, is secreted by CFT073 in a calcium-dependant manner. A CFT073 *hlyA* mutant is significantly more sensitive to 50% serum than the wild-type, implicating haemolysin in the response of CFT073 to serum. In addition to acting as a toxin upon secretion, it has been shown that HlyA forms a complex with LPS and can modulate host immune responses whilst cell associated. The effect of the LPS-HlyA complex on capsule expression and serum resistance was examined and characterised. This study is the first to identify haemolysin as a virulence factor promoting resistance to serum in CFT073, acting as both a secreted factor and whilst associated with the cell.

P080

Responses of human neutrophils to *Fusobacterium nucleatum* subspecies grown planktonically and in biofilm

Maria Muchova, Sarah A. Kuehne, Melissa Grant, Iain Chapple, Josefine Hirschfeld

University of Birmingham, Birmingham, United Kingdom

Abstract

Periodontitis is caused by a combination of oral biofilms and host immune responses mounted towards pathogens in these biofilms. Neutrophils are the dominant innate immune cells in gingival tissues and the oral cavity, where their antimicrobial responses not only kill bacteria, but also cause collateral tissue damage, promoting chronic inflammation. *Fusobacterium nucleatum*, an anaerobic opportunistic pathogen, is a key structural organism in periodontal biofilms. Five subspecies are known to date: *animalis, fusiforme, nucleatum, polymorphum* and *vincentii*. While the subspecies-specific immunogenicity has been investigated in neutrophil-like cell lines, immune responses by human neutrophils are largely understudied.

Human neutrophils isolated from peripheral blood were stimulated with planktonic and biofilm-grown *F. nucleatum* subspecies. Generation of intra- and extracellular reactive oxygen species (ROS) was quantified, as well as formation of neutrophil extracellular traps (NETs). Secretion of anti- and proinflammatory cytokines, neutrophil elastase and matrix metalloproteinase-9 was quantified by enzyme-linked immunosorbent assay (ELISA). Phagocytosis by neutrophils was assessed using flow cytometry.

ROS and NETs production stimulated by planktonic and biofilm-resident *F. nucleatum* subspecies will be discussed. Differential cytokine profiles as well as production of antimicrobial enzymes by neutrophils will be shown. Phagocytic ability of neutrophils will also be presented.

Apart from its association with periodontitis, *F. nucleatum* has also been described in systemic diseases (colorectal cancer for example). Understanding subspecies-specific immunogenicity and pathogenicity of *F. nucleatum* can help unveil novel bacterial and/or biofilm targets for antimicrobial therapies as well as neutrophil targets to mitigate tissue damage in periodontitis and systemic diseases.

P081

Antimicrobial evaluation of mucoadhesive systems containing morin, against periodontal pathogens

Luciana Solera Sales[1,2], Fernanda Lourenção Brighenti[2], Melissa M. Grant[1], Sarah A. Kuehne[1], Michael R. Milward[1]

[1]University of Birmingham - School of Dentistry, Birmingham, United Kingdom. [2]São Paulo State University (UNESP), School of Dentistry, Araraquara, Brazil

Abstract

Periodontitis is the leading cause of tooth loss in adulthood, driven and propagated by a dysbiotic shift in the subgingival pathogenic plaque biofilm and characterized by a dysregulated and excessive inflammatory host response. Morin, a natural botanical substance, has been widely shown to have antimicrobial properties, and therefore offers potential for use in the management of periodontitis. This study aims to evaluate the antimicrobial activity of mucoadhesive systems containing morin, against periodontal pathogens. These systems improve morin's solubility stability and allow sustained release of morin, thereby enhancing the therapeutic potential. Periodontal bacteria, *Porphyromonas gingivalis* (W83) and *Fusobacterium nucleatum* (Fn/23), grown both as a biofilm and planktonic culture, were treated with the system. For both, the system containing morin (2mg/mL of morin) and control (system without morin) were added to respective wells at the beginning of the experiment. For planktonic cultures any inhibitory effects were determined with both turbidity assessment and by counting colony forming units (CFU). A live/dead staining assay and CFU counting were utilized to determine the effect of the system on bacterial viability in mature biofilms. Microbial viability for both *P. gingivalis* and *F. nucleatum* (Log CFU/mL – planktonic culture and mature biofilm) and the turbidity (planktonic culture) reduced after exposure to the morin system compared with the control. Images of mature biofilms using the live/dead staining assay and confocal microscopy, confirmed the CFU/mL findings. Results suggest that the system containing morin is capable of inhibiting two key periodontal pathogens and offers therapeutic potential.

P082

Infection responsive coatings to combat *Proteus mirabilis* crystalline biofilm formation on urinary catheters.

Anthony Slate ORCID iD[1], Ocean Clarke[1], Mina Kerio[2], Jonathan Nzakizwanayo[1], Bhavik Patel[2], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Brighton, Brighton, United Kingdom

Abstract

Indwelling urinary catheters are used extensively for long-term bladder management. However, they are associated with high infection rates; catheter associated urinary tract infections are the most prevalent source of healthcare-associated infection. A primary cause of catheter blockage is colonisation of the catheterised urinary tract by the urease producing bacteria *Proteus mirabilis*, which forms extensive crystalline biofilms. Blockage can initiate the onset of serious medical complications. In response, we have developed a novel infection-responsive catheter coating, to simultaneously provide early warning of blockage, and reduce crystalline biofilm formation. The coating comprises of a pH sensitive upper layer of poly(methyl methacrylate-co-methacrylic acid) and a base layer of poly(vinyl alcohol) loaded with therapeutic agents and a fluorescent dye (5(6)-Carboxyfluorescein). Elevation of urinary pH due to *P. mirabilis* urease activity results in the dissolution of the upper layer and release of the therapeutic agents and dye contained in the base layer. Release of the dye into the urine provides a visual indicator, and an early warning of potential catheter blockage. An *In vitro* model of the catheterised urinary tract was used to evaluate the efficacy of coatings loaded with either acetohydroxamic acid (a urease inhibitor) or ciprofloxacin (as therapeutic agents). A significant increase in blockage time was observed when 5 mg mL^{-1} acetohydroxamic acid and 5 mg mL^{-1} ciprofloxacin were individually incorporated into the coatings. This theragnostic coating constitutes a promising strategy for the localised delivery of therapeutic agents to delay urinary catheter blockage by reducing crystalline biofilm formation.

P083

Effects of increased M cell abundance on early entry of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) into host gut organoids.

Omar Alfituri ORCID iD, Neil Mabbott ORCID iD, Jayne Hope ORCID iD, Joanne Stevens

The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Mycobacterium avium subsp. *paratuberculosis* (MAP) is a contagious bacterium that causes the chronic and enteric Johne's disease in ruminants such as cattle. Johne's disease affects the small intestines and is characterised by gut inflammation and diarrhea. MAP is transmitted via the faecal-to-oral route and invades the gut epithelium of the small intestines through poorly characterised mechanisms. Microfold (M) cells are specialised immune cells of the gut epithelium that have been described as portals of entry for several gut pathogens. These cells can be induced in culture through receptor activator of nuclear factor-κB ligand (RANKL) stimulation. We used a murine 3D enteroid model to show that M cell abundance does not correlate with increased MAP infection. RT-qPCRs from *in vitro* enteroid experiments showed that markers for M cell expression (GP2, SpiB, Sox8 and CCL20) were significantly increased following RANKL treatment. GP2 and bacterial invasion were also visualised by immunohistochemistry and confocal microscopy. Increased M cell abundance did not correlate with increased MAP infection.
However, another invasive enteric bacterial pathogen, *Salmonella enterica* serovar Typhimurium, did exhibit increased intracellular bacteria in enteroids when M cells were stimulated. This suggests that *Salmonella* Typhimurium uses M cells for increased invasion of enteroids. Our data suggest that MAP uptake and survival is not M cell-dependent, and that MAP may be taken up by other cells. A thorough understanding of early entry mechanisms of MAP into the host gut may aid the development of novel strategies to reduce transmission and prevalence of Johne's disease.

P084

Extensive re-modelling of the cell wall during the development of *Staphylococcus aureus* bacteraemia

Nathanael Palk[1], Edward Douglas[2], Ruth Massey[1]

[1]University of Bristol, Bristol, United Kingdom. [2]University of Bath, Bath, United Kingdom

Abstract

Staphylococcus aureus (*S. aureus*) is a leading cause of bacteraemia, a life-threatening infection of the bloodstream. For the last two decades, the mortality rate from *S. aureus* bacteraemia has remained consistently high, highlighting the need for novel therapeutics to improve patient outcome. In previous work, we used a combination of functional genomics and in vivo competition assays to identify the TcaA protein as a novel virulence factor critical for the ability of *S. aureus* to cause bacteraemia. Here, we show that expression of TcaA is induced upon exposure to human serum and that this mediates an increase in wall teichoic acid (WTA) content in the cell wall. We found that this response alters the sensitivity of *S. aureus* to several antimicrobial agents, including peptides and fatty acids present in human serum, and several antibiotics with distinct mechanisms of action. Currently, we are investigating how this response contributes to virulence, our hypothesis being that this response increases the ability of *S. aureus* to adhere to epithelial and endothelial cell lines under flow conditions, promoting survival in the bloodstream and organ dissemination. To understand how TcaA contributes to WTA biosynthesis upon entry to the bloodstream, we are performing co-immunoprecipitation (Co-IP) experiments on cultures uninduced and cultures where *tcaA* expression is induced by serum. Collectively, our data demonstrates that TcaA contributes to *S. aureus* virulence by altering cell wall architecture, a process which is critical for full virulence of *S. aureus* during bacteraemia.

P085

Proteomic investigation of the mode of action of the silver (I) compound SBC3 against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Magdalena Piatek ORCID iD[1], Cillian O'Beirne[2], Zoe Beato[2], Matthias Tacke[2], Kevin Kavanagh[1]

[1]SSPC Pharma Research Centre, Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland. [2]University College Dublin, School of Chemistry, Belfield, Dublin, Ireland

Abstract

Pseudomonas aeruginosa and *Staphylococcus aureus* have been deemed critical and high priority pathogens, respectively, due to high levels of resistance (WHO, 2017). The novel *N*-heterocyclic carbene (NHC) silver(I) acetate complex, SBC3, derived from 1,3-dibenzyl-4,5-diphenylimidazol-2-ylidene (NHC*), has previously shown strong activity against bacterial pathogens, yet little is known about the mode of action.

Label-free quantitative proteomic analysis was employed to ascertain protein abundance changes in *P. aeruginosa* (Gram-negative) and *S. aureus* (Gram-positive) exposed to SBC3. Following protein extraction and peptide purification, peptides were loaded onto a QExactive LC-MS/MS mass spectrometer. MS/MS data were processed through the Andromeda Search engine MaxQuant v.1.6.3.4 using *P. aeruginosa* PAO1 and *S. aureus* NCTC 8325 databases. Statistical and graphical analyses were carried out by processing the resulting LFQ values through the statistical software tool, Perseus v.1.6.6.0.

Both cell types demonstrated increased abundances of outer membrane and cell wall organization/synthesis proteins. Increased abundances of drug efflux, alginate biosynthesis and cell redox homeostasis in *P. aeruginosa* were indicative of a cell stress response. *S. aureus* showed increased abundance of proteins associated with cell redox homeostasis and DNA repair. Anaerobic respiration, the type III secretion system, twitching motility and ABC transport were significantly decreased in SBC3-treated *P. aeruginosa*. Protein synthesis, cell redox homeostasis, protein lipoylation and virulence protein pathways were significantly decreased in *S. aureus*.

The results provide insight into the differential mode(s) of action of a novel silver-based formulation and highlights the potential for the treatment of Gram-positive and -negative mono or dual-infections.

P086

Polyunsaturated fatty acids impact viability and virulence of the food-borne pathogen *Cronobacter sakazakii*

Katie Phair ORCID iD[1], Damien Brady[1], Carmel Kealey[2]

[1]South East Technological University, Carlow, Ireland. [2]Technological University of the Shannon: Midlands Midwest, Athlone, Ireland

Abstract

Background: *Cronobacter sakazakii* is an opportunistic food-borne pathogen predominately associated with infant formula, with current inhibition methods reliant on manufacture and reconstitution guidelines. Fatty acids are gaining interest as potent antimicrobials which are safe to consume. This research explores the efficacy of fatty acids as antimicrobials to combat *C. sakazakii.*

Methods: Oleic acid, linoleic acid and α-linolenic acid ranging from 31.25µM to 1000µM, were individually introduced to overnight *C. sakazakii* cultures. The impact of each exogenous fatty acid was observed in terms of bacterial viability and virulence. Growth over 24-hours was recorded using a FilterMax™ F5 Microplate Reader at 37°C. Biofilm formation, a key virulence trait, was assessed using the Crystal Violet assay. All results were statistically examined and graphed using Minitab™.

Results: α-linolenic acid concentrations as low as 31.25µM reduced growth when compared to the positive control, while oleic acid and linoleic acid were not shown to significantly impact growth. A 1-log reduction of the bacterial culture was observed following incubation with 1000µM α-linolenic acid. This is theorized to be due to reduced membrane integrity leading to cell lysis. Oleic acid and linoleic acid at 125µM reduced biofilm formation by 47.9% and 46.8% respectively, while the same concentration of α-linolenic acid resulted in a 52.5% reduction when compared to the positive control.

Conclusion: Exogenous polyunsaturated fatty acids including α-linolenic acid reduce the growth and biofilm formation of the food-borne pathogen *C. sakazakii.* This data may inform novel inhibition approaches.

P087

Mycobiome diversity analysis in adult cystic fibrosis (CF) patients: analysis of samples from the OligoG phase 2b clinical trials

Abdullah Aseeri[1,2], Rebecca Weiser[1], Philip Rye[3], Eshwar Mahenthiralingam[1]

[1]Cardiff School of Biosciences, Cardiff, United Kingdom. [2]King Khalid University, Abha, Saudi Arabia. [3]AlgiPharma AS, Sandvika, Norway

Abstract

1.Introduction:

CF lung infections are polymicrobial in nature. Traditional diagnosis of CF infection identifies priority microbial pathogens after growth which does not capture the full diversity of microorganisms present. We used culture-independent approaches to explore fungal community composition in adult CF patients who had participated in the OligoG phase 2b clinical trials.

2.Methods:

To examine the fungal diversity, total sputum DNA from 45 Pseudomonas aeruginosa (PA) and 13 Burkholderia cepacia complex (BCC) infected individuals was analysed by next generation sequencing (Illumina) of the ITS2 region. ITS2 sequences were processed using QIIME2. Downstream analysis was conducted with R statistical software.

3.Results:

A total of 2600 different Amplicon Sequence Variants (ASVs) were identified across the dataset indicating the presence of multiple fungal taxa. Filtering to a threshold of 4000 sequence reads resulted in 2 samples being excluded; 1510 ASVs were present across the remaining 56 samples. Comparison of the PA versus BCC samples demonstrated less fungal diversity in CF adults infected with BCC (Simpson index; p-value 0.04). Non-metric multidimensional scaling analysis of the fungal community composition also demonstrated fungal diversity differences between the BCC and PA infected patients. Candida albicans, Candida dublinensis, Candida parasilosis, Aspergillus, Diutina, Fusarium, Podoshaera, and Aurobasium species were the most common fungal taxa encountered in both PA and BCC infected individuals.

4. Conclusion:

Initial mycobiome analysis of CF individuals who participated in the OligoG clinical trial shows that P. aeruginosa infected CF patients harbour a different fungal diversity to B. cepacia complex positive individuals.

P088

Hostile takeover factors of *Pseudomonas*: contact dependent inhibition systems

Alice Collins ORCID iD[1], Nicholas Yuen[1], Jane Davies ORCID iD[1,2], Luke Allsopp ORCID iD[1]

[1]National Heart and Lung Institute, Imperial College London, London, United Kingdom. [2]Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, Sydney Street, London, United Kingdom

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic pulmonary infections in Cystic Fibrosis patients, dominating and damaging the lung environment. *P. aeruginosa* can deliver bacterial-derived toxins into competing neighbours to inhibit growth or kill via the contact-dependent inhibition (CDI) systems. However, the role of CDI systems in *Pseudomonas* infection in Cystic Fibrosis clinical isolates remains unclear. Here we bioinformatically and experimentally investigate the CDI systems encoded in *P. aeruginosa* strain PA14. Using bioinformatics, genetic domains were elucidated and evaluated to determine putative toxin-immunity module functions. We show that PA14 encodes two CDI systems with putative tRNase activities and demonstrate that both CDI systems encode a functional toxin/immunity pair, further supporting their role in antibacterial activity. We constructed whole operon deletion mutants and see no impact of these systems upon motility or biofilm formation. However, we show a role for both systems in interbacterial competition. Finally, we identified regions conserved between and within genetically distinct system types to produce recombinant proteins for antibody production for use as a tool to determine CDI activation status in clinical isolates. Our study further delineates the genomic organisation and role of CDI systems in *P. aeruginosa* interbacterial competition which may contribute to the niche dominance observed in *P. aeruginosa* infection. We propose the use of competitive systems as a mechanism of *P. aeruginosa* dominance in the Cystic Fibrosis lung.

P089

Pyrones as cross species modulators of virulence potential

Muireann Carmody ORCID iD, Gerard McGlacken ORCID iD, F. Jerry Reen ORCID iD

University College Cork, Cork, Ireland

Abstract

Understanding of the complexity of microbial signalling and communication networks has grown in recent decades following disruptive discoveries in quorum sensing, interspecies, and interkingdom communication. However, the field of interspecies and interkingdom communication remains a challenging interface where signalling, coercion, and cues occur spatiotemporally to influence the dynamics of mixed microbial communities in their host ecosystems. In gram-negative bacteria, the classical quorum sensing (QS) system usually consists of a LuxI-type autoinducer synthase that produces a small signalling molecule and a LuxR-type receptor that detects this small molecule to control expression of specific genes. The era of genomics revealed that many species of bacteria encode proteins with homology to LuxR receptors but do not encode the paired LuxI. Initially it was thought that these LuxR orphans enable bacteria to listen for the signal, without themselves producing. However, more recently, alternative small molecule activators of LuxR receptors have been reported, suggesting alternative synthases may exist in these genomes.

The orphan LuxR-type receptor, PluR, in *Photorhabdus luminescens* can detect α-pyrones, specifically derivatives of 3-alkyl-4-hydroxy-6-isobutyl-2H-pyran-2-one. We investigated how QS molecules produced by *P. luminescens* could be interpreted by other species of bacteria to determine if a form of interspecies communication was prevalent for this chemical class of signal. Pyrones (both natural and derived) were found to alter multicellular phenotypic behaviour in gram negative and gram positive pathogens. These data suggest pyrones may play a role in shaping microbial community function, with a role for LuxR receptors in competing species yet to be elucidated.

P090

Development of antimicrobial impregnated sol-gel coatings for urinary catheters to prevent uropathogenic *Escherichia coli* infections.

Kelly Capper-Parkin, Tim Nichol, Tom Smith, Melissa Lacey, Sarah Forbes

Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Uropathogenic Escherichia coli (UPEC) are a predominant cause of catheter associated urinary tract infections (CAUTI). UPEC often form biofilms on the catheter surface, which are recalcitrant to antibiotic therapy. Existing anti-infective catheter coatings have been shown to display limited efficacy and may select for resistance due to the use of a single biocide. Furthermore, at biofilm eradication concentrations, biocides often display cytotoxicity, limiting their antiseptic potential. Quorum sensing inhibitors (QSIs) provide a novel anti-infective approach to disrupt biofilm formation on the catheter surface and help prevent CAUTI.

This work aimed to 1) identify effective antimicrobial concentrations with minimal cytotoxicity of biocides combined with QSIs, 2) profile the release of these agents from a sol-gel coating and 3) quantify biofilm formation upon the combined antimicrobial impregnated sol-gel coatings.

From the panel of antimicrobials, biocides: polyhexamethylene biguanide, benzalkonium chloride and silver nitrate in combination with QSI trans-cinnamaldehyde or furanone-C30, demonstrated antimicrobial synergism against UPEC biofilms. However, furanone-C30 was cytotoxic at concentrations below those required for bacteriostatic activity. Elution of the antimicrobials from the sol-gel coating demonstrated distinct profiles, silver nitrate eluted rapidly from the coating within 24 hours, whereas benzalkonium chloride did not elute. When biocides with QSIs were combined in the sol-gel coating, there was a significant reduction in biofilm formation.

Of the combinations tested, polyhexamethylene biguanide and silver nitrate in combination with trans-cinnamaldehyde demonstrated synergistic antimicrobial activity at non-cytotoxic concentrations. These antimicrobials eluted from the sol-gel coating and inhibited biofilm formation, making them a potential for use in further studies.

P091

The importance of copper hypertolerance genes for *Staphylococcus aureus* survival in neutrophils

Laura Hollis[1], Joan Geoghegan[1,2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Trinity College Dublin, Dublin, Ireland

Abstract

Despite being a persistent member of the nasal microbiome in around 30% of humans, *Staphylococcus aureus* can cause severe skin and soft tissue infections, as well as life-threatening infections such as sepsis and pneumonia. The *copB-mco* operon encodes the CopB P-type ATPase and Mco multicopper oxidase that contribute to copper hypertolerance and increase survival of *S. aureus* within macrophages and in whole human blood. As the neutrophil is an important defence against *S. aureus* bloodstream infection, we hypothesised that *copB* and *mco* promote survival of *S. aureus* within the neutrophil. Here we aimed to determine if the *copB-mco* operon is upregulated when *S. aureus* is internalised within the neutrophil phagosome. A fluorescent reporter construct consisting of the *copB-mco* promoter fused to GFP was generated in *S. aureus*. This enabled detection of *copB-mco* promoter activation in conditions relevant to those experienced within the phagosome, using Western immunoblotting and flow cytometry. This reporter system will be a valuable tool for understanding when and where the *copB-mco* genes are expressed both in *in vitro* and *ex vivo* infection models of *S. aureus* infection.

P092

How to wake up bacteria that like to play dead?

Sariqa Wagley, Abi Brader

University of Exeter, Exeter, United Kingdom

Abstract

Bacterial dormancy occurs in over 80 bacterial species and is usually induced in response to environmental stress. When bacteria are exposed to prolonged environmental stress many cells in a population will not cope with those prolonged conditions and will die unless that stress is removed. Some cells (<1%) will turn into a dormant sleep like state. These dormant cells are metabolically active but are unable to form colonies on standard culture media making them hard to detect. Once the environmental stress has been removed those dormant cells can 'resuscitate' and become culturable once more, restoring their ability to grow on media. The ability of these dormant cells to go undetected by conventional microbiological practices could lead to an underestimation of total viable cells in environmental and clinical samples. In this project, we tested seafood samples for the presence of culturable and dormant *Vibrio* cells and whether sodium lactate can help recover dormant *Vibrio* cells into a culturable form. *Vibrio* species are the most common cause of seafood associated gastroenteritis and their presence in seafood samples is a concern for the seafood industry. Thus, our ability to detect dormant *Vibrio* cells in the environment and understand how to wake them up from a dormant state will be important to predict and prevent disease potential in the future.

P093

Metabolism of host-associated sugars drives virulence in Enterohaemorrhagic *Escherichia coli*.

Curtis Cottam ORCID iD, James Connolly ORCID iD

Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) is a major foodborne pathogen, and a significant cause of zoonotic disease. EHEC has a remarkably low infectious dose, resulting in outbreaks that manifest in bloody diarrhoea, and in extreme cases, acute renal failure. Treatments against EHEC are limited, as antibiotics exacerbate infection by triggering the expression of toxins that lead to renal failure. Understanding EHEC pathogenesis is therefore crucial to the development of novel treatments. EHEC sense environmental nutrients as "signals" in the gut to fine-tune the expression of their primary virulence factor, the Type 3 Secretion System (T3SS), which is essential for effector protein delivery into host cells. However, the mechanisms underlying how EHEC exploit different nutrients are poorly understood.

L-arabinose is an abundant sugar found widely in plant cell walls and can support EHEC growth as a sole carbon source. Here, we found that growth on L-arabinose also significantly enhanced T3SS expression. Deletion of the genes required for arabinose uptake, metabolism and associated regulation revealed that this phenotype relies upon L-arabinose breakdown and not just merely "sensing" its presence. Furthermore, transcriptomic analyses revealed that L-arabinose specifically regulates a novel carbohydrate uptake system, that is encoded on a horizontally acquired genetic element and is significantly enriched in EHEC strains across the *E. coli* phylogeny. We propose that additional nutrient uptake systems allow EHEC to outcompete the native gut microbiota as well as contribute to virulence regulation. Deciphering nutrition-related pathogenic mechanisms could lead to novel treatments, including dietary interventions, as an alternative to antibiotics.

P094

Early Host and Bacterial transcriptomic Responses During Intracellular Staphylococcus aureus Osteoblast Infection

JJ Awodipe[1,2,3], Meera Unnikrishnan ORCID iD[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Medical Research Council (MRC), London, United Kingdom. [3]Medical and Life Sciences Research Fund, Warwickshire, United Kingdom

Abstract

Bone and joint infections (BJIs) are severe infections with 18-73% of cases caused by Staphylococcus aureus. BJIs have high rates of recurrence which has been associated with the persistence of S. aureus inside bone cells, where it evades host immune responses, antibiotics and detection. Understanding the host and bacterial factors that control intracellular infection is thus crucial for developing better treatment and diagnostic strategies.

To examine bacterial and host responses during intracellular infection of bone-forming osteoblasts, we first used live-cell imaging to track S. aureus persistence in MG-63 human osteoblasts. Dual RNA sequencing analysis was then performed at early times during S. aureus osteoblast invasion and intracellular replication (1.5 and 5 h post-infection) to simultaneously analyse bacterial and host transcriptional responses. Following cell infection, S. aureus upregulated known adhesin genes (ecb, efb, fnbp) while toxins (-hemolysin, enterotoxin A) and agr family proteins (agrA, agrB, agrC) were downregulated. Metabolic pathways, including glucose, amino acid and nucleotide metabolism, plus previously uncharacterised surface proteins and regulators, also changed significantly in intracellular S. aureus. In contrast, only a few genes in infected osteoblasts showed significant change compared to the control cells. Notably, SQSTM1, TAP1 and IFNGR2, which participate in host immune responses, were downregulated in infected osteoblasts along with RNA splicing genes and small nuclear RNAs such as U6atac small nuclear. Experiments to characterise interesting bacterial proteins involved in adhesion and regulation of intracellular infection are ongoing. Overall, this study provides new insights into early host-pathogen interactions between intracellular S. aureus and infected osteoblasts.

P095

Activity of Cefiderocol against First Isolates of *Pseudomonas aeruginosa* in a Cystic Fibrosis Population

Saied Ali ORCID iD, Sinead McDermott

St Vincent's University Hospital, Dublin, Ireland

Abstract

Background

Pseudomonas aeruginosa is an opportunistic pathogen that commonly infects the cystic fibrosis (CF) lung, promoting an accelerated decline of pulmonary function. It exhibits significant resistance to innate immune effectors and antimicrobials by expressing specific virulence factors and by acquiring adaptive mutations during chronic infection. Cefiderocol is a novel siderophore cephalosporin with activity against Gram-negative pathogens, including *P. aeruginosa*. We aimed to assess the in vitro efficacy of cefiderocol against *P. aeruginosa* isolates in a CF population.

Methods

The study was conducted in a large tertiary hospital with a specialist adult CF service caring for approximately 300 patients. All first isolates of significant respiratory pathogens among this cohort are cryopreserved at -80°C. Antimicrobial susceptibility testing to cefiderocol was performed as per EUCAST Disk-Diffusion (Version 10) for all first isolates of *P. aeruginosa* from 2017-2022 inclusive.

Results

Forty-one isolates were tested. None were categorised as multi-drug resistant (MDR) or pan-drug resistant (PDR) strains. The average age of patients was 27 years; with the average age at which *P. aeruginosa* was first isolated being 24 years (IQR 18-28). As per interpretative breakpoints, with a zone of ≥22 mm indicating susceptibility to cefiderocol, only 3 isolates were deemed resistant, with an overall average disk-diffusion zone of 27.1 mm.

Conclusion

Cefiderocol exhibited excellent in vitro anti-Pseudomonal activity. Further studies are necessary to evaluate its efficacy against MDR and PDR strains.

P096

Clinical profile of ICU patients with Colistin resistant *Klebsiella pneumoniae* infection and their antimicrobial susceptibility pattern

Rushika Saksena, Shiva Verma, Rajni Gaind

VMMC and Safdarjung Hospital, Delhi, India

Abstract

Introduction: Colistin is a last resort antibiotic to treat infections caused by multi-drug resistant *Klebsiella pneumoniae* in the ICU. However, resistance to colistin is being increasingly reported.

Material and Methods: The retrospective study was conducted in mixed medical-surgical ICUs of a tertiary-care hospital in Delhi, India from January to December 2019. Forty-four adult patients with at least one culture positive for colistin-resistant *Klebsiella pneumoniae* (CRKP) were included. Complete demographics, clinical history, management and outcome available for 20 patients was evaluated. Bacterial identification and antimicrobial susceptibility testing was performed by Vitek-2. Colistin MIC was determined by broth microdilution.

Results: Forty-seven CRKP were isolated from 44 patients. Twenty-six strains were isolated from blood, ten from endotracheal aspirates, seven from urine and four from pus samples. All but one isolates were resistant to third-generation cephalosporins, betalactam+betalactam inhibitor combinations, fluoroquinolones, carbapenems whereas 6.3% isolates showed susceptibility to aminoglycosides. All isolates were negative for mcr-1, mcr-2, mcr-3 and mcr-4 genes. Eleven patients had a history of surgery and three of previous ICU admission in past 30 days. Nineteen patients had an indwelling central venous catheter, 19 a urinary catheter and 18 were on mechanical ventilation. Eighteen out of 20 patients had received colistin therapy before isolation of CRKP. CRKP was isolated after a median 11 days of ICU admission and 7.5 days of colistin therapy. A 60% mortality rate was observed.

Conclusion: Colistin resistance *Klebsiella pneumoniae* show resistance to all other group of antibiotics leading to difficult to treat infections in ICU and high mortality.

P097

Fluorinated galactose analogues inhibit pneumococcal growth and biofilm formation

Charlotte Fairchild[1], Abbie O'Connor[1], Jeremy Webb[2], Saul Faust[2], Bruno Linclau[3], Raymond Allan ORCID iD[1]

[1]De Montfort University, Leicester, United Kingdom. [2]University of Southampton, Southampton, United Kingdom. [3]Ghent University, Ghent, Belgium

Abstract

Streptococcus pneumoniae is a genetically diverse pathobiont capable of adapting its metabolism to survive in the nutritionally-challenging nasopharyngeal niche. Galactose, the most abundant carbohydrate in the nasopharynx, supports pneumococcal colonisation and biofilm formation on the mucosa, acting as both an attachment site and metabolic source. Targeting pneumococcal galactose utilisation therefore represents an attractive opportunity for the development of novel therapeutics. In this study, we assessed the potential of fluorinated galactose analogues (Gal-3F, Gal-4F, Gal-6F) against pneumococcal type strains (D39, TIGR4) and clinical isolates (serotypes 3, 6B, 19F). Biofilm formation and established 48-hour biofilms were assessed by crystal violet staining (biomass) and colony forming unit enumeration (viability), with validation through confocal imaging (live/dead) and scanning electron microscopy (SEM). Adherence to Detroit 562 epithelial cells was measured by cfu enumeration, and planktonic metabolism assessed by ^{1}H-NMR. Initial testing using type strain D39 revealed that treatment with 1-2% w/v Gal-3F significantly reduced biofilm formation ($p \leq 0.05$), with 2% Gal-4F showing only a small reduction, and Gal-6F having no impact, suggesting that the site of fluorination is important. Furthermore, 2% Gal-3F impaired biofilm formation of all pneumococcal isolates tested. Confocal and SEM imaging confirmed a reduction in biomass, with biofilms demonstrating disrupted architecture and evidence of cellular distress. Viability was also reduced 2-log ($p \leq 0.01$). Whilst epithelial adhesion was not affected, preliminary ^{1}H-NMR analyses indicate that Gal-3F elicits a change in metabolic activity. Overall, these data highlight the potential of fluorinated galactose analogues as novel treatments for prevention of pneumococcal biofilm infection.

P098

The role of the Type Six Secretion System of *V. vulnificus* in bacterial killing

Jemma Harford ORCID iD, Cameron Hubert, Chrsitian Fitch, Stephen Michell ORCID iD

University of Exeter, Exeter, United Kingdom

Abstract

Vibrio vulnificus infection is a leading cause of seafood related deaths, either through primary septicaemia or neurotic wound infection. *V. vulnificus* contains two type 6 secretion systems (T6SS); T6SS1 and T6SS2. T6SSs are protein nanomachines which can facilitate killing of non-kin bacterial species. Previous *V. vulnificus* co-culture killing assays using T6SS mutants observed T6SS1 and T6SS2's role in temperature dependent killing. This study further tests these mechanisms using *Vibrio fluvialis* as the 'prey' bacteria. In vitro co-culture assays were set up using *V. vulnificus* and mutants in T6SS1 and T6SS2 as the attacker strain and *V. fluvialis* as prey. After incubation at 30°C and 37°C for 5 hours, Miles and Misra dilutions were carried out and plated on selective agar for CFU calculation. The results demonstrated the role of *V. vulnificus* T6SS in killing of *V. fluvialis* at 30°C. *V. vulnificus* 106-2A mutated for T6SS1 and T6SS2 both failed to kill *V. fluvialis*. No significant killing was observed in co-culture assays at 37°C highlighting the temperature dependent nature of the T6SS's. Future work can test these findings in vivo in a recently developed oyster model of infection to elucidate the interations of these strains in the environment.

P099

The validation of a PCR method for *Streptococcus dysgalactiae* infection in sheep and its application for on-farm disease transmission studies

Louise Jackson[1], Jennifer Duncan[1], Keith Ballingall[2]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Moredun Research Institute, Penicuik, United Kingdom

Abstract

Background: *Streptococcus dysgalactiae* subspecies *dysgalactiae* 'SDSD' is the predominant cause of infectious arthritis (joint ill) in neonatal lambs. Difficult to isolate in mixed bacterial culture, PCR could aid detection and diagnosis. Therefore, this study aims to validate a PCR test for SDSD and test its applicability in field samples from farms with a history of joint ill.

Methods: Five published primer sets for PCR were compared. Rudimentary testing for strain diversity, sensitivity, specificity, and cross-reactivity was conducted. Further confirmation of primer sensitivity and specificity was conducted by DNA quantification and sequencing of the PCR product, respectively. Swabs were collected from ewes (n=350), joint taps were taken from joint ill lambs (n=6), and environmental samples (n=253) were collected across four farms with a history of SDSD joint ill.

Results: Two PCR primer sets produced visible bands via agarose gel electrophoresis up to and including a dilution factor of 10^{-3}, from an original 4.4 ng/µl suspension. The PCRs were screened against a panel of 20 different bacterial species, including a variety of Streptococcal species. No products were formed by one PCR for any bacteria featured. 16s sequencing of the PCR product was conducted, confirming a 100% match to known SDSD genomes. The remaining primer set was adapted for real time PCR, allowing rapid screening of field samples.

Conclusion: Rapid detection of SDSD aids treatment and investigation of transmission routes and bacterial sources, without relying on culture. Following further validation, the PCR described could be a diagnostic tool for SDSD joint ill.

P100

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIMICROBIAL PEPTIDES BASED ON HUMAN β-DEFENSINS FOR BIOMEDICAL APPLICATIONS

Pietro Riccio[1], Sarah Kuehne[2], Artemis Stamboulis[1]

[1]Biomaterials Research group, School of Metallurgy and Materials, University of Birmingham, Birmingham, United Kingdom. [2]School of Dentistry, Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

Biomaterial associated infections have an high failure rate and are not easy to treat with classical antibiotics due to biofilm formation and antibiotic resistance. There is the need to search for new effective antimicrobial agents that can be use directly on implant materials and are less prone to develop resistance. In this context, antimicrobial peptides (AMPs) seem to have great potential because of their broad antimicrobial activity, low toxicity, and ability to induce an immune response.

Using bioinformatic tools, new AMPs were designed modifying the human β-defensins' natural sequences based on physico-chemical properties, such as hydrophobicity and charge, molecular structures, and dynamics. These new AMPs are now being used in the AIMed consortium (https://aimed-itn.eu/) to functionalise several materials creating antimicrobial surfaces and coatings, which will allow for an optimised and localised release of the antimicrobial agent directly to the infection site, without affecting the systemic microbiota.

The new AMPs were produced by Solid-Phase Peptide Synthesis (SPPS) using an automated microwave synthesiser, followed by preparative-HPLC (High Performance Liquid Chromatography) purification, with the aid of Liquid Chromatography-Mass Spectrometry (LC-MS) and Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) to track elution times and molecular masses before and after the purification. Secondary structures were also investigated by Circular Dichroism (CD) spectroscopy.

The antimicrobial and antibiofilm activities of these AMPs were evaluated against a panel of Gram-positive and -negative bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Escherichia coli*. Their cytotoxicity has also been studied on osteoblastic cells.

P101

Development of a simple-to-use and cost-effective tool to indicate the mechanism of action of a novel antimicrobial

Jessica Furner-Pardoe, Charlotte Hind, J. Mark Sutton

UKHSA, Porton Down, United Kingdom

Abstract

Antimicrobial resistance is no longer a looming crisis, but a problem of today. Rapid discovery and development of new drugs is key to saving millions of lives, and understanding an antibiotic's mechanism of action (MoA) and finding novel mechanisms and new classes of antibiotics could be game-changing. There are many techniques to assess MoA, however, with several being time-consuming and expensive, there remains a need for a quick, cost-effective, high-throughput assay. Here, we present a simple-to-use, cost-effective tool to aid the identification of the MoA of a compound through the use of *E. coli* biosensors. We have utilised a published *E. coli* fluorescent reporter strains library where GFP is used to measure promoter activity within living cells. With the use of liquid handling systems, we have screened the entire library (~2,000 biosensors) and characterised *E. coli*'s cellular response to numerous antibiotics with known mechanisms of actions. In doing so, we have determined a 'fingerprint' for different classes of antibiotics. Using this, we have narrowed down key promoters and generated a tool that can be challenged with sub-inhibitory concentrations of a novel compound to see if a known mechanism of action 'fingerprint' is seen. For example, upregulation of recA, lexA and polB is a marker of an antibiotic that affects DNA replication. This tool comes with the advantages of being low cost, easy to use, with minimal chemical hazards and scope to be used for high-throughput, making it a valuable tool to assist drug development of novel antimicrobial compounds.

P102

Novel Models for the Study of Host, Pathogen and Drug interactions in Cystic Fibrosis Lung Infections.

Thomas E. Barton, Joanne L. Fothergill, Aras Kadioglu, Daniel R. Neill

Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, Liverpool, United Kingdom

Abstract

People with cystic fibrosis (CF) face a considerable burden of chronic infection from bacteria such as *P. aeruginosa*. This necessitates a need to develop more efficacious antimicrobial treatments. Current preclinical models used in antimicrobial development translate poorly to the clinic, hindering development. It is necessary to streamline the preclinical development pipeline, to develop models that reflect crucial factors of the CF environment, and can be assessed for treatment-responsive, host-derived biomarkers associated with positive clinical outcomes.

This project aims to optimise *in vitro* and *in vivo* preclinical models that capture essential aspects of the CF host environment, including host factors and biomarkers that are associated with the resolution of airway exacerbation in a clinical setting. Current work involves the optimisation of an *in vitro* cell culture model with infection of *P. aeruginosa*. Planktonic *P. aeruginosa* growth on a lung adenocarcinoma cell (A549) monolayer results in considerable cytotoxicity, a consequence of growth in nutrient-rich cell culture media. As a result, the model has been adapted for A549 growth on cell culture inserts, with cell culture media in the basal compartment and *P. aeruginosa* in the apical compartment, cultured variously at an air-liquid interface or in the presence of CF sputum mimics. Here, we present our optimisation of a cell culture model for CF antimicrobial development.

Streamlining and standardisation of the preclinical antimicrobial development pipeline will increase the efficiency of product progression and facilitate collaboration between clinicians, industry, regulators and academics. This will ultimately improve clinical outcomes for those with CF.

P103

Understanding environmental and biocide survival of *Candida auris* through transcriptional profiling

Alicia Ware ORCID iD[1], Christopher Delaney[2], William Johnston[1], Lesley Price[1], John Butcher[1], Gordon Ramage[2], Ryan Kean[1]

[1]Glasgow Caledonian University, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Background: Candida auris is a problematic fungal pathogen, recently elevated to the Critical Priority group of fungal pathogens by the World Health Organisation. Of key concern is its ability to cause outbreaks within intensive and chronic care units, which is facilitated through its environmental persistence. This current study investigated the role of biofilms in surviving disinfection using a semi-dry surface biofilm (SDB) model and transcriptomic profiling.

Methods: In this study, two phenotypically distinct isolates of C. auris were grown in an optimised SDB model consisting of consecutive 48hr cycles with/without media across a 12 day period. Disinfection was performed using clinically relevant protocols of sodium hypochlorite (NaOCl) treatment and evaluated using conventional plate counting and live/dead qPCR. RNA-sequencing was performed on NaOCl and untreated SDBs in comparison to planktonic cells.

Results: Isolates were found to grow robust biofilms using the SDB protocol, and could tolerate all treatment parameters, with only 2-3 log_{10}-reductions observed at highest concentrations. Transcriptional profiling identified genes corresponding to ABC transporters (CDR1, CDR2), heat shock proteins (HSP10, HSP70) and metal acquisition (SIT1, PGA7) were strongly upregulated in SDBs compared to planktonic cells.

Conclusion: We have optimised a SDB protocol in which C. auris biofilms can mediate tolerance to adverse conditions such as NaOCl disinfection, suggesting a lifestyle through in which this problematic yeast can environmentally persist and transmit. Mechanistically, the upregulation of small-molecule and haeme-iron transport have been identified as a potential survival mechanism.

P104

Does the current practice of intravenous-iron treatment raise the risk of infection by bacterial pathogens?

Sayandini Kirushnamoorthe[1,2], Claire Seeley[2], Sheila MacIntyre[1], Douglas G Barker[2], Simon C Andrews[1]

[1]Health and Life Sciences Building, School of Biological Sciences, University of Reading, United Kingdom, Reading, United Kingdom. [2]Department of Anaesthesia, Royal Berkshire NHS Foundation Trust, Reading, United Kingdom

Abstract

Iron-deficiency anaemia is the most common hematologic problem in the world. Although oral iron supplements are the most common therapy applied, intravenous (IV) iron is considered superior in both efficacy and safety in certain clinical situations. However, since pathogenic bacteria require iron for growth and infection, and blood is (normally) highly iron restricted, there is potential for IV-iron treatment to support growth of bacterial pathogens. Further, catecholamine stress hormones (e.g. norepinephrine, NE) act as iron-mobilisation agents mediating delivery of iron to bacteria. Thus, conditions or treatments that raise catecholamines may increase infection potential particularly when combined with IV-iron. Thus, we have explored whether IV-iron formulae together with NE support growth and virulence of bacterial pathogens under serum-like conditions.

Growth experiments were performed with Salmonella Typhimurium in a non-serum based minimal medium supplemented with a range of iron compounds or commonly available IV-iron formulae (e.g. iron dextran, iron sucrose and ferrous gluconate). In addition, NE and transferrin were included.

The results revealed that partially (15-20%) iron-saturated transferrin (Tf) provides an iron deficient environment by arresting bacterial growth, while greater saturation levels of Tf support growth. Inclusion of NE overcame the bacteriostatic effect of Tf-mediated growth inhibition. Importantly, growth stimulation by second generation of IV-iron formulae was concentration dependent and reversed by apo-Tf, but this effect was countered by NE. Future work will focus on the impact of IV-iron formulae and NE on bacterial growth in serum-based media.

P105

Unravelling extracellular protease regulation in Staphylococcus aureus

Shuxian Li, Maisem Laabei

University of Bath, Bath, United Kingdom

Abstract

Staphylococcus aureus is a virulent and antibiotic-resistant pathogen causing suite of superficial and life-threatening diseases. *S. aureus* pathogenicity involves the secretion of proteases which can act on host proteins, promoting immune evasion and bacterial proteins, altering the stability of key virulence factors central in colonisation and infection.

We determined the proteolytic activity of 134 genome-sequenced USA300 clinical isolates using casein hydrolysis assay (CHA). Our aim was to understand whether protease expression was conserved across these isolates, investigate if infection source impacted expression and finally, using genome-wide association studies (GWAS), to identify loci implicated in protease regulation.

We confirmed the suitability of CHA through screening isogenic protease and virulence regulator mutants previously shown to impact protease activity. Interestingly we observed a significant level of variability in protease activity across the isolates despite high genetic relatedness. We also identified differences in protease expression between different isolation sources, where bacteraemia isolates expressed less protease comparing to isolates from skin carriage or skin and soft tissue infections. Following our GWAS analysis we identified 60 loci that were statistically significantly associated with altered protease activity. Using Nebraska Transposon library, we confirmed that 31 of these loci had altered protease activity when disrupted. These loci were annotated as conserved hypothetical proteins, hydrolases, membrane proteins and drug transporters, permeases and an AraC family DNA-binding response regulator. Future work will narrow in on how these proteins regulate protease expression. This study highlights the complexity of protease regulation in *S. aureus* and deepens our understanding of *S. aureus* pathogenicity.

P106

Evaluation of new tools to determine infection-induced protein complexes

Daniel Lockwood ORCID iD[1], Caleb Logan[1], Iris Lade[1], Karthika Nagalekshmi[1], Jack Penny[2], Ben Collins[2], Gunnar Schroeder[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfas, Belfast, United Kingdom. [2]School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom

Abstract

Legionella pneumophila is an intracellular pathogen responsible for Legionnaire's disease – a severe form of pneumonia. *L. pneumophila* utilises a complex multi-protein machinery, the Dot/Icm Type 4 Secretion System (T4SS), to deliver over 330 effector proteins which manipulate host cells. Many exert their roles through direct interactions with host proteins, modulating their interactomes. Identification of these targets, but also the impact on their interactome is key to dissect the infection process.

Methods such as yeast-two-hybrid screens yielded important insights but do not reflect the state of the infected cell. Its proteome changes due to numerous translocated effectors, host responses and manipulation of translation.

We previously developed a tandem-affinity purification system that relies on biotinylation of a tag (Bio-tag) fused to effectors after delivery into host cells expressing the biotin ligase BirA, enabling determination of effector interactomes, but this system has moderate power to reveal the wider interaction network of the host targets.

Therefore, we tested new systems, which use effectors fused to promiscuous biotin ligases such as TurboID, which efficiently, but unspecifically biotinylate proteins within their close proximity. This allows for isolation of host targets and their interactome by streptavidin pull down and subsequent mass spectrometry analysis. The activity of promiscuous biotin ligase alone or fused to an effector did not compromise bacterial replication. Moreover, we show that the biotin ligase-effector fusions are translocated by the Dot/Icm T4SS and allow biotinylation of different subproteomes, suggesting that they are useful additional tools to dissect *Legionella*-host interactions.

P107

Molecular Identification of Prototheca Infections in UK Cattle

Christopher Shave ORCID iD, Robin May ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Prototheca species are an unusual group of algae that are non-photosynthetic pathogens of diverse mammalian species. They most commonly infect cattle, typically causing mastitis and reducing milk yields. Cases have been reported from around the world, but to date there have been no clinical case reports from the UK. Global prevalence, and thus impact, remains unclear.

We have developed a sensitive PCR-based method to detect the presence of *Prototheca* cells in milk at densities as low as 100 cells per ml. Here we use this method on a set of bulk milk samples to detect, and identify to species level, bovine *Prototheca* infection in the UK. Furthermore, we highlight potential challenges with current *Prototheca* surveillance in the UK in terms of specificity and possibly sensitivity.

We hope that this new method will encourage more detailed analysis of *Prototheca* infections in cattle in order to inform future epidemiological research and reduce the impact of this disease on the UK dairy industry.

P108

ANALYSIS OF PROPHAGES FROM UK *Helicobacter pylori* ISOLATES

Omoyemi Rebecca Ojo, Benjamin Dickins, Jody Winter

Nottingham Trent University, Nottingham, United Kingdom

Abstract

With antibiotic resistance prevalence increasing, there is a need to develop new and more effective therapies to treat *Helicobacter pylori* infection. There is growing interest in the potential for therapeutic use of lytic bacteriophages wholly or in combination with antibiotics for the treatment and eradication of multidrug resistant bacteria. Our study aimed to analyze the prevalence and diversity of prophage genes in a UK collection of *H. pylori* isolates and explore their therapeutic potential.

PHASTER and PhiSpy were used to identify prophage genes in the bacterial genome sequences of 32 *H. pylori* strains isolated from the stomachs of 18 patients. ViPTree was used to generate a proteomic tree based on the genomic sequences of the identified prophages, to determine how related the prophages are and how similar or different they are to other previously identified prophages of *Helicobacter pylori.*

Most of the *H. pylori* genomes we analyzed did not contain prophages. Two prophage clades were identified within the genomes of five of the 32 bacterial strains. One of these clades was similar to the previously reported *H. pylori* phage KHP40 and another is more distantly related although represented by only relatively short sequences. This suggests that the prevalence of prophages in *H. pylori* genomes, and likelihood of finding novel prophages with therapeutic potential using this type of genomic analysis, are relatively low. We are also screening river water samples collected downstream of combined sewage overflows for the presence of phages with lytic activity against *H. pylori* and other bacterial species.

P109

Synthesis of cationic polymers with antimicrobial and antibiofilm activity.

Natasha Reddy, Freya Harrison, Sébastien Perrier

University of Warwick, Coventry, United Kingdom

Abstract

As a result of antimicrobial resistance, we edge closer to a post-antibiotic era and the development of novel antimicrobials is urgently required. Biofilm formation plays a significant role in bacterial persistence and resistance to antibiotics, with up to 80% of bacterial infections being linked to biofilm forming bacteria. Antimicrobial peptides offer a platform to base the development of the next generation of antimicrobials. These small peptides selectively target bacteria, with limited development of resistance. Unfortunately, the cost associated with their production and their toxicity are barriers to their widespread clinical application. By utilising polymer chemistry, specifically using reversible-addition fragmentation chain transfer (RAFT) polymerisation, we have synthesised polymers that mimic aspects of these natural peptides. These polymers are proving to be a promising platform for the development of novel antimicrobials as their antimicrobial effects and toxicity can be tuned, while keeping production costs low. We synthesised an ammonium containing polymer, which has previously been shown to exhibit antimicrobial and antibiofilm activity against *Pseudomonas aeruginosa*. We tested its antimicrobial effects using MIC assays against a panel of 5 bacterial and fungal pathogens of concern, in media mimicking clinically relevant conditions (synthetic wound fluid). Following this we used a Calgary device to determine its biofilm eradication potential. Future work will investigate synergy between the polymer and existing antimicrobials. The goal of this work is to elucidate suitable combinations of polymer and antimicrobial to test for biofilm eradication efficacy in high validity biofilm models.

P110

The effect of growth environment and biofilm formation on the antibiotic resistance of *Pseudomonas aeruginosa*

Jenny Littler, Freya Harrison

University of Warwick, Coventry, United Kingdom

Abstract

Increasing antibiotic resistance rates are a concern for both mortality and the economy. Biofilm formation is implicated in higher levels of antibiotic resistance through preventing diffusion of antibiotics to their targets, and changes in bacterial physiology. *Pseudomonas aeruginosa* is an opportunistic and key pathogen, which is greatly implicated in the lung infections of those with cystic fibrosis. *P. aeruginosa* is also a well-regarded model organism for studying biofilms. I have established that antibiotic susceptibility in planktonic environments varies depending on the media that *P. aeruginosa* is grown in, which can affect the classification of the pathogen as either sensitive or resistant to an antibiotic. I have also investigated antimicrobial resistance in biofilm models including an *ex vivo* pig lung model of cystic fibrosis (EVPL), comparing PA14 WT strain with a PA14 transposon mutant in the gene *pelA*, which cannot make the main PA14 biofilm scaffold polysaccharide and so cannot produce a mature biofilm. Despite this, in the EVPL model, both PA14 WT and *pelA*$^-$ mutant had equally high levels of resistance to colistin and meropenem. This suggests that mechanisms beyond biofilm production aid the increase in antibiotic resistance. Transcriptomics was used to explore the effect that growth environment has on resistance and what factors beyond the biofilm matrix contribute to increased resistance in the EVPL. Key differences in the expression of specific efflux pumps and two-component systems between WT and *pelA*$^-$ were found, these will be further explored to establish the importance of these mechanisms for resistance in the EVPL.

P111

Bloodstream infection-associated dysregulation of haemostasis can be modulated by iron-regulated *Staphylococcus aureus* cell wall elements

James Waterfield[1], Jonathan Gibbins ORCID iD[1,2], Simon Clarke[1]

[1]University of Reading, Reading, United Kingdom. [2]Institute for Cardiovascular and Metabolic Research, Reading, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of bloodstream infections and a significant portion of the morbidity associated with infection can be attributed to the ability of *S. aureus* to dysregulate haemostatic functions through interaction with platelets via Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMs). These cell wall-anchored proteins have been widely studied in nutrient-rich media, but information about their function in nutrient-limited media, such as blood plasma, is limited. As such, we have been studying the effect of iron limitation on the ability of *S. aureus* to modulate platelet function in vitro, with a particular focus on the haem-uptake machinery, Isd. *S. aureus* mutants for two cell wall-anchored Isd proteins, IsdB and IsdA, were precultured in RPMI 1640 medium, followed by an additional overnight culture in RPMI 1640 with or without iron supplementation. Overnight cultures were then introduced to platelet-rich plasma and subsequent platelet aggregation was measured using light-transmission aggregometry. Early data suggests an iron-dependent effect on the lag time between bacterial inoculation and initiation of platelet aggregation, as well as the time for platelets to reach maximum aggregation. These findings may further implicate iron-regulated proteins as an important factor in the haemostatic modulation ability of *S. aureus* in bloodstream infections.

P112

Friend to Foe: Comparison of Paired Nasal and Infection Staphylococcus aureus Isolates from Patients with Invasive Disease,

Kasia M Parfitt ORCID iD[1], Bernadette C Young ORCID iD[2,3], N Claire Gordon ORCID iD[2,3], A Sarah Walker ORCID iD[4], Tim E Peto ORCID iD[5,4], Derrick W Crook ORCID iD[5,4], David H Wyllie[6,7], Daniel J Wilson ORCID iD[1], Justine K Rudkin ORCID iD[1]

[1]Big Data Institute, Nuffield Department of Population Health, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom. [2]Nuffield Department of Medicine, Experimental Medicine Division, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom. [3]Microbiology and Infectious Diseases Department, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, United Kingdom. [4]Nuffield Department of Medicine and NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom. [5]Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom. [6]Nuffield Department of Medicine, Experimental Medicine Division, University of Oxford, Oxford, United Kingdom. [7]Field Service, Public Health England, Addenbrooke's Hospital, Cambridge, United Kingdom

Abstract

Nasal carriage of *Staphylococcus aureus* is a risk factor for invasive disease, with infections often seeded by colonizing strains. Transitioning from commensal to pathogen involves a change of niche, and with different selection pressures. Understanding how *S. aureus* adapts in an infection context can help us to understand how it causes disease and how we might be able to stop it.

Here we investigate differences between carriage and infection associated isolates by characterizing a collection of *S. aureus* strains derived from invasive clinical infections (n=105) and compare them to matched nasal strains isolated from the same patients (n=85). Genetically related strains isolated from a non-infected carrier group termed “population controls” (n=87) are also included for comparison.

When comparing growth dynamics, hemolysis, biofilm formation, and the production of the immune evasion pigment Staphyloxanthin, we see no systematic differences between our carriage and infection isolates. However, there is considerable phenotypic variation between the 277 *S. aureus* isolates, the genetic basis of which we are investigating by GWAS.

P113

First understanding of polymicrobial biofilms of *Candida albicans* and *Pseudomonas aeruginosa* in new ventilator-associated pneumonia model

Joni MacDonald, Freya Harrison, Dean Walsh

University of Warwick, Warwick, United Kingdom

Abstract

Candida albicans and *Pseudomonas aeruginosa* are highly important pathogens capable of causing severe nosocomial infections, including ventilator-associated pneumonia (VAP), which is responsible for high hospital mortality rates. Both species form biofilms, increasing their antimicrobial tolerance and, when cultured together, can positively influence each other's growth. We aimed to grow single and dual-species *C. albicans* and *P. aeruginosa* biofilms in a new media that mimics the airways surface liquid of the airways in VAP. Firstly, the growth of these mixed-species biofilms was optimised within the new VAP model by growing polymicrobial biofilms over different time periods compared to monomicrobial biofilms and then conducting colony counts and Crystal Violet staining. Once optimised, these biofilms can be screened for susceptibility to antimicrobials to determine if increased antimicrobial resistance, which has been observed in other environments, results from growth in the VAP environment. Breaking down extracellular matrix components may restore antibiotic susceptibility in various environments. It may explain the mechanisms of antibiotic tolerance in VAP, previously indicated to be highly correlated to extracellular matrix constituents in *C. albicans* biofilms. Furthermore, this VAP model may help determine the mechanisms behind interspecies interactions and virulence expression in the VAP environment. Our work will shed light on the mechanisms behind the high levels of virulence and antimicrobial tolerance seen in potentially fatal ventilator-associated pneumonia.

P114

Ban on ZnO supplementation in piglet feed: management of post-weaning diarrhoea and the implications for antimicrobial resistance

Nicky Craig[1], Andrew Bease[1], Ilias Kyriazakis[2], Muna Anjum[3], Sam Beechener[4], Sam Connelly[3], Abel Ekiri[5], Roberto La Ragione[5], Sophie Prentice[6], Mark Stevens[1], Nick Wheelhouse[7], Deborah Hoyle[1]

[1]Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom. [3]Animal and Plant Health Agency, Weybridge, United Kingdom. [4]Scotland's Rural College (SRUC), Edinburgh, United Kingdom. [5]School of Veterinary Medicine, University of Surrey, Guildford, United Kingdom. [6]AB Agri Ltd., Peterborough, United Kingdom. [7]Edinburgh Napier University, Edinburgh, United Kingdom

Abstract

Weaning is a critical life period for piglets, during which they are susceptible to post-weaning diarrhoea (PWD), primarily caused by enterotoxigenic *Escherichia coli*. This is both a welfare and an economic concern, resulting in weight loss, morbidity and mortality.

Zinc oxide (ZnO) is added to piglet feed to reduce PWD and boost growth. However, ZnO supplementation may promote antimicrobial resistance (AMR) through linkage of AMR and metal resistance genes, and is an environmental concern due to accumulation of zinc in slurry. From June 2022, the sale of piglet weaner feed supplemented with high ZnO levels was banned in the EU, and will be phased out by 2024 in the UK. There is expectation that PWD will become harder to manage, increasing the already high antibiotic usage within the pig industry.

The aim of this study is to investigate the impact of ZnO withdrawal on piglet health, growth and gut microbiome. We are recruiting 20 farms to a longitudinal study during the ZnO withdrawal UK transition period. Pooled pen faecal samples will be collected from ten pens each per weaner, grower and finisher pig stage before, and at two time-points after, ZnO withdrawal. This biobank of samples will be used to examine changes in bacterial microbiome, AMR and metal resistance gene prevalence, and PWD pathogen carriage, by qPCR, RT-qPCR, and 16S sequencing. These findings will be interpreted, alongside farm level epidemiological data, to identify management factors which may mitigate the impact of ZnO withdrawal on PWD incidence and poor weight gain.

P115

Testing novel therapeutics against the cystic fibrosis lung pathogens *Burkholderia multivorans* and nontuberculous mycobacteria

Lucile Hubert[1], Kasia Parfitt[2], Cedric Berger[1], Eshwar Mahenthiralingam[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Nuffield Department of Population Health, Oxford, United Kingdom

Abstract

Background

Novel therapeutics are needed to treat lung infections in people with cystic fibrosis (CF) caused by problematic antibiotic resistant pathogens. Members of the *Burkholderia* genus are known to produce a variety of specialized metabolites with antimicrobial properties. Two of them, enacyloxin IIa and gladiolin, were investigated as potential novel therapeutics against multidrug-resistant CF pathogens *Burkholderia multivorans* and nontuberculous mycobacteria (NTM).

Methods

Specialized metabolites were extracted following bacterial growth of *Burkholderia ambifaria* and *Burkholderia gladioli* on basal salts medium agar supplemented in glycerol to induce the synthesis of enacyloxin IIa and gladiolin, respectively. Antibiotic susceptibility was evaluated for tobramycin, meropenem and enacyloxin IIa, alone and in combination for a panel of *B. multivorans* strains. Enacyloxin IIa was also tested to determine its impact on virulence functions such as swimming motility in *B. multivorans*.

Results

Antibiotic susceptibility assays indicated that enacyloxin has a lower minimal inhibitory concentration on *B. multivorans* (1-4 µg/mL for MIC_{80}) than meropenem (16 µg/mL for MIC_{80}) and tobramycin (32 – 128 µg/mL for MIC_{80}). Meropenem and enacyloxin IIa appeared to have an additive effect when combined against *B. multivorans*. Adjacent growth of *B. ambifaria* and *B. multivorans* impacted the motility of *B. multivorans* on a strain-dependent level. A collection of CF NTM isolates has been assembled and undergoing testing for susceptibility to gladiolin

Conclusion

Enacyloxin IIa has promising activity against *B. multivorans* compared to existing antimicrobials. Further work is needed to evaluate the activity of gladiolin on NTM.

P116

Type I interferons restrict Salmonella enterica infection of epithelial cells and alter their intracellular lifestyle

Ruth Dickenson ORCID iD[1], Camila Valenzuela Montenegro[2], Jakson Luk[3], Sam Wilson[4], Jost Enninga[2], Chad Swanson[1], Charlotte Odendall[1]

[1]King's College London, London, United Kingdom. [2]Institut Pasteur, Paris, France. [3]The Francis Crick Institute, London, United Kingdom. [4]University of Glasgow, Glasgow, United Kingdom

Abstract

Host recognition of the enteric pathogen *Salmonella enterica* triggers production of cytokines such as interferon (IFN), which induce the expression of IFN-stimulated genes (ISGs). As they are expressed in concert, the individual roles of many ISGs are not well understood. The study of IFNs has predominantly focussed on their role during viral infection and less is known about their effect during bacterial pathogenesis. To address this, we used the non-typhoidal *S*. Typhimurium to infect epithelial cells following treatment with IFNs. We demonstrate that pre-treatment with type I IFNβ significantly decreases bacterial numbers in infected samples. However, there was no significant difference following treatment with type III IFNλ. Furthermore, using flow cytometry, we show that both the percentage of infected cells and the bacterial burdens within infected cells are decreased in IFNβ treated samples.

To study infection in greater detail, we utilised a fluorescent reporter, *Salmonella* INtracellular Analyzer (SINA) and flow cytometry, to investigate the intracellular localisation of S. Typhimurium in IFNb treated samples. We found a decrease in the cytosolic population, which hyper-replicates, and an increase in the dormant population, which does not replicate. These data suggest IFNβ inhibits infection of epithelial cells by preventing invasion and affects bacterial burdens by disrupting the intracellular lifestyle of *S*. Typhimurium. To investigate further, we conducted a screen of over 500 ISGs to determine which mediate the changes observed. We believe the results of this screen will lead to mechanistic insights into how IFN is able to interfere with *S. enterica* infection.

P117

Evaluating lignin derivative-based antimicrobial fabric coatings; an eco-friendly alternative derived from waste materials.

Juliana Martin[1], John G Hardy[1], David Cheneler[1], Stephen Price[2], Michael R Roberts[1]

[1]Lancaster University, Lancaster, United Kingdom. [2]Biotech Services Ltd, Alderley Edge, United Kingdom

Abstract

Staphylococcus aureus is one of the five leading pathogens responsible for bacterial cause of death globally. Prevention of transmission, which can occur through contaminated surfaces including healthcare textiles, represents one approach as a part of a multipronged strategy to tackle this burden. Lignin derivatives are produced as by-products of the paper industry where they are commonly burnt to recover energy from waste, however, they are low-efficiency fuels. The objectives of this study were to validate the antimicrobial activity of lignin derivatives and their efficacy once applied as coatings of textiles used in healthcare.

To examine the antimicrobial activity of lignin derivatives, minimum inhibitory concentration (broth dilution) and bacteria cell viability (alamarBlue™ bioassay) tests were performed against a selection of bacterial species: *S. aureus, Escherichia coli, Pseudomonas syringae and Bacillus pumilus*. For the healthcare textiles evaluation, lignin impregnation into the fabrics was made via free-meniscus coating. The efficacy of the coating treatment was tested against *S. aureus* ATCC® 6538 employing the BS ISO 20743 protocol.

Antimicrobial activity was found to be positively associated with the concentration of lignin derivative used and better efficacy was demonstrated against gram-positive species. For *S. aureus*, there was an 82% of loss of cell viability after 3h incubation in 0.1 g/mL lignin derivative; *S. aureus* cell viability also decreased following incubations of 2-6 h on lignin derivative-coated fabrics.

The findings of this study indicate that the development of lignin derivative treatment of fabrics may present a possible approach to reducing pathogen transmission.

P118

Evaluating the Effectiveness of Cold Atmospheric Plasma in Combating Biofilm and Bone Related Infections

Thomas Thompson[1], Jordanne Maybin[1], Ross Duncan[1], Aled Morton[1], Theresa Freeman[2], Noreen Hickok[2], Brendan Gilmore[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Thomas Jefferson University, Philadelphia, USA

Abstract

Cold atmospheric plasma (CAP) has shown potential as a non-antibiotic treatment option for infections, particularly in orthopaedic procedures where medical devices can provide a site for biofilm formation and the prevalence of antimicrobial-resistant microbes is a concern. In this study, CAP was used to combat methicillin-resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* biofilm models using different plasma devices as a potential treatment for hospital-acquired infections.

With MRSA, the results showed that all devices were effective at reducing the bioburden of biofilms, with some producing up to a 4-fold reduction in 120-sec plasma treatment. In addition to the quantity and presence of specific reactive species, factors such as treatment surface, manipulation of the device, and carrier gas were found to be important contributors to the antimicrobial activity of CAP.

With *P. aeruginosa* the results showed that CAP pre-treatment resulted in a decrease in the minimum biofilm eradication concentration values for all three strains tested, indicating enhanced susceptibility to antimicrobials. Isothermal microcalorimetry showed that the combination of CAP and antibiotics was most effective at increasing time-to-peak metabolism of the biofilms, indicating enhanced susceptibility. Transcriptomic analysis revealed that P. aeruginosa responds to the oxidative stress caused by CAP through the upregulation of enzymes that degrade reactive oxygen and nitrogen species and by triggering a stringent response leading to the formation of persister cells within the biofilm. Overall, these findings suggest that CAP may be a promising alternative for the treatment of bacterial infections, particularly in cases where traditional antibiotics may not be effective.

P119

The impact of artificial sputum media on the minimum inhibitory concentration of antibiotics in Cystic fibrosis associated pathogens

Caitlin Jukes, Kate James, Kirstie Lewis, Yenenesh Tekletsion, Sandy Kimber, Claire Richards, Louise Brackenbury

Charles River Laboratories, Bristol, United Kingdom

Abstract

Pseudomonas aeruginosa and *Burkholderia* species are opportunistic pathogens that can cause infection in cystic fibrosis patients, resulting in worsening clinical outcomes and reduced quality of life. The microenvironment of the CF lung is complex and has been shown to alter the efficacy of antibiotics, with in vitro assays often poorly predictive of antibiotics likely to demonstrate efficacy in a clinical setting. There is a great need to improve *in vitro* assays so that they can better replicate the complex CF lung microenvironment. Improvements in these systems would aid drug discovery, increasing the chance of identifying new antibiotics to treat life threatening and life limiting infections in CF patients.

Traditional MIC screens utilise cation adjusted muller hinton broth, as recommended by the CLSI guidelines. However, it is widely becoming accepted that use of traditional culture media often doesn't reflect the situation in the CF lung. This has resulted in many labs using specialised media types including artificial sputum media (ASM) which has been shown in previous publications to alter the MIC of several key clinical antibiotics in *P. aeruginosa* PAO1. Here we demonstrate the use of an artificial sputum media in an MIC assay, under both aerobic and microaerophilic conditions, to determine the impact of different growth conditions on the MIC of clinically relevant antibiotics. This provides a convenient screening platform in which to select novel antimicrobials relevant to CF-associated pathogens.

P120

SYBR-loop mediated isothermal amplification(LAMP): A simple, rapid, and cost-effective tool for the detection of clinically significant resistant pathogens among ICU-admitted sepsis patients.

AMIT SHARMA ORCID iD[1,2], Mudsser Azam[3], Ruchi Singh[3], Rajni Gaind[2]

[1]University School of Medicine and Paramedical Health Sciences, Guru Gobind Singh Indraprastha University, New Delhi, India. [2]Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. [3]ICMR-National Institute of Pathology, New Delhi, India

Abstract

Background: Sepsis accounts for 20% of death worldwide(WHO) and is a major global health threat, particularly in LMICs due to delays in early diagnosis and prompt management. Carbapenem-resistant significant members of *Acinetobacter calcoaceticus Acinetobacter baumannii*(CR-SM-ACB) complex have emerged as an important cause of sepsis, especially in ICU's and warrants a diagnostic tool that helps in early management of sepsis and prevents overuse and selection of resistance. **Methodology**: A total of 200 patients admitted to ICU, with culture-positive-sepsis were enrolled. Non-duplicate whole-blood and blood culture(BC) broths were collected and directly subjected to LAMP, PCR, and RealAmp assay to detect CR-SM-ACB-complex. Vitek-2 system and conventional PCR were used as confirmatory reference tests. **Results**: Among 200BC, 100 were positive for ACB-complex, and 95 were CR. In 97% of isolates, resistance was mediated by $bla_{\text{OXA-23}}$. Among remaining 100BC, 10 yielded *Acinetobacter* other than ACB-complex, and 90 were positive for diverse pathogens predominantly, *K.pneumoniae*(n=21), followed by CONS(n=18), *Escherichia*(n=9), *Enterococcus*(n=8) and *Candida*(n=6). The overall TAT for identification of CR-SM-ACB-complex using an automated culture system, varied from 28-53 hours. The sensitivity and specificity of LAMP(97,100%) and RealAmp(100,100%) for detection of CR-SM-ACB-complex were better than PCR(87,90%). TAT of LAMP, PCR, and RealAmp was 6-20,9-23,6-20 hours respectively. **Conclusion**: In our study, TAT of LAMP was <24-hours, and depending on emerging pathogens and locally prevalent resistance genes, the assay can be modified. SYBR-LAMP-based diagnosis and early reporting of drug-resistant pathogens will help to optimize therapy in ICU setup.

P121

Investigating interactions between cystic fibrosis (CF) lung pathogens in the presence of antibiotics

Naomi Hughes[1], Cedric Berger[1], Matthew Avison[2], Eshwar Mahenthiralingam[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Background

People with Cystic Fibrosis (CF) develop chronic bacterial infections that occur within the lung microbiota. The influence the CF lung microbiota has on pathogen virulence and antimicrobial susceptibility is not understood. Here we combine pathogens and examine their antibiotic resistance and phenotypic behaviour.

Methods

P. aeruginosa (PA) was the designated dominant pathogen for all assays and interacted with *A. xylosoxidans* (AX), *S. maltophilia* (SM), or *B. multivorans* (BM). Antibiotic susceptibility (EUCAST disc and microbroth dilution) and swimming motility were compared between mono- and co-cultures treated with cefiderocol (FDC) and trimethoprim-sulfamethoxazole (TS).

Results

All three combinations exhibited changes in susceptibility and motility. FDC susceptibility tests showed AX and PA increased and decreased susceptibility, respectively. Motility of the co-culture was greater than that of the two species alone, but was also dependent on the antimicrobial exposure.

SM and PA increased and decreased susceptibility, respectively, to FDC when comparing the monocultures to the co-culture. Reduced motility was found when both mono- and co-cultures were treated with FDC and TS. Co-cultures showed increased motility under both treated and untreated conditions compared to monocultures.

BM and PA increased and reduced susceptibility respectively to TS in co-culture compared to treatment alone. Motility of the co-culture increased under all treatments, however, antibiotic treatment with FDC and TS suppressed motility.

Conclusion

Behavioural and susceptibility changes occur in a species and antimicrobial dependent manner. It is important we improve our understanding of the CF lung as microbial interactions will alter response to treatment.

P122

Hijack of the host: Uncovering how bacteria remodel human cell surface proteins to promote infection

Paige Wolverson ORCID iD, Luke Green, Jonathan Shaw

University of Sheffield, Sheffield, United Kingdom

Abstract

Neisseria meningitidis is the causative agent of meningococcal meningitis and sepsis causing significant morbidity and mortality worldwide. Whilst antibiotics have been an effective therapy, antibiotic resistance is rising across *Neisseria* spp. We have previously demonstrated that interference with a superfamily of host transmembrane proteins, the tetraspanins, can significantly inhibit bacterial adherence to host cells, potentially providing a new avenue for treatment. These proteins do not act as receptors but associate with various interaction partners to form tetraspanin-enriched microdomains (TEMs) able to facilitate a myriad of functions. Tetraspanin expression differs between host cell types therefore defining the full composition, organisation, and function of TEMs during infection has remained elusive. Here, we demonstrate that absence of the tetraspanin, CD9, from epithelial cells significantly reduced meningococcal adherence by 36%. Treatment of epithelial cells with a CD9-derived peptide was also able to reduce meningococcal adherence. We compared the tetraspanin expression profile of CD9 and CD81 across A549 epithelial cells and iAstrocytes, a critical component of the blood:brain barrier. CD9 was abundantly expressed in epithelial cells but lower in iAstrocytes, however CD81 expression was higher across iAstrocytes. Classical meningococcal adhesin receptors demonstrated no differences except CD147 with greater expression in iAstrocytes. Despite these similarities, infection of iAstrocytes was almost ablated compared to epithelial cells. Together, these data suggest that tetraspanins have a crucial role in adhesion of N. meningitidis with specific TEM compositions conferring different infective potential. Ongoing work aims to further discern TEM composition in a range of relevant meningococcal host cell types.

P123

Investigating the Role of Skin Commensals in Mediating Inflammatory Responses to Ultraviolet Radiation

Hannah Serrage ORCID iD[1], Andrew McBain ORCID iD[1], Joanne Pennock ORCID iD[1], Chunting Deng[1], Catherine O'Neill[1,2]

[1]The University of Manchester, Manchester, United Kingdom. [2]Skin BioTherapeutics PLC, Newcastle, United Kingdom

Abstract

Background: Ultraviolet radiation (UVR) modulates the skin immune response via induction of interleukin-10 mediated immunosuppression. In mice, the cutaneous microbiome has proven involvement in modulating UVR-associated immunosuppression via regulation of antimicrobial peptide/chemokine (CCL20) and pro-inflammatory cytokine secretion (IL-6). However, there remains little understanding of whether and if so, which human skin commensals contribute to this response. Here, we present evidence of the skin commensal-dependent manipulation of UVB-associated immune responses.

Methods: Normal-human-epidermal-keratinocytes (NHEKs) were exposed to UVR(10–20mJ/cm², 313nm) ± *Micrococcus luteus* (1x10⁴CFU/ml), *Staphylococcus hominis* (1x10² CFU/ml) and *Cuitbacterium acnes* (1x10⁶CFU/ml). Effects on NHEK viability (trypan blue), and inflammatory mediator secretion (enzyme-linked-immunosorbent-assay; ELISA:IL-10, IL-6 and chemokine;CCL20) were assessed 24h post-irradiation.

Results: Exposure of NHEKs to a single dose of UVR (20mJ/cm²) resulted in a 37% decrease in cell viability ($p<0.05$). *M. luteus* mitigated UVB induced cytotoxicity resulting in a 27% increase in cell viability relative to the 20mJ/cm² treated control ($p<0.05$), whilst *C. acnes* and *S. hominis* exerted no significant effect. *M. luteus*-mediated increased cell viability was accompanied by 39% and 35% decreases in CCL20 ($p<0.05$) and IL-10 ($p<0.0001$) secretion respectively relative to the control. *S. hominis* had no significant effect on cytokine secretion but *C. acnes* elevated IL-6 secretion by 67% (20mJ/cm²;$p<0.05$) and reduced IL-10 secretion by 20% relative to the respective control ($p<0.0001$)

Conclusions: We provide evidence for the modulation of the UVB-induced marker for immunosuppression; IL-10 by skin commensal bacteria. Providing novel insight into the contribution of skin commensals to UVR responses.

P124

Determination of the Antimicrobial effects of Dressings Containing Silver Oxynitrate using a Murine Model

Sajwa Baqader[1,2], Helen Thomason[3], Gavin Humphreys[1], Andrew McBain[1]

[1]Manchester University, Division of Pharmacy & Optometry ,School of Health Sciences, Manchester, United Kingdom. [2]Umm Al-Qura University, Community Nursing and Healthcare, | Faculty of Nursing, Makkah, Saudi Arabia. [3]3M Health Care, Medical Solutions Division, Knutsford, United Kingdom

Abstract

Infection has been strongly associated with delayed wound healing. Slow microbial growth rates are a recognized driver of tolerance towards antimicrobial agents and poor treatment outcomes. We developed a murine infected excisional wound model to study bacterial tolerance and to assess the antimicrobial effectiveness of novel wound dressings. We evaluated the efficacy of Ag Oxynitrate dressings, which produces reactive Ag^{2+} and Ag^{3+} ions, in wounds infected with 10^4 *Staphylococcus aureus* compared to a non-antimicrobial control and to uninoculated wounds. Wound samples were harvested at 1, 3 and 7 d. Wound healing was characterised by quantification of wound width, area and re-epithelialisation from histological sections, and neutrophils/macrophage marker immunohistochemistry. Infection status was characterised by tissue Gram stain, bacterial enumeration, and scanning electron microscopy (SEM). Over seven days, Ag Oxynitrate dressing reduced wound width, area, inflammatory markers, and increased reepithelialisation of *S. aureus* infected wounds compared to the non-antimicrobial comparator dressing. The Ag Oxynitrate dressing significantly reduced *S. aureus* viable counts in infected wounds. SEM imaging showed few bacteria on the wound bed treated with Ag Oxynitrate with disruptions of biofilm extracellular polymeric substance compared to infected wounds treated with non-antimicrobial dressing. In summary, Ag Oxynitrate dressings reduced bacterial bioburden and promoted wound healing of infected murine excisional wounds. This model can be used to evaluate effectiveness of other antimicrobial wound dressing to help inform decisions on biofilm management.

P125

Extensive variation in growth and antibiotic resistance across >16,000 clinical *Pseudomonas aeruginosa* isolates from Non-CF Bronchiectasis lung infections.

Matthew Shepherd[1], Anastasia Kottara[1], Niamh Harrington[2], Kendall Cagney[2], Dylan Childs[3], Jo Fothergill[2], Steve Paterson[2], James Chalmers[4], Michael Brockhurst[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom. [4]University of Dundee, Dundee, United Kingdom

Abstract

Bronchiectasis is a chronic lung disease increasing in global prevalence and likely to become even more widespread due to lung damage following COVID-19 infection. Many bronchiectasis patients suffer recurrent bacterial lung infections, wherein *Pseudomonas aeruginosa* infections in particular are associated with increased morbidity and earlier mortality necessitating long-term antibiotic therapy. Current understanding of the phenotypic adaptation and diversification of *P. aeruginosa* infections and how bacterial traits are associated with disease severity in bronchiectasis are poorly outlined. Determining whether simple bacterial metrics such as growth parameters can predict disease severity and treatment outcome is of clinical interest. Here we present phenotypic data on 16,560 clinical *P. aeruginosa* isolates from 183 bronchiectasis patients who participated in a multi-national randomised phase-3 clinical trial of inhaled liposomal ciprofloxacin therapy (ORBIT-3). We quantified variation in growth parameters and ciprofloxacin resistance before antibiotic treatment to investigate the associations between these bacterial traits, disease severity and treatment outcomes for patients.

P126

Evaluation of a FlpA glycoconjugate vaccine to reduce *Campylobacter jejuni* colonisation in chickens

Ricardo Corona Torres[1], Cosmin Chintoan-Uta[1], Prerna Vohra[2], Abi Bremner[1], Vanessa Terra[3], Marta Mauri[3], Jon Cuccui[3], Lonneke Vervelde[1], Brendan Wren[3], Mark Stevens[1]

[1]1The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom. [2]Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom. [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Infections by *Campylobacter* sp. represent the major cause of food-borne diarrhoea and handling or consumption of contaminated poultry meat is infection key risk factor. The avian host does not commonly develop clinical signs when colonised by *Campylobacter* but it can carry high bacterial loads in the intestines that can contaminate the carcass during processing. Reduction of the bacterial burden through vaccination of chickens has been evaluated in an attempt to improve food safety with mixed success. Glycoconjugate vaccines produced by protein glycan coupling technology (PGCT) are promising candidates given positive results achieved against *Francisella tularensis* and *Streptococcus pneumoniae* in experimental infections. Through PGCT, a glycan is synthesised and transferred to an acceptor protein containing the D/E-X-N-X-S/T motif by E.coli using the *N*-glycosylation system of *C. jejuni*. The fibronectin like adhesin FlpA of *C. jejuni* has been evaluated as glycoconjugate candidate due to reports in the literature of a modest reduction in *C. jejuni* counts conferred by vaccination with a subunit vaccine. In a previous study we evaluated a FlpA glycoconjugate vaccine coupled to two moieties of the *C. jejuni* heptasaccharide but no protection was observed. In the current study, we have increased the number of glycosylation sites in FlpA to 10 (FlpA-10GT) to increase the glycan dose. We additionally evaluated a seeder bird challenge model to mimic natural transmission by in-contact exposure. No protection was conferred with the FlpA-10GT glycoconjugate vaccine, however the study provided valuable data required to refine future vaccine candidates and vaccination trials.

P127

Non-invasive 3D printed sensors for pathogen detection and therapeutic monitoring of wound infection

Erin Magee, Dilidaer Yusufu, Andrew Mills, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

The treatment and management of chronic wounds presents a significant challenge to modern healthcare. For a dressed wound, a small headspace exists between the skin and dressing film that differs in composition from ambient air. When a wound becomes colonised and infected, the headspace composition will change due to microbial respiration and metabolism; hence, an opportunity exists to monitor this change as a marker of infection development.

A formulation containing LDPE, xylenol blue dye, and tetrabutylammonium hydroxide was 3D printed to form a CO_2-sensitive colourimetric indicator film. Porcine skin explants were inoculated with *P. aeruginosa* and sealed in a wound dressing model for headspace monitoring. A photograph was taken hourly to monitor sensor colour change to developing infection. The ability of the sensor for monitoring treatment efficacy was also assessed by treating 24 h biofilms with a range of antibiotic concentrations and packaging in the wound dressing model for sensor monitoring.

The film changed colour from blue to yellow in response to wound infection. The time taken to reach a halfway colour change was found to be directly proportional to the time taken for the microbial load to exceed 10^6 CFU/mL, regardless of initial inoculum. This colour change occurred before a measurable increase in biofilm biomass. Sensors monitoring biofilms exposed to suboptimal antibiotic concentrations changed colour, whilst successfully treated biofilms elicited no colour change.

The indicator film has the potential to improve wound care by allowing healthcare providers to monitor the effectiveness of treatments and intervene as needed.

P128

Investigating a role for the *S. aureus* stringent response in host-pathogen interactions with human macrophages.

Lucy Urwin ORCID iD, Rebecca Corrigan ORCID iD

University of Sheffield, Sheffield, United Kingdom

Abstract

Staphylococcus aureus is an opportunistic pathogen that contributes a major global health burden due to its ability to cause human infections, ranging from superficial skin infections to life-threatening bacteraemia. These infections are often hard-to-treat and recurrent in nature, due in part to the presence of complex bacterial signalling pathways that allow *S. aureus* to adapt to unfavourable conditions within the host environment and evade bacterial killing. We hypothesise that the (p)ppGpp-mediated, stringent response (SR) pathway plays an important role in bacterial pathogenesis as this pathway is known to upregulate genes involved in stress adaptation whilst downregulating genes involved in active growth, potentially contributing to a persister cell phenotype. To test this hypothesis, we have been using primary monocyte-derived macrophages (MDMs) obtained from healthy human donors, to study differences in host-pathogen interactions between wildtype *S. aureus* and staphylococcal SR mutants, which are unable to elicit a functional stringent response. Preliminary data suggests that whilst initial adhesion and invasion of MDMs is unaffected by the SR, this pathway does play an important role in intracellular survival. Bacterial clearance within human macrophages is significantly increased for SR mutants, with a (p)ppGpp-null strain that lacks (p)ppGpp synthetase enzymes, killed at a significantly higher rate than the wildtype over a 3.5 hr infection period. This suggests that the SR may play a critical role in the development of *S. aureus* infections and RNA-seq studies are currently underway to study differences in gene expression for wildtype versus SR mutants, within the macrophage environment.

P129

Development of an *in vitro* pig gut model for evaluating factors driving antimicrobial resistance

Marwa Hassan[1], Maria Getino[1], Joy Leng[1], Mark Chambers[1,2], Roberto La Ragione[1,2]

[1]School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [2]School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Abstract

Background: Antimicrobial resistance (AMR) is a global health threat affecting human and animals. The spread of AMR is further augmented by the selective pressure exerted by the unnecessary use of antibiotics and trace elements as supplements in animal feed. Thus, this study aimed to develop an *in vitro* pig gut model to determine the role of extracellular DNA in the transfer of AMR.

Methods: Static and continuous flow *in vitro* pig gut models were set up and seeded with pig faecal slurry to simulate the intestinal microflora. The models were inoculated with a sodium azide resistant *E. coli* J53 strain, while amplicons of the RNA polymerase β subunit (*rpoB*) gene conferring resistance to rifampicin, bacterial DNA lysate and/or plasmid conferring resistance to beta-lactam antibiotics were used as a source of extracellular DNA.

Results: The continuous flow *in vitro* pig gut model was developed to be composed of six independent vessels under controlled tested anaerobic conditions, pH and temperature. Preliminary natural transformation using *E. coli* J53 as the recipient strain and the *rpoB* DNA amplicon as the donor DNA demonstrated successful recovery of transformants of *E. coli* J53 that were both sodium azide and rifampicin resistant. The study concluded that there is strain competition between the pig microflora and *E. coli* J53 that diminishes with bacterial co-culturing.

Conclusions: An *in vitro* pig gut model was developed and showed potential to be used to determine the role of trace elements, heavy metals and/or antibiotics in the acquisition of AMR-encoding extracellular DNA.

P130

Investigation of Severe Lameness in UK Broiler Flocks

Victoria Smyth ORCID iD, Ryan Devaney, Paula Lagan, Pauline Sheridan

AFBI, Belfast, United Kingdom

Abstract

Background: UK broiler flocks recently presented with rapidly progressing severe lameness, and, in some cases, pericarditis. Mortality was 1.5 - 3% higher than the normal average. Co-infections of avian reovirus (ARV) and enterococcus were confirmed by testing; normally broiler lameness involves single pathogens.

Materials and Methods: Next generation sequencing (NGS) was performed on extracted nucleic acids from viral isolates made from 85 commercial broiler cases with a history of lameness, pericarditis, tenosynovitis, arthritis, and femoral head necrosis. NGS was also performed on colonies from 53 enterococcus culture plates, a total of 40 directly cultured from tissues related to ARV submissions, while the remaining 13 samples displayed similar clinical signs as described above. NGS sequences underwent a pipeline of bioinformatics analysis.

Results: Of 39 ARV field isolates successfully sequenced, amino acid identity ranged between 49.54 – 98.76%, placing ARV isolates into genotype clusters [SV1] [DR2] (GC) 1 – 4. A total of 3 isolates (7.7%) were assigned to GC1, 18 isolates (46.2%) assigned to GC2, 10 isolates (25.6%) assigned to GC3, and 8 isolates (20.5%) assigned to GC4. Enterococcus colonies comprised: 24 samples (47.06%) identified as E.faecium, 14 samples (27.45%) as E.faecalis, 5 samples (9.8%) as E.gallinarum, 2 samples (3.92%) as E.avium, 2 samples (3.92%) as E.hirae, 2 samples (3.92%) as E.durans, and 2 samples (3.92%) as E.casseliflavus.

Conclusion: Three co-infections from leg tendon samples showed ARV GC 2 paired with E.faecium and two cases of ARV GC 4 with E.faecalis. Other tissues showed different combinations suggesting there is not a specific co-infection genotype.

P131

Prolonged exposure of *Staphylococcal spp.* isolates to dermal fibroblast conditioned medium decreases their susceptibility to its anti-staphylococcal activity

Phil Stephens ORCID iD, Aled Clayton, Helen Brown ORCID iD

Cardiff University, Cardiff, United Kingdom

Abstract

Biofilm infections are a serious complication of chronic wounds, with wounds becoming colonised at an early stage. Although, most chronic wounds are colonised, not all will progress to develop clinically diagnosable infections. *Staphylococcus spp*. are commonly identified within chronic wound infections, with isolates typically being highly virulent and tolerant of antimicrobial products. Fibroblasts, a major cell type within the chronic wound produce multiple antimicrobial compounds. We have previously demonstrated that dermal fibroblasts from normal skin and chronic wounds have anti-staphylococcus activity, however not against *Staphylococcus spp.* isolates derived from chronic wounds. Here we aim to understand why some isolates can tolerate dermal fibroblast conditioned medium (CM) when it has such potent activity against others.

Repeated passaging of three susceptible *S. aureus* isolates in increasing concentrations of CM from normal and chronic wound fibroblast indicated that prolonged exposure to CM rapidly increased tolerance. Tolerance was retained following storage and revival of isolates. Interestingly, prolonged exposure of *S. aureus* to the antibiotic gentamicin did not confer any protective effect against CM, even though gentamicin tolerance was increased. The influence of prolonged exposure to CM on virulence and protein expression is currently under investigation, as is whole genome sequencing of the chronic wound derived isolates within our collection. This work highlights that host-microbial interactions, both direct and indirect, within the chronic wound are likely driving evolution of *Staphylococcus spp*. within wounds, potentially contributing to the development of symptomatic infection in some individuals.

P132

An investigation of Clostridioides difficile carriage by Irish pigs and relevance to human disease

Aoife Doyle[1], Lorcan O Neill[2], Rachel Grimes[1], Thomas Rogers[1], Máire McElroy[3]

[1]Trinity College Dublin, Dublin, Ireland. [2]Teagasc, Cork, Ireland. [3]Department of Agriculture, Food and the Marine, Kildare, Ireland

Abstract

Community-associated CDI (CA-CDI) is common, but infection sources are poorly understood. Healthy pigs can carry *C. difficile*. Ribotype (RT) 078 is associated with human CA-CDI cases and is the most common ribotype in pigs in Ireland (Stein et.al.,2017). Moloney et.al.(2021) found genomic relationships between RT078 from Irish pigs and human CDI from Europe, providing evidence for broader epidemiological linkages. The aims were to investigate isolation rates from pigs (birth to slaughter), identify any relationship to in-feed antibiotic use, and investigate Irish pork for presence of *C. difficile*. 50.73% of faeces were positive for *C. difficile*. 95% of sows and 89% of piglets were positive. 78% of pig isolates were RT078 or RT014. 98% of sows and piglets were RT078. Only 7% of weaner isolates (post-antibiotics) were RT078, and RT014 was the most common (44%). There was more diversity in finisher and slaughter isolates- no RT078 or RT014 were found. 0/96 pork products tested positive for *C. difficile. C. difficile* rates varied throughout pigs' life. HAU farms had lower positivity rates (35.33%) than LAU farms (61.89%). RT078 was the most common,varying from 98% in sows to 0% at slaughter. This provides further evidence of the potential of pigs as a reservoir of C. difficile, especially RT078 and RT014. In-feed antibiotics initially reduced *C. difficile* detection rates, but this recovered at slaughter. *C. difficile* absence from pork products may relate to EU protocols that reduce chances of food contamination. These findings warrant further investigation into the pigs' role in the human CDI epidemiology.

P133

Klebsiella pneumoniae adapted to chlorhexidine is stable in a polymicrobial community modelled using *in vitro* bladder models

Isobel Garratt ORCID iD, Anthony J Slate, Jonathan Nzakizwanayo, Brian Jones

University of Bath, Bath, United Kingdom

Abstract

K. pneumoniae is a leading cause of antimicrobial resistance (AMR) attributable death, and a WHO priority pathogen. It is a key uropathogen involved in catheter-associated urinary tract infections (CAUTI) which cause significant burden on healthcare. Up to 85% of patients with indwelling catheters have polymicrobial infections (Stickler *et al.,* 2008). Urinary catheter infection biofilms are often exposed to biocides such as chlorhexidine (CHD) during catheter maintenance and adaptation to working concentrations of biocides in other clinically relevant mixed community biofilms has already been reported. Polymicrobial catheter infections are currently poorly understood, and a greater comprehension is required to fully realise the effect of exposure to biocides. Here, we describe the development of a reproduceable method for studying polymicrobial communities modelled *in vitro* and the application of this model for understanding the effects of CHD adaptation on *K. pneumoniae* in the context of a polymicrobial community. Using *in vitro* bladder model system we investigated the effect of CHD adaptation on *K. pneumoniae*, in polymicrobial communities of *E. faecalis*, *E. coli*, *P. aeruginosa* and *S. aureus*. Our preliminary results have shown that both *K. pneumoniae* pre-adapted to CHD as well as the non-adapted parental strain are able to remain stable in a polymicrobial community of common uropathogens modelled in vitro for 72 hours. These results highlight the effect of biocides on CAUTI polymicrobial biofilm communities and the potential to utilise our developed method for the study of a range of biocides and uropathogens.

P134

Utilising IFN-lambda stimulation and systematic analysis of viral regulation to identify novel effectors of innate immunity

Joanne Kite[1,2], Colin T R Davies[1,2], Cassie M Zerbe[1,2], Robin Antrobus[1], Frank McCaughan[2], Jonas D Albarnaz[1,2], Michael P Weekes[1,2]

[1]Cambridge Institute for Medical Research, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Abstract

Type III interferons, or interferon lambdas (IFNλ), are the first line of defence against viral infections of the epithelia. Despite acting via a distinct receptor, initial reports suggested that they functionally resemble type I interferons. Both activate intracellular signalling pathways and antiviral functions, and both are induced by viral infection. Recent data has hinted that there are type III-specific activities, although these have not been studied at the protein level. Furthermore, whether IFNλ subtypes exhibit distinct activities is unknown.

We have recently established primary-like human bronchial cells, which retain the ability to differentiate, as a tractable, physiologically relevant model for dissecting the IFNλ response. Using a systemic temporal highly multiplexed tandem-mass-tag-based proteomic approach, we have identified common and novel cellular responses to each subset of IFNλ in comparison to IFNα. For example, known modulators of the IFN response, STAT1 and the IFITs were induced by all IFNs, but fascinatingly a sub-set of responders were only induced by certain IFNλ sub-types. Furthermore, as viruses often regulate key cellular antiviral proteins, we compared our data to a compendium of our proteomic studies across multiple different viral infections to discover factors that are both stimulated by IFNλs and regulated by viral infection.

Overall, we have identified novel proteins induced in response to specific IFNλ sub-types, a subset of which are regulated by DNA and/or RNA viruses. Thus, our global, systematic approach has enabled identification of new candidate innate immune factors which will advance our understanding of viral perturbation of this response.

P135

Interplay between skin bacteria and fungi during different stages of wound healing in Epidermolysis bullosa.

Dario Leonardo Balacco ORCID iD, Ajoy Bardhan, Melissa Grant, Sarah Kuehne, Josefine Hirschfeld, Adrian Heagerty, Iain Chapple

University of Birmingham, Birmingham, United Kingdom

Abstract

Epidermolysis bullosa (EB) is a heterogeneous group of inherited bullous disorders characterised by detachment of the epithelium following minimal mechanical trauma. The phenotypic spectrum is varied, with persistent blistering, inflammation, delayed re-epitalisation, abnormal wound healing and often infection, leading to disability and, in the most severe cases, death.

The human skin microbiome has adapted to produce molecules that inhibit the colonisation of pathogenic microorganisms. In EB patients, the skin barrier is broken, and the balance between commensal and pathogenic microorganisms is altered. Understanding shifts in the microbial communities of EB patients may lead to the determination of new therapeutic targets that can improve the management of the skin disorder.

We used whole-genome sequencing to investigate and characterise shifts from a healthy to EB-associated skin microbiome. In particular, we sampled skin from blisters, healthy skin surrounding blisters at the time of blister formation and after 48 hours, from dry skin (hands and forearm) and feet of healthy controls and people with EB. We observed in dry skin of people affected by EB a striking reduction of the skin commensal *Malasezia globosa*, known to limit and attenuate *Staphylococcus aureus* biofilm formation, protecting the host from infection. Importantly, we found that this fungal dysbiosis is associated with an increased relative abundance of *S. aureus* in EB patients on blisters and the surrounding area during the wound healing process.

This shift may be involved with atypical wound healing, and understanding it further may present opportunities for improved intervention, leading to improved quality of life.

P136

Varying levels of adhesion to fibronectin and fibrinogen by *Staphylococcus aureus* bacteremia isolates - the role of genetic variance and associated clinical manifestations

Paula Rożen[1], Marta Zapotoczna[1], Karol Makuch[2], Francesc Coll[3]

[1]Department of Molecular Microbiology, Institute of Microbiology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089, Warsaw, Poland. [2]Department of Soft Condensed Matter at the Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224, Warsaw, Poland. [3]London School of Hygiene & Tropical Medicine, Faculty of Infectious and Tropical Diseases, Department of Infection Biology, WC1E 7HT, London, United Kingdom

Abstract

Staphylococcus aureus is a common source of infections of skin and blood which can lead to life-threatening complications. This study focuses on the microbial surface components recognizing adhesive matrix molecules that are responsible for binding cells to human glycoproteins: fibrinogen (ClfA & ClfB) and fibronectin (FnBPA & FnBPB). The aims of the project are to identify the genetic variance associated with distinctive levels of adhesion and identify associations between bacterial adhesion levels and patients' clinical manifestations.

A newly assembled collection of 237 *S. aureus* bacteremia isolates from three hospitals in Poland was subjected to whole genome sequencing and phenotyped quantitatively to discriminate their ability to adhere to fibrinogen and fibronectin. Measurement of adhesion to fibrinogen was performed using stationary grown isolates whilst adhesion to fibronectin was determined for bacteria in the logarithmic phase. The dose-dependent binding curves were determined for each strain.

The distribution of the adhesion levels across bacteremia isolates was normal. Mean adhesion to fibronectin was different between isolates from different clonal complexes. Increased adhesion to fibronectin was seen for isolates from community acquired infections and correlated with patients inflammatory markers. Mean adhesion to fibrinogen was similar across different clonal complexes. The increased ability to bind to fibrinogen correlated with the same inflammatory markers and reduced risk of patients mortality. These results suggest that adhesion levels are indicators of the isolates ability to induce proinflammatory response.

The ongoing genome wide association study will identify the genetic variance responsible for the varying levels of adhesion.

P137

Non-invasive imaging methods for detection of *in-vivo* wound biofilms

Hadeer Ibrahim ORCID iD[1,2], Sarah Kuehne ORCID iD[3], Josefine Hirschfeld ORCID iD[3], Mohammed Hadis ORCID iD[4], Adrian Heagerty[5], Iain Chapple ORCID iD[6]

[1]School of Dentistry, University of Birmingham, Birmingham, United Kingdom. [2]Dermatology department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt. [3]Oral Microbiology & Periodontal Research Group, Institute of Clinical Sciences, University of Birmingham, Birmingham, United Kingdom. [4]Photobiology Research Group, Institute of Clinical Sciences, University of Birmingham, Birmingham, United Kingdom. [5]Dermatology Research Group, Institute of Inflammation & Ageing, University of Birmingham, Birmingham, United Kingdom. [6]Periodontal & Dermatology Research Groups, Institute of Clinical Sciences, University of Birmingham, Birmingham, United Kingdom

Abstract

Biofilms are consortia of microorganisms that exist within a self-produced matrix, that interact with each other while attached to a surface. Biofilm formation plays a crucial role in many medical conditions, including chronic wounds where biofilms are reported in up to 60%. The presence of biofilms within wounds creates diagnostic and management challenges and impacts upon wound care. Whilst there is no gold-standard diagnostic method for biofilms, direct imaging using different microscopic techniques is a key approach for biofilm identification. The impact of biofilms on skin wounds has not been studied to the same extent as for other organs. To the best of our knowledge, skin-related biofilms have only been diagnosed by skin biopsies, which are invasive.

We have investigated non-invasive methods to sample in-vivo grown biofilms and processed them for diagnosis by imaging. Skin swabs for biofilm detection post-irrigation with saline (to remove planktonic microorganisms) were used as a novel method to collect samples from patients with chronic wounds that showed no visible signs of infection. Samples were fixed and prepared for scanning electron microscopy and stained with different fluorescent stains for confocal laser scanning microscopy.

Images demonstrated clear aggregated cocci and bacilli measuring 0.2-1µm in diameter and resembling bacterial cells within a matrix. Hyphae-like structures and inflammatory cells were observed within the same matrix.

Our results demonstrate, for the first time, that swabs can be used as a non-invasive method for chronic wound sampling, and for biofilm detection with beneficial consequences on skin disease management and future research.

P138

Host signals and strain diversity within mixed microbial populations perturb the pathogen-host interaction

Ay'sha Moore-Machacek ORCID iD, Maite Rekalde, Antje Gloe, Dr Niall O'Leary, Dr F. Jerry Reen

University College Cork, Cork, Ireland

Abstract

Microbes rarely exist in isolation in any niche, instead comprising diverse polymicrobial communities of various bacterial, fungal, archaeal, viral species/strains. The polymicrobial community composition is not static and instead is spatiotemporal and niche specific. The diverse nature of these polymicrobial communities reflects the diversity of possible interactions between various microbes and between microbes and their host. As such, microbe-host interactions are also spatiotemporal and niche-specific in nature, ranging from pathogenic to symbiotic at various stages of the infection cycle, depending on a range of parameters, such as nutrient availability, host health status, host antimicrobial usage, etc. This is the case with regard to the microbial interactions underpinning chronic colonisation and inflammation in respiratory disease as well as in the complex interactomes that sustain plant health in the rhizosphere.

In an attempt to gain some insights into the spatiotemporal dynamics of how species behaviour is altered in the presence of common co-colonising microbes, the behaviour of clinical and model bacterial (incl. *Pseudomonas aeruginosa*) and fungal (incl. *C. albicans* and *C. dubliniensis*) strains were studied in response to host/microbial signals including *N*-acetyl-glucosamine (NAG). NAG is present in all protozoans and metazoans, excluding yeast and is critical in cell signalling events, protein localisation, cell survival under various harsh conditions, etc. Fungal and bacterial pathogens were found to alter their virulence profiles through biofilm formation, EPS production, pigmentation, and toxin production in response to specific host signals, adopting a distinct profile when co-cultured in the presence of competing organisms.

P139

Post-transcriptional regulation of the PQS quorum sensing system in *Pseudomonas aeruginosa*

Dimitra Panagiotopoulou, Natalia Romo Catalan, Miguel Cámara, Stephan Heeb

University of Nottingham, Nottingham, United Kingdom

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen, highly resistant to antibiotics and a major cause of nosocomial infections. The ability to survive in diverse environments, allows *P. aeruginosa* to colonise the lungs of cystic fibrosis patients and establish chronic infections. The presence of three quorum sensing (QS) systems (*rhl/las/pqs*) and a wide range of small non-coding RNAs (sRNAs) have been shown to play an important role in the adaptation of this organism to different environments. sRNAs regulate gene expression at the post-transcriptional level aiding the adaptation of this organism to rapidly changing environments. In this study, we have identified a region encoding a sRNA that overlaps with the promoter of the *pqsABCDE* operon from the *pqs* QS system and named it *pqsX*. After validating the presence of the PqsX transcript by Northern blot in the *P. aeruginosa* model sublines PAO1 and PA14, the secondary structure of the sRNA was predicted *in silico*. The relationship of PqsX with all three QS systems of P. aeruginosa was investigated using a *lux*-based bioreporter system. In addition, the impact of *pqsX* overexpression on the production of QS molecules from the three QS systems was determined. This study highlights the importance of sRNAs in the post-transcriptional regulation of the QS systems adding another layer of complexity to the regulation of virulence in *P. aeruginosa*.

P140

Sustainable antimicrobial fabrics for use within wound care applications.

Jonathan A. Butler ORCID iD[1], Iffat Shahzad[1], Sofyan Ahmed[1], Kirsten Scott[2], Karen Spurgin[2], Anthony J. Slate ORCID iD[3], Prabhuraj D. Venkatraman ORCID iD[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Istituto Marangoni, London, United Kingdom. [3]University of Bath, Bath, United Kingdom

Abstract

Surgical site, soft tissue and wound infections are some of the most prominent causes of healthcare associated infections. The development of antimicrobial textiles and wound dressings is one method of reducing the transmission of bacterial pathogens in healthcare environments, whilst assisting the healing process and promoting localised antisepsis. This study aimed to determine the antimicrobial efficacy of a series of natural Ugandan barkcloths derived exclusively from the *Ficus natalensis* and related tree species, which have been produced for hundreds of years using traditional techniques. This fabric possesses many ideal properties associated with wound dressing technology, including good gaseous transmission, biocompatibility, mechanical protection, biodegradable and cost-effectiveness. Antimicrobial susceptibility and time-kill kinetic assays demonstrated that barkcloth derivatives inhibited the growth of multiple clinically relevant methicillin-resistant *Staphylococcus aureus* (MRSA) strains and acted as bactericidal fabrics. Scanning electron microscopy was used to reveal morphological changes in the MRSA bacterial cell ultrastructure when exposed to different barkcloth derivatives. The observed antimicrobial properties, combined with the physical characteristics elicited by barkcloth, suggest these fabrics are ideally suited for wound and other skin care applications. This is the first example where whole barkcloth products made by traditional methods have been employed as antimicrobial fabrics against MRSA. Barkcloth is a highly sustainable and renewable product and this study presents a major advance in the search for natural fabrics which could be deployed for healthcare applications.

P141

Combatting antimicrobial resistance: Small-molecule inhibitors as precision anti-virulence agents.

Holly Ansell-Downey[1], Laila Ralif[1], Thomas K. Britten ORCID iD[1], Andrew M. Lewis ORCID iD[1], Mark G. McLaughlin ORCID iD[2], Jonathan A. Butler ORCID iD[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Lancaster University, Lancaster, United Kingdom

Abstract

Antimicrobial resistance (AMR) is an ever-increasing global issue and it is estimated that deaths associated with AMR infections will exceed 10 million by 2050. Traditional antibiotics display antimicrobial activity through direct targeting of key bacterial cellular processes such as cell wall formation, which are essential for viability but are susceptible to resistance evolution. In contrast, one approach to combatting AMR is the development of novel Small-Molecule Inhibitors (SMIs) as anti-virulence agents, which target pathogen specific virulence-related traits, such as enzymes involved in the generation of post-translational modifications, biofilm formation and toxin production. This study aimed to determine the antimicrobial and anti-virulence activity of a novel library of SMIs designed for precision targeting of essential epigenetic bacterial targets including phosphorylation and methylation. Antimicrobial susceptibility and time-kill kinetic assays identified lead candidates which demonstrated activity at <5 µM against multiple clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, with one lead SMI able to partially resolve MRSA infections in a *Galleria mellonella in vivo* model. At biologically relevant concentrations, these SMIs exhibited no cytotoxic activity against mammalian skin cell lines including Normal Human Dermal Fibroblasts. Research is now focused on determining the downstream effects of these SMIs on virulence traits, such as biofilm formation and quorum sensing, in addition to confirming the bacterial cellular target site via affinity chromatography coupled with determining binding activity. This research represents a significant advance in the search for novel antimicrobial agents which target essential bacterial processes beyond those associated with traditional antibiotics.

P142

Changes in tolerance of Pseudomonas aeruginosa to tobramycin following treatment with tobramycin inhalation powder (TIP)

Ross McCleave[1], Gisli Einarsson[1], James Chalmers[2], Stuart Elborn[3], Michael Loebinger[4], Charles Haworth[5], Michael Tunney[1], Laura Sherrard[1]

[1]Queen's University Belfast, School of Pharmacy, Belfast, United Kingdom. [2]Scottish Centre for Respiratory Research, University of Dundee, Nineweels Hospital and Medical School, Dundee, United Kingdom. [3]Queen's University Belfast, School of Medicine, Dentistry and Biomedical Sciences, Belfast, United Kingdom. [4]Host Defence Unit, Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom. [5]Cambridge Centre for Lung Infection, Royal Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom

Abstract

Background

Antimicrobial tolerance is the ability of a bacterial population to survive in antibiotic concentrations exceeding the minimum inhibitory concentration (MIC). There is a lack of studies determining if tolerance develops in vivo in patients receiving antimicrobials. This study determined if Pseudomonas aeruginosa (PA) tolerance to tobramycin increased longitudinally in people with bronchiectasis treated with TIP.

Methods

Paired (identical-genotype) within-participant tobramycin-susceptible isolates (mean MIC: 0.59 mg/L) from a clinical trial (doi:10.1016/j.pupt.2019.101834) were selected: isolates were cultured from patients before and after treatment with TIP (continuous, n=7; cycling [TIP/placebo], n=5) or placebo (n=8) [0 to 85 or 113 days]. Time-kill assays were performed with tobramycin (32 mg/L), with total viable counts enumerated over 7-hours. The time taken (minutes) for a 2-log10 reduction (Minimum Duration of Killing, MDK99) was determined. The Wilcoxon signed-rank test was used for comparisons.

Results

The median MDK99 trended towards a statistically significant increase from 24.4-minutes before to 32.8-minutes after treatment (p=0.05) in isolates from participants receiving TIP. When these isolates were segregated into groups based on treatment regimen, increases in the median MDK99 were observed in both groups but neither were statistically significant (continuous: 24.0-minutes before vs 30.6-minutes after treatment, p=0.2; cycling: 26.7-minutes before vs 34.9-minutes after treatment, p=0.3). No significant difference was detected in the MDK99 of isolates from participants receiving the placebo (median: 28.8-minutes before vs 24.0-minutes after treatment, p=0.4).

Conclusion

A modest increase in tobramycin tolerance was observed in PA from patients treated with TIP. Further work will investigate biofilm-tolerance longitudinally.

P143

Prophage degradation enhances stress resistance in serotype M4 group A Streptococcus

Josh Rand[1], Claire Turner[2], Nicola Lynskey[1]

[1]Roslin Institute, Edinburgh, United Kingdom. [2]The University of Sheffield, Sheffield, United Kingdom

Abstract

Group A Streptococcus (GAS) is a gram-positive bacterium and human pathobiont, and is the causative agent of a diverse array of infections including scarlet fever, necrotising fasciitis and rheumatic fever. A major driver of changes to GAS epidemiology and virulence is clonal replacement, a process by which new variants of an existing lineage emerge. A new clonal lineage of serotype M4 GAS has recently been reported in Europe and the US, and is associated with enhanced virulence, however the mechanisms underpinning this change remain poorly understood. A key feature of the emergent M4 lineage is degradation of all associated prophages, the impact of which on GAS virulence remains unknown.

The ability for degraded prophage to excise from the bacterial chromosome in a process known as induction was quantified. Three ancestral isolates and three emergent isolates were treated with the prophage inducing agent mitomycin C. This revealed that the complete prophage could be induced, whereas the degraded prophage could no longer excise from the bacterial chromosome rendering them “cryptic”. Growth curves assessing the impact of prophage induction on bacterial cell lysis were performed and showed that the transition of phage to a cryptic state resulted in a significant reduction in bacterial cell lysis compared to the ancestral lineage, providing a potential fitness advantage to the new clone.

Overall, we demonstrate that degradation of all prophage in an emergent linage of serotype M4 GAS results in inhibition of prophage induction and subsequent survival advantage via a reduction in prophage-mediated bacterial cell lysis.

P144

Control of avian pathogenic *Escherichia coli* (APEC) through postbiotic modulation of chicken macrophages

James Adams ORCID iD[1,2], Josue Diaz Delgado[1], Jai Mehat ORCID iD[1], Shahriar Behboudi[2], Roberto La Ragione ORCID iD[1]

[1]University of Surrey, Guildford, United Kingdom. [2]The Pirbright Institute, Pirbright, United Kingdom

Abstract

Background

Avian pathogenic *Escherichia coli* (APEC) significantly impacts animal welfare and economic productivity within the poultry industry. Historically, APEC has been managed using vaccination and antibiotics. However, resistance and legislative barriers have led to an urgent requirement for alternatives, with innate immunomodulation showing promise as a potential alternative control strategy.

Methods

In this study, the postbiotic butyrate's ability to enhance the HD11 chicken macrophage cell line's antibacterial activity was investigated. Intracellular survival of serotype O78 and O111 APEC, as well as *Salmonella* Typhimurium was determined by gentamicin protection assays, with cell viability quantified through trypan blue staining. Nitric oxide (NO) and reactive oxygen species (ROS) generation following infection was determined using a Griess assay and 2',7'-dichlorofluorescein, respectively. Finally, changes in phagocytosis were determined by measurement of the uptake of GFP-tagged K12 *E. coli*.

Results

Butyrate priming significantly reduced intracellular survival of both APEC serotypes and *S*. Typhimurium within HD11 cells ($p \leq 0.05$), while having no effect on cell viability. This enhanced antibacterial activity did not correlate with increased NO production following infection with APEC O78. However, priming led to significantly increased ROS generation in butyrate primed cells, both at rest and following infection, alongside greatly enhanced phagocytosis of GFP-tagged K12 *E. coli* ($p \leq 0.05$).

Conclusions

Collectively, the data suggests that butyrate exposure modulates chicken macrophages, improving their antimicrobial activity against multiple APEC lineages and enhancing phagocytic ability. This study highlights the potential utility of targeting the innate immune system as a novel infectious disease management strategy.

P145

Transcriptomic Analysis of the Activity and Mechanism of Action of a Ruthenium(II)-Based Antimicrobial That Induces Minimal Evolution of Pathogen Resistance

Adam Varney

Nottingham Trent University, Nottingham, United Kingdom

Abstract

The World Health Organisation has declared antimicrobial resistance (AMR) as one of the top 10 global health threats and estimates 10 million deaths annually attributed to AMR by 2050. Mono and di-nuclear ruthenium complex offer a novel solution to relieve the burden posed by AMR infections showing excellent antimicrobial activity against multi-drug resistant pathogens including Escherichia coli EC958.

Minimum inhibitory/bactericidal concentrations assays used to determine antimicrobial activity of ruthenium compounds. Time-kill assays and various imaging techniques including structured illumination microscopy (SIM)/TEM and STEM performed to probe the effect on actively growing EC958.

Significant activity is shown against clinically isolated multidrug resistant pathogens with concentrations comparable to standard antibiotics (2.9mg/L [1.6μM] against EC958). Both compounds are also effective across a broad pH range (4-9). Time-kill assays demonstrate a lag-phase (~30-60min) after introduction of compound where no change in turbidity is seen with structured illumination microscopy demonstrating di nuclear ruthenium accumulates within the cell membrane 20 min post exposure before concentrating inside the cell.

Ruthenium complexes showed potent antimicrobial activity with a broad range both across bacterial species and across pH ranges, demonstrating the compounds effectiveness across a wide range of infections where environmental conditions may change throughout the infection time-course. We later aim to establish a greater understanding of the mechanism of action through a full transcriptomic study.

P146

Ruthenium metallotherapeutic agents: novel approaches to combatting *Pseudomonas aeruginosa*.

Nicole S. Britten ORCID iD, Jonathan A. Butler ORCID iD

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen which is highly resistant to antibiotics and biocidal products. There is an ongoing need to develop novel approaches for combatting antimicrobial resistant infections caused by *P. aeruginosa*. Metals have been used as antimicrobial agents throughout history for a broad range of applications. Ruthenium (Ru) metallotherapeutic compounds have potent antimicrobial properties and in contrast to traditional antibiotics, these are thought to elicit antibacterial activity at multiple sites within the bacterial cell, thereby reducing the possibility of resistance evolution. Minimum inhibitory and bactericidal concentration (MIC / MBC) assays, coupled with disc diffusion assays were used to screen a library of Ru metallotherapeutics. One lead compound was identified which was highly active at inhibiting growth of multiple clinical strains of *P. aeruginosa* at ≤32 µg mL^{-1}, with loss of viability occurring within 6 h. Crystal violet biofilm assays showed a decrease in biomass following exposure of *P. aeruginosa* biofilms over a 24 h period. Scanning electron microscopy was used to reveal morphological changes in the bacterial cell ultrastructure after exposure, with evidence of membrane perturbation which supported a proposed mechanism of antimicrobial activity. Cell culture *in vitro* scratch assays and 3D skin full thickness wound infection modelling were used to demonstrate the wound healing potential of the lead Ru metallotherapeutic. These findings make a significant contribution towards the search for novel bactericidal agents and further research is now focussed on determining the potential for use as novel adjuvants within medicinal applications.

P147

The cystic fibrosis pathogen *A. xylosoxidans* survives intracellularly in human macrophages within a pre-lysosomal compartment

Hannah J. Parks, Keren Turton, Miguel A. Valvano

Wellcome-Wolfson Institute for Experimental Medicine at Queen's University, Belfast, United Kingdom

Abstract

Achromobacter xylosoxidans is an emerging opportunistic bacterium that causes chronic, life-threatening infections in immunocompromised individuals including people with cystic fibrosis (PWCF). In PWCF, *A. xylosoxidans* infection is associated with disease severity like infection with *Pseudomonas aeruginosa*. However, how *A. xylosoxidans* interacts with innate immune cells, such as macrophages is poorly understood. In this study, we have investigated the course of infection of an *A. xylosoxidans* CF clinical isolate and its intracellular traffic in human macrophages. We demonstrate that the bacterium traffics to EEA1-positive vacuoles that mature into LAMP-1-positive phagolysosomes that do not colocalise with the cathepsin D lysosomal marker, suggesting the bacteria interfere with the end stages of the normal phagosome maturation, by preventing fusion with lysosomes. The *A. xylosoxidans* containing vacuole (AxCV) does not appear to traffic in a LC3b-dependent autophagosomal pathway but co-localises with dextran fluorescein in phagolysosomal compartments. Macrophages infected with *A. xylosoxidans* displayed significant cytotoxicity peaking at 8 hours post-infection, as evidenced by morphological changes and loss of integrity of the cell membrane, which are due to pyroptosis induction, as previously demonstrated in our laboratory. Experiments with the actin inhibitor cytochalasin D demonstrated that multiple *A. xylosoxidans* clinical isolates adhere to the cells and additionally induce cytotoxicity extracellularly. Together, we conclude that intracellular survival of *A. xylosoxidans* in human macrophages occurs within a phagolysosome that does not progress into a lysosome and does not interact with the autophagy pathway.

P148

A DNA supercoiling-induced secretory phenotype enhances tight junction disruption, adherens junction breakdown and actin remodelling in 3D intestinal Spheroids for *Campylobacter jejuni*

Matthew Whelan ORCID iD[1], Jeremy Simpson[2], Tadhg O Croinin[3]

[1]London, London, United Kingdom. [2]University College Dublin, Dublin, Ireland. [3]University College London, Dublin, Ireland

Abstract

Campylobacter jejuni is the leading cause of bacterial gastroenteritis. Although *C. jejuni* is a prolific human pathogen, the mechanisms of the induction of virulence within human intestine and consequences for host-epithelia are poorly understood, particularly in a 3D tissue context. Previously, we demonstrated a role for DNA supercoiling as a global regulator of C. jejuni pathogenesis. Drug-induced relaxation of chromosomal supercoiling resulted in an inducible secretory phenotype (*C. jejuni*(S)), associated with increased invasion of human epithelial cells, biofilm formation and survival in oxygen-rich conditions. We found that this DNA supercoiling modulated invasive phenotype was associated with disruption of tight-junctions(TJ) and adherence junctions(AJ) within polarised HT29 monolayers at a significantly faster rate than non-primed *C. jejuni*. This correlated with observations of increased paracellular transport not attributable to bacterial autoagglutination. We implemented High-Content Screening microscopy, challenging polarised HT29 intestinal spheroids with *C. jejuni* and *C. jejuni*(S) to compare bacterial total-association, TJ, AJ and actin cytoskeleton perturbation over 24h infection. *C. jejuni*(S) was far more efficient at the disruption of organoid TJ, with total loss of TJ observed at 4hpi. AJ disruption and loss of F-actin was also enhanced for *C. jejuni*(S), evident at 24hpi, and was associated with increased penetrance of viable *C. jejuni*(S) into spheroids. Infection of spheroids with pharmacologically inhibited cytoskeleton remodelling revealed that actin remodelling was necessary for *C. jejuni*(S) disruption of TJ but only partially needed for dismantling AJ. These results hint that environmental stimuli (e.g.high oxygen) may prime *C.jejuni* for virulence.

P149

Galleria mellonella as a burn wound and infection model to identify and study potential wound probiotics.

Evgenia Maslova, Shanga Osman, Ronan McCarthy ORCID iD

Brunel University London, London, United Kingdom

Abstract

Burn wound infections are the leading cause of mortality among burn patients. One of the most commonly isolated burn wound pathogens is *Pseudomonas aeruginosa*, which is a notorious multidrug resistant nosocomial bacterium. Due to the antibiotic resistance crisis, there is an urgent need for the development of alternative treatments for burn infections. One of the potential wound infection prevention measures is to treat the wound with probiotic bacteria. *Lactobacillus spp*, are well-known commensals that have been reported to display antimicrobial activity against Gram-negative and -positive pathogens largely in the gut. Several different *Lactobacillus* species have been shown to inhibit the growth of wound pathogens and to enhance wound healing processes, highlighting its probiotic potential. Due to the complex nature of burn wounds, they need to be studied *in vivo*. However, *in vivo* models, such as mouse and rabbit model, are challenging, strictly ethically regulated and do not facilitate screening approaches. The recently established *G. mellonella* burn wound and infection model addresses several of these challenges, such as affordability, maintenance, and lack of ethical regulations. This study shows that in the *G. mellonella* burn wound model treatments with *Lactobacillus* colonies and supernatant have improved the survival of *P. aeruginosa* infection. It also confirms that the *Lactobacillus spp* treatment is non-pathogenic to the host. Furthermore, this study validates the capacity of this model to facilitate screening for potential probiotics by demonstrating that *Lactobacillus spp* treatment can limit *P. aeruginosa* burn wound infection and improve the survival prognosis in this model.

P150

Characterising the association between macrophages and *S. pneumoniae* in an *ex vivo* human spleen infection model

Neama Alnabati[1], Shiying Tang[1], Trisha Kanani[1,2], Ryan Hames[1], Wen Chung[2], Ashley Dennison[2], Marco Oggioni[1,3]

[1]University of Leicester, Leicester, United Kingdom. [2]University Hospitals of Leicester, Leicester, United Kingdom. [3]University of Bologna, Bologna, Italy

Abstract

Streptococcus pneumoniae (*S. pneumoniae*) is Gram-positive bacterium associated with lower respiratory tract infections and can cause Invasive pneumococcal disease (IPD) which is linked with a high morbidity and mortality rate. The human spleen is located in the left upper quadrant of the abdomen and serves a role in innate and adaptive immune responses as it contains macrophages that are involved in the defence and clearance of pathogens. Despite the progress in clinical testing, there is still a major gap in our understating of how *S. pneumoniae* replicates and evades tissue macrophage clearance, especially during the early phase of infections. Therefore, we aimed to test the uptake of bacteria by different macrophage subsets by utilising an *ex vivo* human spleen model. In a Clinical Trial (NCT04620824), spleens were collected, perfused *ex vivo* for 6 hours and infected with a mix of *S. pneumoniae* serotypes. Physiological blood gas was monitored, adjusted accordingly, and CFU/ml or CFU/g of perfusate and biopsies were counted. Data showed that bacteria were found more in the spleen than in the perfusate. Immunofluorescence staining microscopy analysis showed that there is a higher number of bacteria associated with red pulp macrophages followed by perifollicular sheath macrophages and capillary sheathed macrophages and regardless of the strain. Additionally, opsonisation with antibodies allowed for rapid ingestion by phagocytes where serotype 4 was reduced over time which indicates an antibody-dependent clearance by macrophages. Our data are the first indication of the dynamics of bacterial clearance within the main human immune organ.

P151

Molecular Epidemiology of *Staphylococcus aureus* ST152 in Nigeria

Anderson Osemuahu Oaikhena ORCID iD[1,2], Odion O. Ikhimiukor[1], Aaron O Aboderin[3], Veronica O Ogunleye[4], Aderemi Kehinde[4], Ayorinde O Afolayan[1], Erkison E Odih[1,5], Ifeanyi E Mba[1], Gabriel T Sunmonu[1], Dorothy Cyril-Okoh[1], Abeeb Adeniyi[1], Adewale A Amupitan[3], Oyekola V Abiri[3], Temitope O Obadare[3], Olatunde F Olabisi[3], Olanipekun A Bashirat[6], Abayomi Fadeyi[6], Oyinlola O Oduyebo[7], Josephine F Ojo[8], Abolaji T Adeyemo[8], Charles Elikwu[9], Adebayo O Shittu[3], Ifeoluwa Komolafe[1], Philip O Oshun[7], Victoria A Aikhonu[7], Anthony Underwood[10], Silvia Argimón[10], Mihir Kekre[10], Abiodun Egwenu[11], Tochi Okwor[11], Chikwe Ihekweazu[11], David M Aanensen[10,12], Iruka N Okeke[1]

[1]University of Ibadan, Ibadan, Nigeria. [2]Technical University of Graz, Graz, Austria. [3]Obafemi Awolowo University Teaching Hospital Complexes, Ile-Ife, Nigeria. [4]University College Hospital, Ibadan, Nigeria. [5]University of Copenhagen, Copenhagen, Denmark. [6]University of Ilorin Teaching Hospital, Ilorin, Nigeria. [7]Lagos University Teaching Hospital, Lagos, Nigeria. [8]Osun State University Teaching Hospital, Osogbo, Nigeria. [9]Babcock University Teaching Hospital, Ilishan, Nigeria. [10]Oxford University, Oxford, United Kingdom. [11]Nigeria Centre for Disease Control, Abuja, Nigeria. [12]Wellcome Genome Campus, Hinxton, United Kingdom

Abstract

Staphylococcus aureus is a major cause of bloodstream infections in Nigeria. ST152, typically community-associated and *mecA*-negative, is increasingly isolated in hospitals with *mecA*-positive genotypes. We characterized invasive *S. aureus* isolates from the Nigerian reference laboratory and determined clonal structure, antimicrobial resistance and virulence, focusing on ST152.

The *S. aureus* were isolated from blood cultures at six tertiary institutions in Nigeria. They were verified and antimicrobial susceptibility tested at the reference laboratory using VITEK-2 before sequencing on an Illumina platform. SNP phylogeny was analyzed by a publicly available nextflo pipeline. SCC*mec*-, *spa*-, and Sequence types (STs) were derived with SCC*mec*Finder, *spa*Typer, and ARIBA, respectively. Furthermore, NCBI's AMRFinderPlus, PlasmidFinder, and VirulenceFinder were utilized for antimicrobial resistance, virulence, and plasmid prediction, respectively.

Of the 137 *S. aureus* sequenced, 48 (35%) belonged to ST152. Other predominant clones were ST772 (21; 15.3%), ST8 (16; 11.6%), and ST789 (13; 9.5%). Sixty-two percent (85/137) of isolates were *mecA*-positive overall and 27 of 48 ST152 isolates had the *mecA*. Four SCC*mec* types (mainly SCC*mec* Vc) and 26 spa types (mainly t4690) were detected. ST152 was detected at four of the six sentinels and formed two major sub-clades with most (25; 52.1%) isolates belonging to the ST152-t4690-MRSA-Vc lineage. Although the ST152 *S. aureus* isolates were not resistant to multiple antibiotics, 93.8% (45/48) of ST152 were PVL-positive, compared to 53.9% (48/89) non-ST152.

The predominance of ST152 with high *mecA* and PVL positivity rates observed in this study, indicates need for active surveillance of these and other staphylococci in Nigeria.

P152

Antiretrovirals as antibiotics: The effect *in vitro* of HIV nucleoside reverse transcriptase inhibitors (NRTIs) on vaginal microbiota

Veronica Georgiana Preda[1], Lauren A. Roberts[2], Rachael A. Quinlan[3], Charlotte-Eve S. Short[1], Graham P. Taylor[1], Julian Marchesi[2]

[1]Section of Virology, Department of Infectious Disease, Imperial College London, London, United Kingdom. [2]Liver & Anti Viral Unit, 10th Floor, QEQM, St Mary's Hospital Campus, Imperial College London, London, United Kingdom. [3]March of Dimes European Prematurity Research Centre & Section of Virology, Department of Infectious Disease, Imperial College London, London, United Kingdom

Abstract

Background: HIV protease inhibitors are associated with two-fold increase in pre-term delivery (PTD) rates, the main cause of neonatal death, whereas Zidovudine monotherapy, a NRTI, is associated with decreased PTD. The mechanism is unknown. Bacterial vaginosis (BV), which is associated with PTD, is characterized by abundant biofilm microbes, e.g. *Gardnerella vaginalis* and *Lactobacillus iners* and lack of protective lactobacilli, e.g. *L. crispatus*. Certain antiretrovirals inhibit gut pathobionts growth. We hypothesize that antiretrovirals inhibit BV-associated bacteria (BVAB).

Methods: Broth microdilution method was used to determine the MICs and IC50 values for Metronidazole and Ciprofloxacin (controls), against *G. vaginalis* DSMZ 4944, *L. iners* DSMZ 13335 and *L. crispatus* DSMZ 20854 at eight concentrations. MICs and IC50s of current NRTIs were compared to these antibiotics.

Results: Metronidazole inhibited *G. vaginalis* growth at MIC of 1.8 µg/mL (IC50 6.1 µg/mL). Ciprofloxacin inhibited *L. iners* with an MIC of 0.24 µg/mL (IC50 1.3µg/mL). Zidovudine MIC against *G. vaginalis* was 39.9 µg/mL (IC50 142.6 µg/mL) and against *L. iners* 5.5 µg/mL (IC50 19 µg/mL). Abacavir MIC of 4.0 µg/mL (IC50 13.7 µg/mL) against *G. vaginalis* was two-fold higher than Metronidazole. Emtricitabine inhibited *L. iners* (MIC 78.7 µg/mL, IC50 136.7 µg/mL), but not *G. vaginalis*. Tenofovir (TDF) and Lamivudine showed no inhibition against *G. vaginalis* and *L. iners*. None of the tested compounds inhibited *L. crispatus*.

Conclusion: This direct effect in vitro of selected NRTIs against BVAB offers insight into the potential protective effect in vivo on PTD risk observed with zidovudine.

P153

Infection responsive coatings for urinary catheters

Anabella Kazubska, Jonathan Nzakizwanayo ORCID iD, Nazia Merhban, Anthony Slate ORCID iD, Brian Jones ORCID iD

University of Bath, Bath, United Kingdom

Abstract

Catheter associated urinary tract infections (CAUTIs) are amongst the most common infections acquired in a healthcare setting. *Proteus mirabilis* infections are particularly problematic for catheterised patients, and often responsible for blockage of the catheter. Around 50 % of patients with long-term catheters experience blockage, which often leads to the onset of serious clinical complications such as pyelonephritis, septicaemia and subsequently death. Crystalline biofilm formation is driven by the potent *P. mirabilis* urease enzyme, which converts urea into ammonia leading to an increase in urinary pH. Alkaline conditions allow crystals of magnesium ammonium phosphate (struvite) and calcium phosphate (hydroxyapatite) to amalgamate into the developing biofilm in a process called ureolytic biomineralization. This results in encrustation of the catheter and occlusion of urine flow. Key targets for control, prevention and detection of blockage therefore include inhibition of biofilm formation, inhibition of urease activity, and detection of pH elevation to provide advanced warning of catheter blockage. We have recently developed two distinct but complementary approaches that each work on a key target for controlling blockage: repurposing available drugs as anti-biofilm agents and novel coatings that can deliver agents in response to infection. This project will combine these strategies to further optimise a developed infection-responsive, “theranostic” catheter coating. Optimisation of this coating will enhance early warning detection of catheter blockage and actively delay this process. In conjunction, we will trial novel polymicrobial CAUTI models to better understand coating performance under representative clinical conditions, and provide robust proof-of-concept data in order to reduce catheter blockage.

P154

A pangenome analysis comparing and contrasting *Salmonella enterica* subspecies *enterica* and *Salmonella enterica* subspecies *salamae* isolated from households in Malawi.

Catherine Wilson ORCID iD[1], Patrick Musicha ORCID iD[2], Mathew Beale ORCID iD[1], Yohane Diness[2], Oscar Kanjerwa[3], Nicholas Feasey[4,5], Eric Fèvre[6,7], Gina Pinchbeck[6], Nicholas Thomson[8]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Malawi Liverpool Wellcome Trust Research Programme, Blantyre, Malawi. [3]Malawi Liverpool Wellcome Trust Research Programme, Blantyre, United Kingdom. [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [5]Malawi Liverpool Wellcome Trust Clinical Research Programme, Blantyre, Malawi. [6]University of Liverpool, Liverpool, United Kingdom. [7]International Livestock Research Institute, Nairobi, Kenya. [8]Wellcome Sanger Institue, Cambridge, United Kingdom

Abstract

Few studies have reported the genomic difference between *Salmonella enterica* subspecies *enterica* (*S. enterica*) and *Salmonella enterica* subspecies *salamae* (*S. salamae*). This study adds to our understanding the genomics and pathogenic potential of *S. salamae* in comparison to *S. enterica*.

102 *S. salamae* genomes and 125 *S. enterica* genomes cultured from the stool of healthy humans, animals and environmental samples at households in Malawi. Roary and Panstripe were used to assess the pangenome. Antimicrobial resistance (AMR) determinants, plasmids and virulence genes were assessed using AMRFinderPlus, MOB_suite and Abricate.

S. enterica and *S. salamae* were isolated from a range of host species and environmental sites. The core genome of the 227 genomes was 3,106 genes, *S. salamae* 3,460 and *S. enterica* 3,491 genes. The pangenome of the collection was 16,626 genes, *S. salamae* 11,250 and *S. enterica* 10,712 genes. Both subspecies had an open pangenome and different rates of gene exchange within the population. Thirty-one virulence genes were determined within *S. enterica* genomes only, mainly associated with *Salmonella* Type 3 secretion system proteins. No virulence genes were detected within the *S. salamae* population alone. Overall, there was a low number of AMR determinants within the population. *S. salamae* carried only chromosomal AMR determinants. Only IncFII and Inc gamma K1 plasmids were shared across the whole population of *Salmonella*.

This is the first-time a comparative analysis of *S. enterica* and *S. salamae* genomes has documented differences in pan- and core genome size, plasmids, virulence genes and AMR determinants between the two populations.

P155

Filamentous phages released from *Pseudomonas aeruginosa* modulate immune response of human lung epithelial cells

Krzysztof Fiedoruk, Magdalena Zakrzewska, Ewelina Piktel, Urszula Wnorowska, Tamara Daniluk, Robert Bucki

Medical University of Bialystok, Białystok, Poland

Abstract

Pseudomonas aeruginosa filamentous bacteriophages (Pf) are essential virulence factors of this archetypal cystic fibrosis (CF) pathogen, which contribute to chronic lung infection via promotion of biofilm formation, antibiotic resistance, and immune suppression. However, Pf phages constitute a heterogeneous group of viruses with distinct structural properties. Therefore, we aimed to determine the immune response of human lung epithelial cells after exposure to various Pf phages.

Pf phages were purified from *P. aeruginosa* strains isolated from CF patients using standard PEG/NaCl precipitation method. In addition, Pf1 phage suspension (ASLA Biotech AB, Latvia) was used as the reference. Pf phages in concentrations ranging from 10^8 to 10^1 PFU/mL, alone and in combination with LPS, were used to stimulate A549 human lung epithelial cells. Subsequently, RNA was isolated from the cells to estimate the expression of genes encoding immune response factors, including TNF-α, IL-6, IL-8, and IFNs, with SYBR Green RT-qPCR technique.

Altered expression of several genes was recorded in the presence of Pf phages, in particular at concentrations ranging from 10^6 to 10^4 PFU/mL. For instance, Pf1 phage induced the expression of IFNB1 gene mostly at concentration of 10^4 PFU/mL. In addition, the expression of TNF-α gene was modulated by Pf phages in cells simultaneously stimulated with LPS compared with the cells treated with LPS only.

P. aeruginosa Pf phages interfere with the expression of genes encoding immune factors controlling anti-bacterial and anti-viral responses.

Research funding: HARMONIA 10 grant (2018/30/M/NZ6/00502), National Science Center (Poland).

P156

Decreased efficacy of antimicrobial agents in a polymicrobial environment

Wendy Figueroa, Thomas O'brien, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

The airways of people with cystic fibrosis (CF) are exceptionally susceptible to infections. These infections are often polymicrobial and include bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as fungal species like *Candida albicans*. These airway infections can be impossible to resolve through antibiotic intervention, even though isolates of the individual species present are susceptible to the treatment when tested in vitro. In this work, we used a polymicrobial model to investigate how key CF-associated pathogens respond to treatment with species-specific antimicrobial agents such as colistin, fusidic acid, and fluconazole. We found that growth in a polymicrobial environment protects the target microorganism (sometimes by several orders of magnitude) from the effect(s) of the antimicrobial agent. This decreased antimicrobial efficacy was found to have both non-heritable (physiological) and heritable (genetic) components. Whole-genome sequencing of the colistin-resistant *P. aeruginosa* isolates revealed mutations in genes associated with the O-antigen biosynthesis pathway and/or pilus biogenesis, revealing a previously undescribed colistin resistance mechanism. Using genetic analyses, we confirmed the involvement of such pathways in the resistance. Moreover, by analysing a collection of nearly 1000 *P. aeruginosa* genomes, we found a correlation between the introduction of colistin to the clinic and the emergence of mutations in those genes. Our findings indicate that the polymicrobial nature of the CF airways is likely to have a significant impact on the clinical response to antimicrobial therapy.

P157

Investigating the role of the staphylococcal type VII secretion system in cell surface integrity

Victoria Smith, Arnaud Kengmo Tchoupa, Ramón Garcia Maset, Meera Unnikrishnan

University of Warwick, Coventry, United Kingdom

Abstract

Staphylococcus aureus is a successful human pathogen with a notable ability to acquire resistance to many antibiotics. *S. aureus* encodes a specialised type VII secretion system (T7SS), which is a key virulence factor and a putative vaccine candidate, hence it is considered to be a good drug target. However, the role of the T7SS in bacterial physiology and infection remains unclear. We recently reported that T7SS mutants are more sensitive to fatty acids, which was attributed to altered cell membrane integrity. Here we demonstrate that *S. aureus* strains lacking T7SS effector, EsxC, show an altered cell surface morphology using scanning electron microscopy. LC/MS analysis showed altered protein profiles of T7SS mutant membrane preparations compared to the wild type (WT). Localisation of cell membrane proteins such as flotillin were also altered in the mutants. Additionally, the whole cell surface charge of *esxC* mutants was less positive compared to WT. To investigate if the surface changes would impact bacterial sensitivity to membrane targeting antibiotics, we studied effects of the last resort drug, daptomycin, on T7SS mutants. We found that T7SS mutants displayed defective growth *in vitro* in the presence of daptomycin compared to the WT. Microscopic analysis of propidium iodide stained, daptomycin-treated bacteria showed an increased cell death in the mutants. Furthermore, in a murine skin infection model, *esxC* mutants were killed better when treated with daptomycin. Thus, our data suggest that the staphylococcal T7SS contributes to the maintenance of cell surface integrity, which affects sensitivity to membrane-acting drugs such as daptomycin.

P158

Characterising the bovine humoral response to *Cryptosporidium parvum.*

Ellen-Alana Tiffney[1], Janine L. Coombes[2,1], Joseph W. Angell[3,1], Robin J. Flynn[4,1], Richard A. Urbanowicz[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Aberdeen's Robert Gordon University, Aberdeen, United Kingdom. [3]Wern Veterinary Surgeons, Ruthin, United Kingdom. [4]South-East Technological University, Waterford, Ireland

Abstract

Cryptosporidium parvum is a zoonotic parasite with no effective treatments or vaccines. Recent outbreaks in Wales have seen a 30% mortality rate in bovine neonates. Surviving neonates often have long-term impacts on their weight gain and milk production. Therefore, preventative vaccines or more effective treatments, such as monoclonal antibodies, are needed. Fortunately, successful passive transfer of maternally derived antibodies, through colostrum, has been shown to reduce severity of *C. parvum* infections in bovine neonates. In light of this, our research focused on defining the antibody composition of these successfully transferred maternally derived antibodies in the serum from bovine neonates.

Our data indicate a difference in IgG subclass composition in neonates with and without disease, as well as a reduction in binding in those that succumbed to disease. Using purified IgG from neonates that survived, we have developed a novel *in vitro* neutralisation assay, that demonstrate the antibodies can block *C. parvum* entry.

Taken together this provides data that these antibodies not only bind, but sterically hinder entry of the parasite, thereby providing protection against infection. Further work is now underway to isolate the relevant B cells to enable production of these antibodies, with the aim to use them as a therapeutic agent.

P159

Multidrug resistant members *Cupriavidus spp*.: underrecognized causes of lower respiratory tract infection?

Sean Kelly[1], James Butler[1], Katie Muddiman[1], Dervla Kenna[2], Heather Green[3], Andrew Jones[3], Sumita Pai[4], Gyorgy Fejer[1], Mathew Upton[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]National Infection Service, London, United Kingdom. [3]Manchester Adult Cystic Fibrosis Centre, Manchester, United Kingdom. [4]Papworth Hospital, Cambridge, United Kingdom

Abstract

Cupriavidus species are primarily environmental organisms, but some (*C. pauculus and C. gilardii*) cause human infections. We recently reported the genomic and phenotypic characterisation of a strain of *C. pauculus* recovered from a hospital sink trap, devices that have been linked to outbreaks of (respiratory tract) nosocomial infections.

Three clinical isolates were identified as members of Cupriavidus using gyrB and 16S rRNA gene sequencing. They were recovered from patients with cystic fibrosis in the Southeast (isolates H18C1 and H18C2; 2018) and Northwest (isolate H19C3; 2019) of England. We characterised the isolates using hybrid genome sequencing, susceptibility profiling and investigation of biofilm formation.

The draft genomes were 5.6 Mb to 7.2 Mb and autoMLST indicated a close relationship of H18C1 to *C. gilardii* but did not give species identifications for H18C2 and H19C3. Low average nucleotide identity and digital DNA-DNA hybridisation indicate that these are members of a novel species.

Eight antibiotic resistance genes were identified, including beta-lactamases and efflux pumps. High levels of resistance to multiple antibiotics was seen (meropenem, tobramycin, amikacin and colistin). Fifteen virulence factors were observed. Biofilm formation in the isolates was strongly strain-dependent, with strain H18C1 exceeding levels seen for the environmental isolate MF1 and higher than for the known biofilm forming Pseudomonas aeruginosa strain PAO1.

These clinical isolates are potentially novel species of multi-drug resistant *Cupriavidus* causing human infection in PWCF and data suggest that they may be acquired directly from (nosocomial) environments. High levels of antibiotic resistance may impact treatment outcomes.

P160

Development of a novel topical antifungal for the treatment of onychomycosis

Grace Lawler[1], Carmel Kealey[1], Damien Brady[2], James Roche[1]

[1]Technological University of the Shannon Midland Campus Athlone, Athlone Co. Westmeath, Ireland.
[2]South East Technological University, Carlow, Ireland

Abstract

Background: To determine and evaluate the mechanism of action of a modified coconut oil that has previously shown promising antimicrobial activity against the opportunistic infection Trichophyton rubrum.

Methods: Clinical isolates of Trichophyton rubrum and Candida albicans have been cultivated, isolated and quantified by microbial methods. A modified coconut oil formulation has been prepared according to a proprietary procedure with components determined by physiochemical, chromatographic and spectroscopic techniques. C. albicans and T. rubrum were challenged with the modified oil through in vitro bioassays (minimum inhibitory concentration, time-kill and disc diffusion assays). Microscopy techniques used such as, scanning electron microscopy and transmission electron microscopy reveal the morphological effects on the
fungus.

Expected outcomes: To uncover the mechanism of action by establishing the low concentration ingredients in the oil that are attributing to the antifungal action. By carefully determining the nature of these entities, the reproducibility of their occurrence and their stability in the oil, allowing an understanding of the heretofore unexplained antifungal action. Microscopy techniques will establish what makes T. rubrum susceptible to this promising antifungal
agent.

Conclusion: With limited number of antifungal treatments available on the current market and the widespread use of a small selection of such agents, there is a fear of a potential rise in antifungal resistance and therefore a demand for novel antifungal therapeutic agents. Once the modification process of the coconut oil is proved/validated, by identifying the components that allow the oil to prevail over the destructive infection, a promising antifungal market contender exists

P161

Novel T-cell based vaccines to fight ESKAPE infections globally

Paulina Zarodkiewicz ORCID iD, Rebecca Ingram, Miguel Valvano

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antibiotic resistance is a major public health concern worldwide. Novel approaches are required to curtail the emergence of multi-drug antibiotic resistant pathogens. One appealing strategy is vaccination, which can reduce infection incidence, lower use of antibiotics and limit resistance spread. We hypothesise that T cell-mediated immunization is protective for bacterial pathogens such as *Klebsiella pneumoniae* and *Enterobacter cloacae* infections. These bacteria are members of the ESKAPE pathogens. To test our hypothesis we implemented a reverse vaccinology approach. Bioinformatic tools were used to construct the core pan-proteomes of *K. pneumoniae* and *E. cloacae* using proteomes available on UniProt. Further, a reverse vaccinology pipeline was implemented to filter proteins based on their probability to be antigens, homology to human and mouse and size, followed by prediction of T cell epitopes. At the final stage of the pipeline 346 and 444 putative antigens were identified for *K. pneumoniae*, and *E. cloacae*, respectively. Further, 248 antigens were shared in common, which have the potential to be cross-protective candidates. The antigens were subjected to molecular cloning prioritising proteins with the highest antigenic probability rank. Screening over 100 candidates in a murine *in vivo* model allowed us to identify top antigenic candidates based on IFNγ responses. We show that our top candidate proteins are immunogenic and cross-protective against *K. pneumoniae* and *E. cloacae* challenge with a significant reduction of bacterial load and inflammation, thus validating our hypothesis and paving the way for the development of T-cell-based vaccines against ESKAPE pathogens.

P162

Mutagenesis identifies caf1R binding consensus required for biogenesis of Yersinia pestis F1 antigen

Sayandini Kirushnamoorthe[1], Dharmender K Gahlot[2], Sheila Macintyre[1]

[1]School of Biological Sciences, Health and Life Sciences Building, Reading, United Kingdom. [2]S Umea Centre for Microbial Research (UCMR), Umeå University, 901 87 Umeå, Umea, Sweden

Abstract

Yersinia pestis, the bacterium responsible for bubonic and pneumonic plague, can be transmitted from flea to human host (bubonic plague) or by respiratory transmission from person to person (pneumonic plague). During human infection, the bacterium is surrounded by F1 antigen, a protective proteinaceous capsule formed by thin fibrillae of polymers of the Caf1 subunit. Caf1R, a transcriptional activator belonging to the AraC/XylS (A/X) regulator family, controls expression of the caf1MA1 operon.

Based on bioinformatic analysis, protein-DNA modelling and promoter-fusions, promoter Pcaf1M and Caf1R binding site were defined. Mutagenesis of Caf1R defined amino acid residues that were critical to DNA binding. Mutagenesis of the Caf1R binding site upstream of Pcaf1M confirmed absolute requirement of both binding sites, BS1 and BS2, within this sequence, for production of F1.

Additional Caf1R binding consensus sequences were identified, within the intergenic region, upstream of caf1R. Highly conserved residues within each motif were mutated and the impact on F1 biosynthesis monitored. Two of these additional repeat motifs were required for F1 expression, with greatest impact following mutation of DNA binding site 2 (BS2). This is consistent with earlier evidence of autoregulation of caf1R.

These results characterising basic properties of the regulatory regions of the caf fimbrial cluster, provide a solid basis to understand the impact of temperature and other environmental factors on expression of the caf locus and how this interplays with expression of other key virulence determinants and plague disease in humans.

P163

What drives synergistic interactions in fungal-bacterial co-infections?

Seána Duggan ORCID iD

MRC CMM University of Exeter, Exeter, United Kingdom

Abstract

Co-infections caused by the fungus *Candida albicans* and the bacterium *Staphylococcus aureus* result in prolonged treatment and poorer patient outcome due to a phenomenon termed “lethal synergy” where *C. albicans* and *S. aureus* interact to enhance virulence resulting in worse disease. To understand how these two microbes interact during disease, this work investigates *C. albicans* and *S. aureus* co-infections using a combination of co-culture and primary human cell infection models, cytometry, multi-omics, and microscopy. Biofilms of both *C. albicans* and *S. aureus* have greater biomass than respective single species biofilms and mass spectrometry proteomics on *C. albicans* and *S. aureus* mature biofilms revealed proteins with functions in stress responses, quorum sensing, gluconeogenesis and cell wall structure contributing to increased biofilm. This work considers not only the microbes, but also the human host cells which play an important role in disease onset. Employing primary human neutrophils, a co-culture infection model has been established and interrogated to reveal how human cells recognise, become activated and phagocytose co-infecting microbes. Compared to infections caused by a single microbes, human neutrophils are attracted to co-infections at a greater rate and have distinct activation patterns. RNAseq is currently underway and will provide mechanistic insight to synergies and interferences between microbes and host cells which can be dissected using functional genomics. This work demonstrates both host and infecting microbes play a role in the outcome of co-infection and this knowledge has the potential to unveil avenues to rationally interfere with synergism in co-infections.

P164

The *Staphylococcus aureus* CamS lipoprotein is a repressor of toxins

Katrin Schilcher[1,2], Morgan Severn[1], Christian Jenul[1,2], Young-Saeng Cho[1], Rebecca Keogh[1], Alexander Horswill[1]

[1]Department of Immunology and Microbiology, University of Colorado, Anschutz Medical Campus, Aurora, USA. [2]Department of Genetics and Genome Biology, University of Leicester, Leicester, United Kingdom

Abstract

Lipoproteins (Lpp) are present in all bacteria and play important roles in bacterial physiology and virulence. Due to their localization at the interface of the bacterial surface and the extracellular environment, they are often involved in nutrient transport and signal transduction. Lpp consist of three distinct parts, a lipid moiety that anchors the Lpp to the bacterial membrane, a protein part with diverse binding and enzymatic activities and a small linear peptide derived from the secretion signal sequence of the Lpp precursor.

We employed bacterial genetics, transcriptomics, functional assays and murine infection models to investigate the role of the CamS Lpp in *S. aureus* toxin production and virulence.

In this study, we demonstrate that the protein component of the CamS Lpp acts as a repressor of toxin production in *S. aureus*, and therefore has a distinct biological function from its associated linear peptide *staph*-cAM373. Mutation of the protein component of CamS, increases cytotoxin mediated hemolysis of rabbit red blood cells, cytotoxicity towards human polymorphonuclear leukocytes and leads to a significant increase in virulence in a murine skin and soft tissue infection model and bloodstream infections *in vivo*. Our discoveries establish CamS as a repressor of staphylococcal cytotoxins and provide the first evidence for distinct biological functions of a Lpp protein moiety and its associated linear peptide.

P165

Prevalence and molecular characterization of *Listeria monocytogenes* in cattle of Pakistani Punjab Province

Qaiser Akram

UVAS, Lahore, Lahore, Pakistan

Abstract

Aim and background: This study was designed to find out the identification and prevalence of Listeria monocytogenes in milk and meat samples from different cities in Punjab, Pakistan.

Methods: A total of 800 samples were collected including farm milk, market milk, slaughter-house meat, and market meat samples (n=200 each). Pre-screening of L. monocytogenes was performed through rapid testing strips. Strip-positive samples were subjected to PCR analysis for molecular confirmation.

Results: The overall prevalence of L. monocytogenes through the strip-based method was 6.3%. However, PCR confirmed the prevalence of 2.25%. Statistical analysis revealed that the difference in overall prevalence in meat and milk samples from different cities was not significant ($p>0.5$). The prevalence of isolated L. monocytogenes from marketed milk and meatwas different significantly.

Conclusion: Phylogenetic analysis indicated the similarity of the L. monocytogenes strain with Chinese and Indian strains.

Keywords: Listeria monocytogenes, Strip Testing, Phylogenetic analysis, Foodborne listeriosis, Immuno-prevalence

P166

Molecular Characterisation of most important Pathogenic Bacteria by Multiplex PCR and its comparison with the conventional method for diagnosis of Subclinical Mastitis in Cattle and Buffalo

SUDEEP SOLANKI ORCID iD, DURGA DEVI

RAJUVAS, BIKANER, India

Abstract

Background: Subclinical mastitis is a potential threat to the dairy sector and possesses a great challenge for its earliest detection of pathogens responsible and its proactive treatment.

Objective: The study was aimed to access and analyse the conventional method and multiplex PCR assays (MPCR) for the simultaneous detection of significant pathogens of sub-clinical mastitis.

Methods: 200 pooled milk samples were collected from Cattle and Buffaloes in Rajasthan from 2020 to 2021. The major bacterial pathogens were identified by conventional method using culture and biochemical tests and multiplex PCR from milk samples.

Results: The conventional method obtained 97 isolates from 200 pooled milk samples of cattle and buffalo. Prevalence was detected as Staphylococcus aureus 54 (27%), Streptococcus spp. 30 (15%), and E. coli 13 (6.5%), respectively, as single or mixed infections. The predominant pathogen detected was Staphylococcus aureus, Streptococcus and E. coli simultaneously. Multiplex PCR revealed simultaneous detection of Staphylococcus 65 (32.5%), Streptococcus 37 (18.5%), E. coli, and 16 (8 %) directly from milk samples.

Conclusion: Investigation results exhibited multiplex PCR assay have higher specificity and sensitivity making it more reliable assay than conventional method. The multiplex PCR adopted in the present investigation was simple and rapid assay to determine major pathogens and simultaneously systematic analysis of milk by multiplex PCR adopted in the current study have potential to become a valuable asset for determining the status of herd for detection of contagious and pathogens causing environmental mastitis.

P167

Generation and characterisation of an interaction dataset of uropathogenic *Escherichia coli* strains and a panel of bacteriophage for 'smarter' phage therapy treatments.

Alison Low, Marianne Keith, Alba Park De La Torriente, Antonia Chalka, Adriana Vallejo-Trujillo, Sean McAteer, Gavin Paterson, David Gally

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Urinary tract infections (UTI) are one of the most common infectious diseases in dogs. While many UTIs are short-lived, some become chronic with life-limiting complications including kidney damage and sepsis. Antibiotics are the routine treatment for UTI infections but with increasing levels of antibiotic resistance there is traction for the development of phage therapy as an alternative treatment. The most common cause of UTI is uropathogenic *Escherichia coli* (UPEC) and we have collected, sequenced and generated an interaction dataset with 300 UPEC strains and 31 phage. This interaction dataset has been used alongside the whole genome sequences of the bacterial strains to generate machine learning models that can predict the most effective phage to use against a particular infecting bacterial strain. In addition, the interaction dataset has allowed us to determine phage activity groupings and with further phage characterisation and identification of bacterial resistance determinants we can create effective phage cocktails with the phage predicted to be active from our models. Key to this work has been using realistic '*in vivo*-like' conditions to measure phage activity thereby increasing the likelihood the phage will work in vivo. This work aims to pave the way to use phage therapy for treatment of dogs suffering from unresponsive chronic UTI infections.

Session Topic: SARS-CoV2 and retroviruses

P168

Four cellular proteins depletion reduces the SARS-CoV-2 replication

ALAA ABED, Joshua Lee, I'ah Donovan-Banfield, David Matthews, Andrew Davidson

University of Bristol, Bristol, United Kingdom

Abstract

The Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are Betacoronaviruses capable of causing fatal human infections. Both viruses are believed to have emerged from bats via an intermediate host (camels for MERS-CoV, unknown for SARS-CoV-2) into the human population.

MERS-CoV and SARS-CoV-2 are enveloped positive-sense RNA viruses which encode four structural proteins (envelope (E), nucleocapsid, spike, and membrane (M)). The E and M proteins are involved in virus assembly, budding, envelope formation, and pathogenesis. Finding cellular protein interactors for these viral proteins, conserved across species, will increase our understanding of the coronavirus lifecycle and identify targets for antiviral development.

Three cell lines (human HEK293, bat Pteropus alecto PaKiT and Camelus dromedarius Dubca) were used for transient expression of the MERS-CoV and SARS-CoV-2 E and M proteins (FLAG epitope-tagged) followed by co-immunoprecipitation (co-IP) and high-throughput mass spectrometry-based interactomic analysis. There were 33 high-confidence cellular interaction proteins conserved amongst the different cell lines and viruses ($p < 0.05$, $0 > \log2$ fold change compared to the controls). Before that, the Selection of 11 cellular proteins was found to interact with the MERS-CoV E and/or M proteins (stably expressed in HEK293 cells) by LC-MS/MS. To determine the importance of these 44 proteins in the virus lifecycle, functional validation was done by siRNA depletion in human cells, followed by infection with SARS-CoV-2. Four proteins have been identified (UBA52, CERS2, LPCAT1 and TM9SF2) when depleted by siRNA knockdown, reliably reduced SARS-CoV-2 replication.

P169

Development of a Pipeline for Phenotyping Virulence of SARS-CoV-2 variants in Golden Syrian Hamsters

Gavin Meehan ORCID iD, Vanessa Herder ORCID iD, Georgios Ilia, Jay Allan, Kyriaki Nomikou ORCID iD, Karen Kerr, Sergi Molina Arias, Quan Gu ORCID iD, Giuditta De Lorenzo ORCID iD, Wilhelm Furnorn ORCID iD, Vanessa Cowton, Nicole Upfold, Xinyi Huang, Mohan Chen, Ana Da Silva Filipe ORCID iD, Arvind Patel ORCID iD, Massimo Palmarini ORCID iD

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

SARS-CoV-2 has continued to evolve throughout the COVID-19 pandemic, giving rise to multiple variants of concern (VOCs). These VOCs differ in transmissibility, immune evasion, and virulence with BA.1 (Omicron), and its sub lineages, overtaking Delta to become the dominant strain. Omicron induces a milder disease, which has been attributed to it preferentially infecting tissues of the upper respiratory tract.

Multiple studies have examined VOCs in rodent models. However, these have often been limited to comparing virus replication, weight loss and basic histopathology in animals infected with different variants. Here, we aimed to (i) gain a better understanding of the complex immunopathology of SARS-CoV-2 infection, and (ii) develop quantitative methods that could be used to assess virulence in the hamster model. We used various approaches including RNA in situ hybridization, immunohistochemistry, bulk RNAseq and RT-qPCR to quantify virus replication, extent of pulmonary lesions and host responses. We observed higher numbers of Delta-infected cells throughout the respiratory tract and found that Omicron displayed a limited ability to invade the lung parenchyma. Upregulation of interferon stimulated genes was directly related to the presence of virus-infected cells. Importantly, the degree of pulmonary lesions, quantified by assessing type-II pneumocyte proliferation and macrophage infiltrates, was considerably higher in Delta- compared to Omicron-infected animals.

This data provided an experimental pipeline that we subsequently used to quantify the virulence of recent Omicron sub-lineages. This pipeline should prove invaluable for rapidly assessing newly emerging variants and their potential to cause severe disease as the pandemic progresses.

P170

Phage display screening of bovine ultralong antibodies to SARS-CoV-2

Emily Park[1], Theocharis Tsoleridis[1], Joshua Ducan[1], Joseph Chappell[1], Patrick McClure[1], Alexander Tarr[1], Richard Urbanowicz[2], Jonathan Ball[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

The urgency for therapeutics in the arms race against SARS-CoV-2 increases with the emergence of each novel variant. Broadly neutralising antibodies are highly adept at binding conserved epitopes, and, with a short developmental timescale, they are ideal candidates for antibody therapies. A subset of naturally occurring bovine antibodies possess the unique ability to bind to conserved epitopes by utilising unusual paratope structures, courtesy of an ultralong heavy chain complementary determining region 3 (CDRH3). A high throughput screening method is therefore desirable for the isolation of antibody candidates from the bovine antibody repertoire. Antibody phage display has been a powerful tool since the 1980's for the isolation and identification of monoclonal antibodies (mAbs). During validation of a newly developed bovine Fab phage display library, we identified two ultralong mAbs derived from PBMCs of *Bos taurus* immunised with various forms SARS-CoV-2 lineage A spike. These two mAbs bound to the lineage A variant receptor binding domain (RBD) and neutralise SARS-CoV-2 pseudotypes supplemented with spike from multiple variants of concern. Using this approach to tap into the bovine immune repertoire for ultralong antibodies highlights a powerful tool for combating rapidly emerging pathogens.

P171

The SKI complex is a broad-spectrum host-directed antiviral drug target

Stuart Weston[1], Lauren Baracco[1], Louis Taylor[1], Alison Scott[2], Paul Shapiro[3], Alexander MacKerell[3], Matthew Frieman[1]

[1]University of Maryland, School of Medicine, Baltimore, USA. [2]University of Maryland, School of Dentistry, Baltimore, USA. [3]University of Maryland, School of Pharmacy, Baltimore, USA

Abstract

The SARS-CoV-2 pandemic has highlighted many deficiencies in our ability to respond to emerging pathogens. A huge amount of scientific progress has been made during the pandemic with multiple vaccines and approved drugs now available. To be better equipped for the next viral pandemic, it is imperative to develop broad-acting antiviral compounds that can be rapidly utilized against known or novel pathogens. To that end, we have been working to develop a host-directed therapy targeting the SKI complex. Host-directed therapies have the advantage of minimizing viral resistance through adaptive evolution and have a greater potential for broad activity with many viruses utilizing overlapping systems within cells. The human SKI complex is an RNA helicase that works in concert with the RNA exosome to degrade cytosolic RNA. We have previously reported various chemicals that were *in silico* modelled to bind the SKI complex have antiviral activity, inhibiting replication of pathogenic human coronaviruses, influenza virus, and the filoviruses Ebola and Marburg virus. We are now working to develop these chemicals towards potential antiviral drugs with continued structural-activity relationship screening and *in vivo* studies showing antiviral activity in mice. Along with the drug development work, we are also studying how targeting this cellular RNA helicase complex can inhibit a broad array of viruses, with results from transcriptomic and mass-spectrometry studies demonstrating the involvement of cholesterol biosynthesis pathways. We are therefore working towards developing an understanding of the underlying mechanism of antiviral activity, and better understanding the cell biological function of the SKI complex.

P172

Using Ultravision™ to Capture and Inactivate Viral Particles from Bioaerosols.

Hannah Preston[1], Rebecca Bayliss[1], Nigel Temperton[2], Martin Mayora Neto[3], Dominic Griffiths[4], Jason Brewer[4], Alan Parker[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]Medway School of Pharmacy, Kent, United Kingdom. [3]Medway School of Pharmacy, kent, United Kingdom. [4]Alesi Surgical Ltd, Cardiff, United Kingdom

Abstract

Ultravision™ technology is used to clear surgical smoke during laparoscopic surgery. Ultravision™ operates by 'electrostatic precipitation' (EP), whereby solid particles are removed from a gas via electrical energy. Previous studies have shown that EP can capture airborne pathogens. However, little is known regarding the effects of EP on viral activity. Capturing and inactivating aerosolised viral particles using Ultravision™ may limit the spread of pathogens that are transmissible by aerosol, enabling elective laparoscopic surgeries to continue during periods of viral pandemics.

To mimic the release of surgical bioaerosols, model systems resembling open and closed surgery were constructed. Two viruses were used to evaluate the effects of Ultravision™ on enveloped and non-enveloped virus particles: a Lenti-SARS Pseudovirus and Adenovirus serotype 5. A known concentration of each virus was aerosolised into the model systems, exposed to Ultravision™, and collected from a BioSampler for experimental analysis. Physical parameters affecting the efficiency of EP were altered to identify optimal conditions for Ultravision™ usage. Samples were analysed for viral presence by qPCR and viral activity by transduction and plaque assays.

Virus particles were successfully captured and inactivated by Ultravision™, in both model systems. Ultravision™ functioned most efficiently at 10kV, or when combining two discharge electrodes at 8kV.

This study highlights Ultravision™ as an efficient device for reducing pathogen transmission, however the exact mechanisms underpinning viral inactivation remain unknown. It was hypothesised that virucidal reactive species generated by Ultravision™ degrade aerosolised viral particles. Future work using more representative models is required to confirm findings from this study.

P173

Measuring SARS-CoV-2 persistence on surfaces to predict the possibility of fomite transmission contributing to meat processing plant outbreaks and determine virus survival on contaminated foods

Tristan Russell ORCID iD, Gerald Barry ORCID iD

School of Veterinary Medicine, University College Dublin, Dublin, Ireland

Abstract

The meat processing industry was particularly badly affected by COVID-19 with outbreaks occurring in meat processing plants (MPPs) and some evidence suggesting contaminated foods can transport SARS-CoV-2 between countries during shipment. Conditions maintained in MPPs appear to increase the risk of SARS-CoV-2 transmission with prolonged viability of SARS-CoV-2 outside the body in these environments potentially contributing to this. This study investigated persistence of SARS-CoV-2 on surfaces by inoculating relevant materials with virus and incubating under conditions reflective of Irish MPPs or conditions used for shipping foods. Viable SARS-CoV-2 recovered from surfaces was measured overtime using cell culture based techniques. When incubated at temperatures used for shipping, SARS-CoV-2 recovery from beef, pork meat, pork fat, salmon flesh and salmon scales was temperature sensitive and significant variation between some surfaces was measured. Similarly, surface type also impacted SARS-CoV-2 recovery following incubation at higher temperatures with more rapid loss of viability observed for abiotic surfaces compared to foods. Fomite transmission during MPP outbreaks cannot be ruled out due to the recovery of SARS-CoV-2 from stainless steel and fabric for at least ten hours, and conditions maintained in plants were found to increase viability. This data supports a multi-layered approach to reducing the risk of airborne infections, such as SARS-CoV-2, which mitigates against fomite transmission as well as reducing the risk of aerosol or respiratory droplet transmission in MPPs. Recommendations based on these findings include routine disinfection of surfaces and, where possible, increasing environmental temperatures.

P174

Digital pathology pipelines to assess virulence of SARS-CoV-2 variants and host responses in hamsters

Vanessa Herder ORCID iD, Gavin R. Meehan, Jay Allan, Georgios Ilia, Xinyi Huang, Kyriaki Nomikou, Quan Gu, Karen Kerr, Sergi Molina Arias, Giuditta De Lorenzo, Wilhelm Furnon, Vanessa Cowton, Mohan Chen, Ana Da Silva Filipe, Arvind Patel, Massimo Palmarini

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

The virulence of SARS-CoV-2 varies between distinct variants of concern (VOCs). For example, delta (B.1.617.2) is more virulent than the original B.1 (Wuhan D614G), while omicron (B.1.1.529) has a relatively attenuated phenotype. As SARS-CoV-2 continues to evolve, it is important to monitor the virulence of newly emerging variants. Importantly, virulence of VOCs in the human population can be, so far, experimentally recapitulated in animal models such as the golden hamster. Hence, developing robust methods to experimentally assess virulence of SARS-CoV-2 variants can be used in risk-assessment frameworks for new emerging VOCs. Here, we used various experimental digital pathology platforms to assess the virulence of SARS-CoV-2 in hamsters. All our analyses were performed on whole scanned tissue slides by quantifying the number of specific cells or size of areas stained with antibodies or RNA probes in bright field microscopy to comparatively assess lesions in the respiratory tract of infected hamsters. To overcome the challenge of relying on semiquantitative scoring systems for histopathology findings, we applied software-assisted analysis procedures to (i) minimize bias and to (ii) increase the speed of data-acquisition. For a deeper understanding of the mechanistic immunological differences between VOCs we complemented our analysis with spatial phenotyping including high-plex protein-staining for studying the complexity of tissue microenvironment as well as spatial transcriptomics. As a result of this study, we generated a reliable, standardised, and reproducible pathology-based pipeline to assess the virulence of SARS-COV-2 VOCs in hamsters, including intermediate phenotypes between highly virulent and attenuated VOCs.

P175

The use of pseudotyped lentivirus to pre-screen neutralising antibodies ahead of BSL-3 testing

Charlotte Cumper[1], Andrew Davidson[2], Dan Rocca[1], Sandy Kimber[1]

[1]Charles River Laboratories, Portishead, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Background: SARS-CoV-2 is a biosafety level 3 pathogen and requires specialist laboratory facilities to handle virus stocks and test anti-viral compounds. We investigated the use of pseudotyping technology to screen therapeutic anti-SARS-CoV-2 antibodies at biosafety level 2.

Methods: Antibodies were mixed with a GFP-expressing lentivirus harbouring the SARS-CoV-2 spike protein. BSL-2 assays were run using HEK293 cells overexpressing viral target receptors ACE2 and TMPRSS2. GFP expression was measured by IncuCyte Zoom and used to calculate percentage infection. BSL-3 assays were run using Vero E6 cells overexpressing the TMPRSS2 receptor and three strains of SARS-CoV-2 (Wuhan, Delta and Omicron). Cells were stained with anti-SARS-CoV-2 nucleocapsid antibody and imaged to quantify percentage infection.

Results: An anti-SARS-CoV-2 nanobody inhibited infection with both pseudovirus and the Wuhan strain of SARS-CoV-2. Pooled SARS-CoV-2 convalescent plasma (WHO standard) inhibited infection at low concentrations in the pseudovirus assay. Results were mirrored in assays against the Wuhan and Delta strains of SARS-CoV-2. The WHO standard showed inhibition of the Omicron strain at the highest concentrations only. To control for non-specific effects an isotype control was used. This did not inhibit infection with either the pseudotyped virus or authentic SARS-CoV-2.

Conclusions: In summary, we have found that virus neutralisation assays using lentivirus pseudotyped with the SARS-CoV-2 spike protein under BSL-2 conditions were directly comparable to assays run in a BSL-3 lab using live SARS-CoV-2. The ability to identify potential neutralising compounds of BSL-3 viruses in a BSL-2 setting could reduce the costs for these early screening phases.

P176

Discovery of a bovine broadly neutralising monoclonal antibody against SARS-CoV-2

Theocharis Tsoleridis ORCID iD[1,2], Joseph G Chappell[1,2], Joshua D Duncan[1,2], Parul Sharma[3], Eleanor G Bentley[3], Adam Kirby[3], C Patrick McClure[1,2], James P Stewart[3], Richard A Urbanowicz[3], Jonathan K Ball[1,2]

[1]Wolfson Centre for Global Virus Research, University of Nottingham, A Floor, West Block, Queen's Medical Centre, Derby Road, Nottingham NG7 2UH, UK., Nottingham, United Kingdom. [2]School of Life Sciences, University of Nottingham, A Floor, West Block, Queen's Medical Centre, Derby Road, Nottingham NG7 2UH, UK., Nottingham, United Kingdom. [3]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, United Kingdom, Liverpool, United Kingdom

Abstract

The emergence of SARS-CoV-2 has caused millions of deaths worldwide. Although current vaccines provide protection against severe disease in the majority of the population, there is still a great need for therapeutics for immunocompromised and severely ill patients. The design of therapeutics is challenging due to new variants emerging every few months. In this study we present the discovery of a bovine broadly neutralising monoclonal antibody which can neutralise, *in vitro*, all the SARS-CoV-2 variants to date. We performed consecutive immunisations on two cows with Lineage A SARS-CoV-2 spike and peripheral blood mononuclear cells were isolated from the last bleed. Using flow cytometry, single spike-reactive B-cells were sorted, their IgH and IgL sequences were retrieved with PCR, then cloned into a human antibody cassette and expressed in mammalian cells. A panel of 31 monoclonal antibodies was generated of which 21 bound to SARS-CoV-2 spike. A full characterisation was performed with binding and neutralisation assays using spikes from different variants to assess breadth and potency. One antibody was selected based on the best EC_{50} and IC_{50} value across all variants and was tested in an *in vivo* hamster model, showing lung protection against Lineage A live virus comparable to the commercially available Ronapreve. Further *in vivo* tests will be performed against new variants compared to commercial current therapeutics to determine its clinical potential.

P177

Investigating the role of SARS-CoV-2 NTD Spike mutations in viral entry, transmission and evading host immunity.

Nazia Thakur[1,2], Joseph Newman[1], Ahmed ME Elrefaey[1], Jeffrey Seow[3], Katie Doores[3], Dalan Bailey[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. [3]Department of Infectious Diseases, School of Immunology and Microbial Sciences, King's College London, London, United Kingdom

Abstract

Mutations within the SARS-CoV-2 genome have arisen throughout the COVID-19 pandemic, and in certain instances, these have given rise to variants leading to repeated epidemic waves of infection (i.e., Alpha, Delta, Omicron). Mutations within the Spike protein are of particular significance as Spike is the main target for neutralising antibodies (nAbs), a fundamental component of vaccine and infection derived immunity. The majority of nAbs are targeted against the S1 domain of Spike, especially the receptor binding domain (RBD) or regions closely adjacent to the ACE2-binding interface. Antibodies are also able to potently bind a “supersite” on the N-terminal domain (NTD) within S1, but this mode of action is less well understood. To investigate this, we synthesised a database of individual or combinatorial extant Spike NTD mutations or deletions, which were subsequently used to generate lentiviral-based pseudotypes. We first tested whether any of these mutations or deletions were essential for productive viral infection. We then screened functionally active pseudotypes against sera and monoclonal antibodies from vaccinated and/or infected individuals exposed to different SARS-CoV-2 variants to look for specific effects of NTD mutations on nAb responses. Elucidating the effect of RBD and NTD-specific mutations on nAb responses, either alone or in combination, is an important element of continued SARS-CoV-2 surveillance.

P178

Importance of molecular epidemiological surveillance: updated picture of SARS-CoV-2 mutations in a northeast Mexican population.

Jose Agustin Gutierrez Gomez, Soraya Sarahi Mendoza Olazaran ORCID iD, Jocabed Eunice Rubio Salazar, Valeria Veloz Moralez

Universidad de Monterrey, Monterrey, Mexico

Abstract

Background

SARS-CoV-2 is incessantly mutating, producing variants that become a challenge for public health maintenance. In Mexico, its molecular epidemiological description has been limited. This study aims to determine the temporal distribution of SARS-CoV-2 mutations and demonstrate the importance of maintaining its surveillance.

Methods

We included 557 samples from subjects who presented a positive nasopharyngeal swab against SARS-CoV-2 with a CT ≤30. These samples were collected from March 2020 to July 2022 by the Molecular Diagnostic Laboratory at the University of Monterrey (UDEM) in Mexico, and the variants were determined by real-time PCR.

Results

Of the 557 positive samples, 79.3% (442) were wild-type strains and 20.7% (115) were non-wild-type strains. These latter included Alpha, Beta, Delta, Gamma, Omicron, and some that were not identified (0.8%). The three variants with the highest frequency were Alpha (30.4%), Omicron (27.8%), and Delta (18.3%). Our studied population had an expected turn from wild-type (at the beginning of the pandemic) to non-wild-type variants (as the pandemic progressed). It is essential to denote, that Alpha, Beta, Delta, and Gamma variants, were detected in our laboratory before they were officially reported in the epidemiological surveillance sites in Mexico.

Conclusions

Like many other viruses, SARS-CoV-2 mutates at a rate exceeding our early detection capacity. Epidemiological molecular surveillance makes it possible to know the ongoing variants and provide effective sanitary measures.

P179

African-Rapid Immuno-surveillance system for epidemic response (ARISE): Lessons from COVID-19 pandemic in Nigeria

Chinedu A. Ugwu[1,2], Kazeem Akano[2,1], JOHNSON OKOLI[1], Philomena Eromon[1], Grace John[1], Blossom Akinnawo[1], Israel Ajayi[1], Ooreofe Odebode[1], Peter Olaitan[3], Ajibola Olagunoye[3], Ibukun Adebayo[3], Victor Adebayo[3], Elizabeth Babalola[3], Omowumi Abioye[3], Nnennaya Ajayi[4], Emeka Ogah[4], Kingsley Ukwaja[4], Sylvanus Okoro[4], Ogbonnaya Oje[4], Chiedozie Ojide[4], Matthew Eke[4], Venatius Onyia[4], Olivia Achondu-atijegbeo[1], Oluwafemi Ayodeji[5], Chukwuyem Abejegah[5], Samson Owhin[5], Nicholas Oyejide[1], Sylvester Abah[5,1], Winifred Ingbian[1], Emmanuel Osoba[1], Ahmed Alebiosu[1], Angalee Nadesalingam[6], George Carnell[6], Nina Krause[6], Andrew Chan[6], Charlotte George[6], Paul Tonks[6], Ernest Aguinam[6], Nigel Temperton[7], Jonathan Heeney[6], Christian Happi[1,2]

[1]The Africa Centre of Genomics of Infectious Diseases (ACEGID), Redeemer's University Nigeria, Osun state, Nigeria. [2]Department of Biological sciences, Faculty of Natural sciences, Redeemer's University Nigeria, Osun state, Nigeria. [3]Ladoke Akintola University Teaching Hospital Osogbo Nigeria, Osun state, Nigeria. [4]Alex Ekwueme Federal University Teaching Hospital (AEFUTHA) Abakaliki Nigeria, Abakaliki, Nigeria. [5]Federal Medical Centre Owo, Nigeria, Owo, Nigeria. [6]Department of Veterinary Medicine, University of Cambridge, Cambridge, CB30ES, UK., Cambridge, United Kingdom. [7]Medway School of Pharmacy, The Universities of Greenwich and Kent Medway Anson Building Central Avenue Chatham Maritime Chatham Kent, ME4 4TB, UK., Kent, United Kingdom

Abstract

Given the pace of SARS-CoV-2 transmission and its relatively high mortality rate, COVID-19 has become the most severe pandemic in recent times. Nigeria with many densely populated cities and poor health infrastructure presents a unique situation for the explosive spread of SARS-CoV-2. ACEGID is at the centre of the genomic revolution in Africa having provided over 50% of SARS-CoV-2 genomic sequences from Nigeria. Taking advantage of the progress made in genomic surveillance on the continent, we established the Africa rapid immunosurveillance system for epidemic response (ARISE), with the aim to generate immunologic data to understand the impact of these emerging variants on both natural and vaccine-induced immunity. We used ELISA and/or Luminex/MAGPix bead-based multiplex antibody assays to screen sera for antibodies against antigens from different SARS-CoV-2 variants. Generating full-length spike genes for each of the sequenced variants enabled us to determine if immune sera could neutralise pseudotyped viruses expressing variant spike genes in real-time. Our results showed that while both convalescent and vaccine sera induced strong cross-reactive binding and neutralizing antibody responses to the circulating SARS-CoV-2 variants, sera from asymptomatic, seropositive participants did not bind or neutralised the Omicron variant. The overarching goal of this project is to contribute to new knowledge by generating immune data to match PCR-confirmed variants sera. This will inform governmental (NCDC, AfricaCDC) vaccine response policy. By combining genomic and immunologic technologies to combat SARS-CoV-2, we will have an informative platform that will inform African global health strategies to combat future infectious disease threats.

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Using whole-genome sequencing in the rapid response to SARS-CoV-2 in Aotearoa New Zealand

Rhys White ORCID iD[1], David Winter ORCID iD[1], ESR Genomics Team[1], COVID-19 WGS Team[1], Michael Bunce ORCID iD[1], Sam Carr[1], Jordan Douglas ORCID iD[2], Nigel French ORCID iD[3,4], Jemma Geoghegan ORCID iD[5,6], James Hadfield ORCID iD[7], Andrea McNeill ORCID iD[1], Matt Storey[1], David Welch ORCID iD[2], Joep de Ligt ORCID iD[1]

[1]Institute of Environmental Science and Research, Porirua, New Zealand. [2]The University of Auckland, School of Computer Science, Centre for Computational Evolution, Auckland, New Zealand. [3]Massey University, Tāwharau Ora/School of Veterinary Science, Palmerston North, New Zealand. [4]Institute of Environmental Science and Research, Te Niwha, Infectious Diseases Research Platform, Palmerston North, New Zealand. [5]Institute of Environmental Science and Research,, Porirua, New Zealand. [6]University of Otago, Department of Microbiology and Immunology, Dunedin, New Zealand. [7]Fred Hutchinson Cancer Research Centre, Seattle, USA

Abstract

Whole-genome sequencing has been critical for Aotearoa New Zealand's response to the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent behind the coronavirus disease 2019 (COVID-19) pandemic. Early in the pandemic, the rapid and distributed approach to sequencing made possible by Oxford Nanopore Technologies provided near-real-time data for contact tracing and supported the elimination of SARS-CoV-2 from New Zealand. While New Zealand's international borders were closed, sequencing of cases among returning travellers provided vital data on the transmission of SARS-CoV-2, including some of the most robust early evidence for transmission on aeroplanes and via aerosols indoors. As New Zealand moved out of the elimination phase to managing COVID-19 in the community, whole-genome sequencing became a key part of the surveillance of COVID-19. Here, we present a summary of the changing role of whole-genome sequencing for COVID-19 in New Zealand. We focus on how the early success of whole-genome sequencing in managing SARS-CoV-2 contributed to fundamental research and how the infrastructure developed early in the response is now being used to support surveillance of SARS-CoV-2 and other viruses.

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Detection of IgG antibodies titers against SARS-CoV-2 among Mexican population with Ad5-nCoV vaccine (CanSinoBio) and BNT162b2 vaccine (BioNTech/Pfizer)

Soraya Mendoza-Olazarán, Obed Medina-Macías ORCID iD

Department of Basic Sciences, Faculty of Medicine, University of Monterrey, Monterrey, Mexico

Abstract

Background

Addressing the limited published information regarding the immunogenicity of the CanSinoBio vaccine in our population, we analyzed immunological data of our faculty staff.

Materials

An observational study was performed including population of the northeast region of Mexico. A brief survey was performed, subsequently a comparison of SARS-CoV-2 spike 1-2 IgG antibody titers was carried according to the SARS-CoV-2 infection history.

Results

A total of 665 participants, age (IQR) 44 (36-52) years, 38.3% men, were included. From those, 72.2% were immunized with Ad5-nCoV, 15.8% received BNT162b2, and 12% of the participants received a different vaccine. The participants vaccinated with Ad5-nCoV showed a seropositivity proportion of 80.2%. Participants with a positive history of SARS-CoV-2 infection had a median (IQR) titer of antibodies of 804 (465-1250), those with a negative history had a median of 48.2 (17.22-146.75) (p=<0.001) after a median of 87 (62-91) days from immunization. In participants with BNT162b2 vaccine the median titer of antibodies was 1144 (387.5-2737.5) vs. a median of 602 (239-1030) in participants without a previous SARS-CoV-2 infection (p=0.011) after 54 (36.5-87) days from fully immunization.

Conclusions

A positive history of COVID-19 showed an induction of higher levels of SARS-CoV2 spike 1-2 IgG antibody titers. The Ad5-nCoV vaccine had a lower seropositivity and antibody titers however the participants with this vaccine had the longest time from the immunization.

P182

Differences in Airborne Stability of SARS-CoV-2 Variants of Concern is Impacted by Alkalinity of Surrogates of Respiratory Aerosol

Allen Haddrell, Mara Otero-Fernandez, Henry Oswin, Tristan Cogan, James Bazire, Jianghan Tian, Robert Alexander, Jamie Mann, Darryl Hill, Adam Finn, Andrew Davidson, Jonathan Reid

University of Bristol, Bristol, United Kingdom

Abstract

In this study we explored some of the underlying mechanisms hypothesised to be key drivers for the loss of viral infectivity whilst in the aerosol phase. A next generation bioaerosol technology was used to explore the aero-stability of the of several of the variants of concern of SARS-CoV-2. Viral infectivity was measured after aerosolization and levitation at high (90%), medium (65%) and low (40%) relative humidities (RH), at intervals over 40 minutes. When compared to the ancestral strain, the Delta variant infectivity displayed distinctly different decay profiles for the first 60 seconds, after which they became identical. At low RH, a loss of viral infectivity of ~55% was observed over the initial 5 seconds. Regardless of RH and variant, >95% of the viral infectivity was lost after 40 minutes of aerosolization. At time scales below 5 minutes the Delta variant was found to be less aero-stable than the Beta variant, which itself was less aero-stable than the Alpha variant and ancestral strain. Aero-stability of the variants correlated with their sensitivities to alkaline pH. Removal of all acid vapour dramatically increased the rate of virus infection decay, with 90% loss after 2 minutes of aerosolization. A model to predict loss of viral infectivity is proposed: at high RH, the high pH of the aerosol droplet drives the loss of viral infectivity; at low RH, the salt content limits the loss of viral infectivity. A similarity to the infectivity decay profiles in droplets consisting of artificial saliva and growth medium is confirmed.

P183

An ante-mortem assay to identify Jaagsiekte Sheep Retrovirus from whole blood samples

Kevin Purves ORCID iD[1], Chris Cousens ORCID iD[2], Nicola F Fletcher ORCID iD[1]

[1]University College Dublin, Dublin, Ireland. [2]Moredun Institute, Edinburgh, United Kingdom

Abstract

Ovine Pulmonary Adenocarcinoma (OPA) is an infectious lung tumour of sheep caused by Jaagsiekte Sheep Retrovirus (JSRV). Found in flocks across the UK and Ireland, OPA causes significant economic losses in affected flocks. There are currently no treatments or vaccines to mitigate infection. While thoracic ultrasound is currently the best available screening test for OPA in live animals, it requires highly trained veterinarians and a long-term approach to eliminate OPA from infected flocks. The primary sites of replication and neoplastic transformation of JSRV are Type II pneumocytes of the lung, resulting in transmission of virus mainly through close contact between animals. Viral nucleic acid has previously been detected in lymphocytes and monocytes/macrophages. Recent studies have indicated that viral nucleic acid is present in extracellular vesicles/exosomes within peripheral blood of retrovirus-infected individuals, including HIV infection. Due to the low abundance of JSRV proviral nucleic acid within peripheral blood, current PCR-based assays have poor sensitivity, particularly in early stages of infection, which has resulted in significant challenges to the development of diagnostic blood tests for pre-clinical OPA. We have designed improved PCR assays and liquid biopsy techniques with the aim of developing more sensitive and specific assays for diagnosis of OPA using a blood sampling approach. We will present the findings of a proof-of-principle assay using peripheral blood mononuclear cells and plasma-derived exosomes from experimentally- and naturally infected sheep.

P184

SARS-CoV-2 surveillance in UK wildlife

Shweta Shukla ORCID iD[1], Rebecca Shipley[1], Amanda H. Seekings[1], Ruth Cox[2], Alexander M.P. Byrne[1], Amber Martyn[1], Victoria Gould[1], Richard Delahay[2], Ian H Brown[1], Anthony R Fooks[1], Lorraine McElhinney[1], Sharon M Brookes[1]

[1]Virology Department, Animal and Plant Health Agency, Weybridge, United Kingdom. [2]National Wildlife Management Centre, Animal and Plant Health Agency, Woodchester Park, Nympsfield, United Kingdom

Abstract

Since the start of the COVID-19 pandemic there have been several instances of reverse zoonotic transmission. However, significant gaps remain in our understanding of the role of non-human species in the transmission of SARS-CoV-2, and the potential for wild animals to act as reservoirs and/or amplifying hosts. We conducted opportunistic sampling of wild mammals across the UK, using a network of contacts involved in wildlife management, rehabilitation, conservation and research. Swab samples collected from badgers (n=181), foxes (n=214), mink (n=18), grey squirrels (n=2), bats (n=84), stoats (n=11) and deer (n=40) tested negative for SARS-CoV-2 RNA by E-gene RT-PCR. Bat swabs were also tested by Pan-CoV PCR which yielded four positives (n=3 for alphacoronavirus in Daubenton's, Natterer's and Common pipistrelle bats and n=1 for betacoronavirus in a Brown long eared bat). Serum samples from a badger (1/251), a fallow deer (1/9), and a fox (1/4) tested positive by ELISA and virus neutralisation test (VNT) against delta and/or omicron variants. In addition, five of 251 (2.3%) badger sera samples tested non-negative by ELISA, but negative by virus neutralisation test (VNT) against delta and omicron variants. Serological investigations to identify the variants are underway. The results do not confirm active infection in these wild species, but they are consistent with exposure to SARS-CoV-2. Surveillance of wild species is ongoing. Identification of exposed and/or infected species can inform disease surveillance and risk management strategies.

P185

Characterisation of cytokine responses in hamster lung and nasal turbinate on SARS CoV-2 infection with Beta and Omicron variants.

Claire Ham, Sarah Kempster, Deborah Ferguson, Jo Hall, Neil Berry, Neil Almond

MHRA, Potter's Bar, United Kingdom

Abstract

The golden hamster (Mesocricetus auratus) model of SARS CoV-2 infection is frequently used in immunological studies. These studies are designed to test the breadth and persistence of vaccine or innate protection from subsequent infection. As different viral variants have emerged, differences in replication site and cell specificities in infected subjects have been noted.

Here we examine the innate transcription profile of hamster tissues from pairs of animals actively infected with beta and omicron variants using qPCR. We identify pathways that are differentially expressed in tissues and correlate these with blood cytokine levels. We compare with signatures seen in tissues some weeks after resolution of infection to attempt to recapitulate histochemical evidence of lung damage. Further resolution of lymphoid cells from these tissues will be elucidated using single-cell analysis.

Understanding those pathways differentially affected will improve modelling of human disease states and may establish cytokine correlates of protection and disease severity.

P186

In vitro analysis of the antiviral potential of peptides derived from Bothropstoxin-I against SARS-CoV-2

Tamara De Carvalho ORCID iD[1,2], Paulo Ricardo da Silva Sanches[3], Cintia Bittar[4,1], Andres Merits[5], Eduardo Maffud Cilli[3], Paula Rahal[1], Marilia de Freitas Calmon[1], Mark Harris[2]

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho", Sao Jose do Rio Preto, Brazil. [2]University of Leeds, Leeds, United Kingdom. [3]Universidade Estadual Paulista "Júlio de Mesquita Filho", Araraquara, Brazil. [4]Rockefeller University, New York, USA. [5]University of Tartu, Tartu, Estonia

Abstract

Given the global public health problem that SARS-CoV-2 still represents, the present work aimed to identify and investigate the potential of peptides derived from Bothropstoxin-I, a myotoxin from the Bothrops jararacussu snake, to inhibit SARS-CoV-2 infection. Eight synthetic peptides were analyzed, developed with modifications on their structure from a peptide sequence (MR1903) that had already been shown to possess antimicrobial activity. For this study, we determined the maximum non-cytotoxic concentrations for the peptides on Vero and BHK-21 cells, then investigated the activity in different phases of the virus replication cycle. Seven peptides showed acceptably low levels of cell toxicity and were taken forward for an initial antiviral trial. Four peptides (MC1937, MC1947, PE1940, and MR1903) exhibited antiviral potential, inhibiting up to 95% of SARS-CoV-2 infectivity. Time of addition studies showed that MC1937, MC1947, and PE1940 exhibited significant inhibition of both entry and post-entry events, together with virucidal activity. On the other hand, MR1903 demonstrated inhibition only for entry and post-entry events. By the results obtained so far, it is possible to conclude that four of the peptides selected present promising *in vitro* results as potential antivirals against SARS-COV-2. Further experiments to elucidate the mechanisms of action of the peptides will be presented.

P187

A SARS-CoV-2 replicon tool-box to investigate antivirals and intracellular viral replication structures.

Maximilian Erdmann[1], Maia Kavanagh Williamson Kavanagh Williamson[1], Tuksin Jearanaiwitayakul[1,2], Peter Wing[3], James Bazire[1], Luca Shytaj[1], David A Matthews[1], Andrew D Davidson[1]

[1]University of Bristol, Bristol, United Kingdom. [2]Mahidol University, Bangkok, Thailand. [3]University of Oxford, Oxford, United Kingdom

Abstract

Since SARS-CoV-2 emerged, studies characterizing the virus have been hindered by the necessity to work at containment level 3 (CL3), highlighting the need for a biosafe form of the virus ("replicon") that can be used for investigations at CL2.

SARS -CoV-2 replicons corresponding to Wuhan-Hu-1 and the Delta "variant of concern" were generated by replacing the Spike, Membrane and ORF6/ORF7a genes with puromycin resistance, Renilla luciferase (Rluc) or fluorescent reporter genes respectively. Electroporation of replicon and N-gene in vitro transcripts into permissive cells resulted in transient but not stable replicon replication. Replicon replication was assessed using Rluc activity assays, marker protein fluorescence and immunostaining.

Replicon replication in VeroE6 cells was inhibited by remdesivir at IC50 concentrations in the low-micromolar range, in line with assays using the parental viruses. In human cells such as Caco-2, the IC50 of remdesivir was in the low nanomolar concentration range, demonstrating a highly sensitive surrogate assay for virus replication. As the replicons lack Spike they can be used to identify antivirals that effect viral intracellular replication. This rationale was applied to investigate non-Spike mediated anti-viral effects of drugs including; Avasimibe, Cobicistat and Ritonavir. Using transient replicon replication assays, all three drugs were identified as inhibitors of intracellular replication. Comparison of replicon and parental virus replication complexes using correlative light electron microscopy and tomography is in progress.

The replicon system provides a useful toolbox to investigate inhibitors of SARS-CoV-2 replication as well as virally induced cellular ultrastructures at CL2.

P188

Regulation of influenza A virus and SARS-CoV-2 replication by N6-methyladenosine RNA methylation

Piotr Kwiatkowski, Michelle Holland ORCID iD, Chad Swanson ORCID iD

King's College London, London, United Kingdom

Abstract

N6-methyladenosine (m6A) is the most prevalent internal modification on eukaryotic mRNA. It influences nearly all aspects of mRNA metabolism, and thus functions as an important mechanism for post-transcriptional gene expression regulation. m6A methylation is also found on many viral RNAs. Interestingly, the presence of this modification on viral and cellular RNAs has been shown to have a significant effect on the replication of numerous viruses. These effects were proposed to be mediated through direct effects of m6A on viral RNAs as well as enhanced antiviral innate immune signalling and downstream gene expression in host cells depleted of the m6A methyltransferase METTL3/14. However, exactly how these processes are controlled by m6A remains poorly understood. Furthermore, there is conflicting evidence over the precise roles of m6A in influenza A virus (IAV) and SARS-CoV-2 replication. Here we employ a highly potent and selective small molecule inhibitor of METTL3/14 and show that global suppression of m6A addition restricts replication of IAV and SARS-CoV-2. Our preliminary data indicates that blocking type I interferon (IFN) signalling potently rescues IAV but not SARS-CoV-2 replication in cells with suppressed m6A methylation. This suggests that the type I IFN response is regulated by m6A and contributes to inhibiting IAV replication, while replication of SARS-CoV-2 is regulated by m6A though another mechanism. Collectively, these findings demonstrate that m6A modification is required for replication of the two unrelated respiratory viruses, potentially through distinct mechanisms.

P189

Generation of SARS-CoV-2 fluorescent reporter viruses for live cell analysis of virus replication.

Edward Sullivan, Maia Kavanagh Williamson, Maximillian Erdmann, James Bazire, David Matthews, Andrew Davidson

University of Bristol, Bristol, United Kingdom

Abstract

SARS-CoV-2 remains a worldwide health concern, and a major focus of research; particularly with the continual emergence of new variants.

Using a yeast based reverse genetics system we generated a panel of SARS-CoV-2 viruses expressing fluorescent reporters in place of non-structural open reading frames (ORFs) allowing the use of live cell imaging to visualise viral replication and spread. Initially replacements were introduced into ORFs 6, 7, and 8 of the parental Wuhan-Hu-1 virus, to test which of the gene replacements was most disruptive. Analysis of the growth kinetics of the viruses in Vero cells expressing TMPRSS2 (VTN) and A549 cells expressing ACE2 and TMPRSS2 (A549-AT) compared to the parental virus showed little difference in virus replication in VTN cells but ORF7 and 8 replacement viruses demonstrated attenuated replication in A549-AT cells. Transcriptomic analysis of A549-AT cells 18 hours post infection with either the parental or fluorescent reporter viruses has been done to investigate the basis of viral attenuation.

Recently, recombinant viruses containing the equivalent gene replacements in the backbone of the Omicron BA.5 variant of concern have been rescued, allowing direct comparison between these and the initial Wuhan strain.

In addition to basic replication studies, the fluorescent reporter viruses are being used for antiviral testing and analysis after initial benchmarking against the parental virus using remdesivir.

We believe that our fluorescent SARS-COV-2 viruses provide a valuable tool for continuing research into SARS-CoV-2, both in our understanding of the virus as an entity, but also in efforts to identify effective interventions.

P190

ANTI-SARS-COV-2 ACTIVITY OF FAC2: AN ACRIDONE-LIKE COMPOUND SYNTHESIZED BASED ON BRAZILIAN NATURAL SCAFFOLDS

Igor Andrade Santos[1,2], Victória Riquena Grosche[1,3], Rodrigo Paolo Flores Abuna[4,5], João Santana da Silva da Silva[4,5], Luis Octávio Regasini[6], Andres Merits[7], Mark Harris[2], Ana Carolina Gomes Jardim[1,3]

[1]Laboratory of Antiviral Research (LAPAV), Institute of Biomedical Sciences, Federal University of Uberlândia (UFU), Uberlândia, Brazil. [2]School of Molecular and Cellular Biology – University of Leeds, LS2 9JT, Leeds, United Kingdom. [3]São Paulo State University, IBILCE, São José do Rio Preto, Brazil. [4]Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil. [5]Fundação Oswaldo Cruz Bi-institucional, Riberão Preto, Brazil. [6]Laboratory of Antibiotics and Chemotherapeutics (LAC), Institute of Biosciences, Humanities, and Exact Sciences (IBILCE), São Paulo State University (Unesp), São José do Rio Preto, Brazil. [7]Institute of Technology, University of Tartu, Tartu, Estonia

Abstract

Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19 (Coronavirus Disease 2019), has spread around the world, causing more than 649 million confirmed cases and 6.6 million deaths. Despite vaccination, SARS-CoV-2 infection still poses a threat to individuals and public health systems due to both Long-COVID syndrome and the potential for emergence of new SARS-CoV-2 variants. Therefore, it is essential to identify antiviral molecules that can abrogate viral infection. The use of synthetic compounds based on natural scaffolds, such as those from the acridone family, has attracted attention due to their reported activities against several RNA viruses, the possibility of chemical modifications, large-scale production, and patentability. Here we evaluated the inhibiting potential of FAC2, an acridone synthesized based on a natural scaffold from Brazilian plants, against SARS-CoV-2 infection. Methods: FAC2 was screened for its cytotoxicity in BHK-21 and Vero E6 cells, and the highest non-cytotoxic concentration was selected to evaluate the effect on SARS-CoV-2 entry and replication using: i) a VSV-pseudotyped viruses expressing the SARS-CoV-2 S protein (VSV-S-SARS-CoV-2) at 100 pfu/well and ii) a SARS-CoV-2 subgenomic replicon. Results: FAC2 at 10 µM inhibited 47% of VSV-V-SARS-CoV-2 infection in Vero E6 cells, and inhibited 67.4% of SARS-CoV-2 subgenomic replicon at 50 µM in BHK-21 cells. Conclusion: Data suggest that FAC2 has the potential to inhibit the SARS-CoV-2 replicative cycle through different modes of action.

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X-ray irradiation inactivates SARS-CoV-2 variants, while retaining structural integrity of virus antigens.

Elle Campbell, Laura Bonney, Mollie Curran-French, Jane Burton, Ellie Drinkwater, Babak Afrough, Roger Hewson

UKHSA, Salisbury, United Kingdom

Abstract

The establishment of a robust and flexible workflow to quickly adapt and respond to new variants of concern (VOCs) is the key to protecting public health during pandemics. Utilisation of X-ray technology to inactivate SARS-CoV-2 variants provided an avenue to reduce reliance on cumbersome high containment facilities and increase throughput. Non-infectious whole virus stocks of high titre VOCs were produced on the commercially available MultiRad 225 X-ray irradiator using established methodology. All irradiated samples were confirmed non-infectious through three rounds of consecutive 1-week tissue culture passage in Vero E6 cells. No signs of cytopathic effect were seen in any flasks infected with irradiated material and no increase in genome copies was detected for irradiated samples through real-time PCR. Furthermore, irradiated samples were also seen to show no difference in LFD sensitivity compared to live stocks and inactivated whole virus particles were confirmed through TEM imaging. These results confirm that X-ray irradiation can preserve structural and antigenic integrity of samples, while producing inactivated material suitable for multiple downstream applications including antigen detection assays (our material was widely used for LFD development, validation and rollout during the pandemic response). This is a capability not shared with many other conventional inactivation techniques, except from gamma irradiation. However, unlike gamma radiation, X-ray sources do not suffer from the same security and costs associated with ‘live’ radiologic materials. Therefore, these results support the notion of X-ray irradiation being a safer and cheaper alternative to producing structurally pristine inactivated material within pandemic environments.

P192

Repurposing approved drugs as potent antiviral combinations to treat COVID-19 disease

Olivier Touzelet[1], Ahlam Ali[1], May Al Adwan[2], Jo Sharp[2], Jack Mellors[3], Ken Mills[4], Andrew Owen[2], James Stewart[2], Miles Carroll[3], Ultan Power[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom. [2]University of Liverpool, Institute of Translational Medicine, Liverpool, United Kingdom. [3]High Consequence Emerging Viruses Group, Pandemic Sciences Institute & Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. [4]Patrick G Johnston Centre for Cancer Research, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom

Abstract

Most antiviral treatments for Covid-19 are monotherapies administered when individuals are hospitalized with severe disease. These therapies do not address the global clinical unmet need of treating early mild to moderate disease, which would diminish disease progression and the impact of long-covid. Treating earlier in the disease course with new combinations of approved drugs at home is the UK government's preferred focus for identifying new covid-19 therapeutics. Novel drug combinations may enhance antiviral activities, diminish the risk of escape mutants, and potentially improve clinical outcomes compared to monotherapies.

From the literature, we identified 133 re-purposed drugs with significant antiviral activity against SARS-CoV-2 in pre-clinical testing. Our MuSIC platform was used to screen this customized library to identify synergistic interacting compounds with SARS-COV-2 antiviral activity. Five compounds per well were assigned by the algorithm and 'hits' which showed ≥90% inhibition in cytopathic effects (CPE) in Calu-3 and VAT (Vero overexpressing human ACE2 and TMPRSS2) cells were de-convoluted. CPE induced by SARS-CoV-2 Delta variant was determined 3 dpi using a cytotoxicity Assay.

Our results revealed 103 selected hits overlapped in the two cell lines, which consisted of 18 double and 162 triple combinations. Successful hits were characterized against 3 other variants (Alpha, Omicron BA.1 and BA.5), and IC50s/IC90s were determined in both cell lines. Optimal dosing ratios for the combination treatments were established. Hits were further ranked based on drug synergism and clinical profiling.

We identified potential combination therapies for SARS-CoV-2 and are currently preforming virology studies to validate promising combinations.

P193

Mutations in Spike gene of SARS-CoV-2 Omicron that confer altered tissue tropism and cell entry and determine attenuation, are distinct from those that encode antigenic distance

Jonathan Brown, Thomas Peacock, Jie Zhou, Wendy Barclay

Imperial College London, London, United Kingdom

Abstract

During the course of the COVID-19 pandemic new variants of SARS-CoV-2 have continuously emerged and some have come to predominate, earning them the designation of Variant of Concern (VOC). Epidemiological data allows us to observe the relative successes of distinct viral genotypes in the population but the phenotypic traits which determine this success have taken more time to elucidate. The ability of a novel SARS-CoV-2 variant to displace the dominant VOC results from a transmission advantage which may owe to any one or a combination of characteristics including enhanced escape from pre-existing immunity, more rapid replication in human host cells and altered cell tropism.

The Omicron BA.1 sublineage emerged in 2021 and rapidly displaced the Delta variant before itself giving way to BA.2, BA.5, XBB and BQ.1.1 sublineages.

We use SARS-CoV-2 virus isolates and recombinant viruses bearing VOC Spike proteins in the background of a precursor variant to show that the Omicron Spike determines a replication advantage over Delta in the human nasal epithelium but a disadvantage in deeper lung epithelial cells, Calu3. We also show with these recombinant viruses and with lentiviral pseudotypes that Omicron Spike, unlike Delta Spike, mediates cell entry via the endosomal route.

Importantly the genetic determinants of Omicron's endosomal cell entry pathway are mapped to the S2 domain of the Spike gene, whereas the mutations that confer its antigenic distance residue in S1. This means that future variants with large antigenic distance from current vaccines would not necessarily display the modest severity of Omicron.

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Comparison of antivirals molnupiravir and PF-07321332 either individually or in combination in immune supressed mice infected with SARS-CoV-2 as a model for severe COVID-19.

Rebekah Penrice-Randal ORCID iD[1], Eleanor G. Bentley[1], Adam Kirby[1], Parul Sharm[1], Anja Kipar[1,2], Daniele F. Mega[1], Chloe Bramwell[1], I'ah Donovan-Banfield[1,3], Xiaofeng Dong[1], Aleksandra M. Rzeszutek[1], Jack Pilgrim[1], Alistair Darby[1], Andrew Owen[4], Julian A. Hiscox[1,3,5], James P. Stewart[1]

[1]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]Laboratory for Animal Model Pathology, Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland. [3]NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom. [4]Department of Pharmacology and Therapeutics, Centre of Excellence in Long-acting Therapeutics (CELT), Liverpool, United Kingdom. [5]A*STAR Infectious Diseases Laboratories (A*STAR ID Labs), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore

Abstract

Immunocompromised patients with a SARS-CoV-2 infection are prioritised for treatment with anti-virals due their risk of developing severe COVID-19. These include compounds that indirectly target virus replication by causing hyper-mutation (molnupiravir) or directly inhibiting viral proteases critical to virus replication (paxlovid). Combination therapy may reduce the emergence of resistant genotypes, particularly in patients who are unable to clear the virus rapidly due to compromised immunity. Anti-virals used for immunocompromised patients may decrease viral loads but enhance genomic plasticity. To investigate this, the genomic variation of SARS-CoV-2 in the immunocompromised host was evaluated, in the absence and presence of single or combination anti-viral treatment with molnupiravir and PF-07321332 (a component of paxlovid). K18-hACE2 transgenic mice were artificially immunocompromised with cyclophosphamide prior to infection and treatment. Anti-viral treatment of infected immunocompromised mice, both individually and in combination, resulted in decreased viral loads and reduced pathology compared to untreated mice. Whole genome amplicon sequencing data confirmed the mechanism of action of molnupiravir, showing an increased transition mutation frequency in SARS-CoV-2 from mice treated with molnupiravir. No specific dominant genomic changes in SARS-CoV-2 were associated with individual or combination anti-viral treatment over the time-course tested. In contrast, mutational frequency was related to the site of sampling, with greater frequency of mutations in SARS-CoV-2 derived from lung or nasal tissue compared to throat swabs. Overall, during acute infection of an immunocompromised mouse model for COVID-19 there were no differences SARS-CoV-2 genomic plasticity observed between immunocompetent and immunocompromised hosts.

P195

UK International Coronavirus Network

Sharon Brookes ORCID iD[1], Paul Digard[2], Lisa Boden[3], James Wood[4], Erica Bickerton[5], Dalan Bailey[5], Julian Hiscox[6]

[1]Animal Plant Health Agency, Weybridge, United Kingdom. [2]The Roslin Institute, Easter Bush, United Kingdom. [3]The University of Edinburgh, Easter Bush, United Kingdom. [4]University of Cambridge, Cambridge, United Kingdom. [5]The Pirbright Institute, Pirbright, United Kingdom. [6]University of Liverpool, Liverpool, United Kingdom

Abstract

The UK International Coronavirus Network (UK-ICN) is a four year research and innovation network (2021-2025) bringing together researchers from animal and human coronavirus communities, funded by BBSRC and DEFRA, UK. Its aim is to provide and support global coordination for the delivery of collaborative scientific research and a sustained long term One Health approach. Global partners include Europe, China, India, North America, Southeast Asia, Middle East, Africa, Oceania with >200 members, join via links

UK-ICN@liverpool.ac.uk, https://www.liverpool.ac.uk/health-and-life-sciences/research/uk-international-coronavirus-network/member/

UK-ICN is led by Prof. Julian Hiscox at the University of Liverpool with co-directors from UK Universities and Institutes (authors). Our themes include: One Health and Zoonoses, Surveillance, Detection and Characterisation, Countermeasures and Interventions, Behaviour and Social Policy and SARS-CoV3 and the Future.

The aim is to provide a community gateway, including a network-of-networks and international bodies (FAO/OIE-WOAH/WHO) to facilitate and co-ordinate interactions focusing on animal-human-environment interface research, to foster cross-fertilisation of ideas, provide expert perspectives, to identify knowledge gaps, create research opportunities and build an evidence-based road map to ensure the sustainability of coronavirus research and disseminate and preserve knowledge to better combat future emerging coronaviruses.

We have a focus on early career scientist (ECS) development, identification of research gaps and future priorities, generating a roadmap (STAR-IDAZ; https://www.star-idaz.net/) for future priorities and actionable intelligence for policy makers.

Upcoming meetings:

International Conference on Livestock, Companion Animals and Wildlife Coronaviruses. 25-26th May 2023 Riddel Hall, Queen's University Belfast, UK.

Abstracts: 20th January, 2023

https://www.liverpool.ac.uk/health-and-life-sciences/research/uk-international-coronavirus-network/events/

Including a Microbiology Society Journals special collection:

https://www.microbiologyresearch.org/content/international-coronavirus-networks-collection-nbsp

P196

Effect of rapamycin on SARS-CoV-2 infectivity

Alisha Reid, Maria Lista Brotos, Rui Pedro Galao, Stuart Neil

King's College London, London, United Kingdom

Abstract

Manipulation of the cellular metabolic status is increasingly viewed as important for virus replication at multiple levels. Rapamycin and related inhibitors of the central metabolic kinases mTORC1 and mTORC2 have been shown to have pleiomorphic and opposing effects on SARS-CoV-2 depending on the experimental readout, cell line, drug dose, and clinical isolate. Here we explore the phenotypic basis for the effects of rapamycin.

Using both pseudotype lentiviral vectors expressing SARS-CoV-2 spike glycoproteins and replication competent SARS-CoV-2 the effect of rapamycin on viral titre, replication, and protein expression was investigated in several lung epithelial cell lines. Low-moderate rapamycin treatment at 2 nM – 2 mM enhanced SARS-CoV-2 Eng-02 titres released from A549-ACE2 cells by 13.9-fold. By contrast, there was either minimal or no significant titre enhancement in A549-ACE2-TMPRSS2, Beas2B-ACE2, or Calu3 cells, which all express high levels of the cell-surface serine protease TMPRSS2. Titre enhancement appeared independent of increased entry as rapamycin had no effect on the infectivity of pseudotype lentiviral vectors expressing SARS-CoV-2 spike glycoproteins, and could be observed with other SARS-CoV-2 variants of concern. While there was a minimal effect of viral RNA production in rapamycin treated cells, enhanced titre could however be attributed to increased protein expression of the SARS-CoV-2 structural proteins in supernatants, suggestive of a late stage effect on viral production modulated by TMPRSS2. We are currently investigating the replication stages of SARS-CoV-2 in more detail to define how rapamycin and mTOR regulate viral production and infectivity, and whether this is related to manipulation of autophagy-related pathways.

P197

Investigating the antiviral activity of volatile compounds from *Nigella sativa* against coronaviruses.

Gemma Cooper, Hesham F I Khodeir, Karrar Kamoona, Ahmed Alalaqi, Parvez I Haris, Maitreyi Shivkumar ORCID iD

Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom

Abstract

The recent emergence of three major coronaviruses and presence of coronaviruses circulating in bats suggests that spillover of new pandemic-potential coronaviruses into humans is likely in future. The development of pan-coronavirus antivirals will be crucial to combat this. Here, we investigated the antiviral activities of *Nigella sativa* (black cumin) oil extracts from various global locations against seasonal human coronaviruses OC43 and 229E, and SARS-CoV-2 pseudoviruses.

Coronavirus-infected cells were directly treated with oil extracts and antiviral activity determined by quantifying viral titres. In diffusion assays, oils were incubated in microwell plates with virus in adjacent wells to investigate the effect of diffused volatile compounds. After incubation over a range of times, infectivity was determined.

Our diffusion assay results indicate that volatile compounds present in *Nigella sativa* extracts show antiviral activities against coronaviruses, with no cytotoxic effect on cells. Significant inhibition of infection was observed after 12 hours incubation, with the most potent oils showing a ≥4 $\log_{10}$ reduction in OC43 infectivity at 24 hours. Interestingly, direct treatment of infected cells with oils showed limited antiviral efficacy, suggesting that the vapour phase may offer higher concentrations of the bioactive compounds without compromising cell viability. We also identified key volatile compounds present in the oil vapour phase; evaluation of the antiviral activity of these volatiles in isolation and in synergy are ongoing.

Overall, this work provides a first step towards identifying novel pan-coronavirus antiviral compounds that can be formulated as sprays or inhalers for direct delivery to the site of infection.

P198

Characterisation of cytokine responses in hamster lung and nasal turbinate on SARS CoV-2 infection with Beta and Omicron variants.

Claire Ham, Sarah Kempster, Deborah Ferguson, Jo Hall, Neil Berry, Neil Almond

MHRA, Potter's Bar, United Kingdom

Abstract

The golden hamster (Mesocricetus auratus) model of SARS CoV-2 infection is frequently used in immunological studies. These studies are designed to test the breadth and persistence of vaccine or innate protection from subsequent infection. As different viral variants have emerged, differences in replication site and cell specificities in infected subjects have been noted.

Here we examine the innate transcription profile of hamster tissues from pairs of animals actively infected with beta and omicron variants using qPCR. We identify pathways that are differentially expressed in tissues and correlate these with blood cytokine levels. We compare with signatures seen in tissues some weeks after resolution of infection to attempt to recapitulate histochemical evidence of lung damage. Further resolution of lymphoid cells from these tissues will be elucidated using single-cell analysis.

Understanding those pathways differentially affected will improve modelling of human disease states and may establish cytokine correlates of protection and disease severity.

P199

Multiple previous exposures to SARS-CoV-2 generates Omicron and Lineage-A spike-reactive IgG$^+$ memory B-cells but reduced IgA$^+$ memory B-cells

Richard Urbanowicz ORCID iD[1], Theocharis Tsoleridis[2], David Onion[2], Jessica Nightingale[3], Anthony Kelly[2], Ana Valdes[2], Benjamin Ollivere[2], Jonathan Ball[2]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom. [3]Nottingham University Hospitals NHS Trust and University of Nottingham, Nottingham, United Kingdom

Abstract

Protection from infection, following either vaccination and/or natural infection has diminished since the advent of Omicron and related variants. However, protection from serious disease has been maintained. In this study we analysed, using multi-parameter flow cytometry, the spike-specific B cell repertoire present in blood obtained from healthcare workers approximately three months following the third dose of BNT162b2 vaccine. These healthcare workers form part of the Panther study cohort, whose vaccination and infection status has been followed extensively since the outbreak of the pandemic and were divided into two groups: previously uninfected and infected. Our data show that although no significant differences were observed in sera IgG or IgA reactivity to Lineage-A or Omicron trimerized-spike, the donors had a significantly lower neutralisation potential against Omicron than Lineage A in a pseudotype system. The frequency of Omicron-spike reactive peripheral IgA+ B cells was significantly lower than Lineage A reactive ones, and the relative proportion of Omicron:Lineage A spike reactive IgG+ memory cells was much higher than IgA+ cells. B-cell subset analysis showed that reactivity was detected in both classical memory and double-negative (DN) B cell populations. Previous studies have shown good correlation between mucosal and peripheral IgA reactivity and specificity. Our finding provides evidence that antibody immunity to the recent variants of concern at the mucosa (IgA) is blunted compared to that in the peripheral (IgG) circulation. This could explain why vaccination or previous infection provides little protection from infection or reinfection, but why protection against serious disease is maintained.

P200

Evaluation of SARS-CoV-2 genomic stability in response to molnupiravir treatment in humans.

I'ah Donovan-Banfield ORCID iD[1,2], Rebekah Penrice-Randal[1], Hannah Goldswain[1], Aleksandra Rzeszutek[3], Jack Pilgrim[3], Katie Bullock[4], Geoffrey Saunders[5], Xiaofeng Dong[1], Yan Ryan[1], Helen Reynolds[6], Saye Khoo[6], Julian Hiscox[7], Thomas Fletcher[8], AGILE CST-2 Study Group[7,9,8,5,10,11,12,13,6]

[1]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom. [3]Department of Evolution, Ecology and Behaviour, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [4]GCPLab Facility, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom. [5]Southampton Clinical Trials Unit, Southampton, United Kingdom. [6]Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom. [7]University of Liverpool, Liverpool, United Kingdom. [8]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [9]University of Southampton, Southampton, United Kingdom. [10]NIHR Royal Liverpool and Broadgreen Clinical Research Facility, Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom. [11]NIHR Manchester Clinical Research Facility, Manchester University NHS Foundation Trust, Manchester, United Kingdom. [12]NIHR Lancashire Clinical Research Facility, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom. [13]NIHR Kings Clinical Research Facility, King's College Hospital NHS Foundation Trust, London, United Kingdom

Abstract

Molnupiravir is an antiviral, currently approved by the UK Medicines and Healthcare products Regulatory Agency (MHRA) for treating at-risk COVID-19 patients, that induces lethal error catastrophe in SARS-CoV-2. How this drug-induced mechanism of action might impact the emergence of resistance mutations is unclear. To investigate this, we used samples from the AGILE Candidate Specific Trial (CST)−2 (clinical trial number NCT04746183). The primary outcomes of AGILE CST-2 were to measure the drug safety and antiviral efficacy of molnupiravir in humans (180 participants randomised 1:1 with placebo). Here, we describe the pre-specified exploratory virological endpoint of CST-2, which was to determine the possible genomic changes in SARS-CoV-2 induced by molnupiravir treatment. We used high-throughput amplicon sequencing and minor variant analysis to characterise viral genomics in each participant whose longitudinal samples (days 1, 3 and 5 post-randomisation) passed the viral genomic quality criteria (n = 59 for molnupiravir and n = 65 for placebo). Over the course of treatment, no specific mutations were associated with molnupiravir treatment. We found that molnupiravir significantly increased the transition:transversion mutation ratio in SARS-CoV-2, consistent with the model of lethal error catastrophe. Additionally, the clinical findings showed that whilst molnupiravir was well-tolerated, the probability that molnupiravir was superior to placebo in reducing time to SARS-CoV-2 PCR negativity was 75.4% - less than the predefined 80% threshold for recommending large-scale evaluation. The genomic study complemented the primary clinical study and highlighted the utility of examining intra-host virus populations to strengthen the prediction, and surveillance, of potential treatment-emergent adaptations.

P201

Receptor binding domain antibodies predominantly contribute to total spike IgG responses elicited by COVID-19 vaccination

Bethany Hicks[1], Sasha Francis[1], Tom Coleman[1], Cathy Rowe[1], Susan Hopkins[2], Victoria Hall[3], Ashley Otter[1]

[1]UK Health and Security Agency, Porton Down, United Kingdom. [2]UK Health and Security Agency, London, United Kingdom. [3]UK Health and Security Agency, Lodon, United Kingdom

Abstract

Development of numerous COVID-19 vaccines has been an integral part in controlling SARS-CoV-2 transmission. Each vaccine elicits an immune response to the spike glycoprotein which contains the receptor binding domain (RBD). We aimed to determine the proportion of RBD and non-RBD spike-directed antibodies in vaccinated or previously infected individuals.

Serum samples from individuals enrolled in the SIREN study were selected for those with multiple BNT162b2 doses with no prior infection, or singular Wuhan or Alpha infections without vaccination. Samples underwent two consecutive depletions using SARS-CoV-2 RBD-coupled magnetic beads and were tested to determine SARS-CoV-2-specific total or IgG titres to several viral proteins.

With multiple rounds of RBD-antibody depletions, >97% of RBD antibodies were removed from sera of vaccinated individuals. In one-dose vaccinated individuals, there was a 91.9% (86.7-97.1) reduction in total spike IgG antibodies. Similar trends were observed in two-dose vaccinated individuals (78.5% (71.7-85.2)) whilst three-dose vaccinated individuals showed a lower reduction of 66.7% (59.6-73.8) after RBD depletion. However, sera from individuals with prior infection displayed a significantly lower percentage reduction in total spike IgG antibodies after RBD-antibody depletion: 51.2% (44.6-57.9) and 41.1% (27.6-54.5), respectively.

We demonstrate that antibodies induced by one or two vaccinations are primarily RBD-focused, with a marked reduction in total spike IgG antibodies after RBD depletion. In comparison, naïve and booster-vaccinated or previously infected individuals display significantly lower IgG reduction after depletion. This suggests that vaccine boosters or infection results in broad spike antibody responses, and that non-RBD antibodies should be considered in future vaccine development.

P202

Variant-specific antibody binding following SARS-CoV-2 infection enables VOC-specific serosurveillance

Hannah Selman[1], Hannah White[1], Bethany Hicks[1], Sarah Foulkes[2], Edward Monk[2], Ellie Cross[1], Silvia D'Arcangelo[1], Tom Coleman[1], Sebastian Millward[1], Maria Zambon[1], Polina Brangel[2], Cathy Rowe[1], Amanda Semper[1], Tim Brooks[1], Susan Hopkins[2], Victoria Hall[2], Ashley Otter[1]

[1]UK Health Security Agency, Porton Down, United Kingdom. [2]UK Health Security Agency, London, United Kingdom

Abstract

During the COVID-19 pandemic, numerous variants of concern (VOC) emerged: Alpha, Delta, and Omicron. Each has distinct mutations within the spike protein, with decreased neutralisation from vaccinated sera and differing antibody binding. Using serum samples from individuals with known VOC infection, we aimed to identify the infecting variant based on antibody binding in the absence of sequencing.

We performed multiplex serology to determine antibody responses in serum samples from VOC-infected individuals to the RBD and spike antigens. The data was analysed using receiver-operator curve analysis to determine optimum assay cut-offs, sensitivity, and specificity.

Using serum samples from ~1,000 individuals enrolled in the SIREN study with PCR-confirmed infection between October 2020-March 2021, we performed geographical and temporal analysis using serology to map the emergence of Alpha in South-East England.

Seroreactivity of individuals' antibodies to VOC-antigens was specific to their infecting strain, except for Omicron, likely due to prior infection confounding seroreactivity.

Alpha-specific seroreactivity accurately mirrored temporal changes of Wuhan/Alpha in national UK sequencing data. Seroreactivity combined with geographical and temporal data demonstrates the emergence of Alpha in South-East England and its eventual spread to other parts of the UK.

Additionally, using longitudinal samples from the SIREN cohort, we have been able to demonstrate prolonged seroreactivity to Wuhan, with Alpha-infected individuals maintaining Alpha-specific antibody binding after multiple subsequent Wuhan-based vaccinations.

This provides a valuable tool for discriminating between VOC infections based on antibody binding, enabling VOC-specific serosurveillance and aiding in the future investigation of immunology of prior infection.

P203

Understanding the role of ORF7a during a SARS-CoV-2 infection.

Sharon Flanagan, Gerald Barry ORCID iD

University College Dublin, Dublin, Ireland

Abstract

ORF7a is a small accessory protein of SARS-CoV-2 that is reported to carry out a number of functions in the cell during infection. To examine the role of ORF7a in detail confocal microscopy was used to examine its localisation in the cell. It localised to a number of areas including the endoplasmic reticulum and golgi. Different reports have suggested that it plays a role in the activation of the unfolded protein response but although the UPR is activated during SARS-CoV-2 infection, ORF7a was not found to contribute to this activation. To investigate the interaction of ORF7a with the innate immune system, ORF7a was expressed with a HA tag in the presence of virus and co-immunoprecipitation followed by mass spectrometry was used to identify protein-protein interactions between ORF7a and cellular proteins. We have further characterised the interactomes of ORF7a from different variants, identifying differences between them and their role in the control of the host innate immune response against infection.

P204

Longitudinal analysis of neutralizing antibody titres in a cohort of hospitalized and outpatients from the Czech Republic.

Martin Mayora Neto ORCID iD[1], Kelly da Costa[2], Tobia Fantoni[3], Mariana Pjechova[4], Donato Zipeto[3], Miroslav Fajfr[5], Andrew Miller[4], Daniel Růžek[6], Nigel Temperton ORCID iD[1]

[1]Viral Pseudotype Unit, Chatham, United Kingdom. [2]University College London, London, United Kingdom. [3]University of Verona, Verona, Italy. [4]Mendel University, Brno, Czech Republic. [5]Institute of Clinical Microbiology University Hospital, Hradec Kralove, Czech Republic. [6]Veterinary Research Institute, Brno, Czech Republic

Abstract

Background: COVID19, the disease caused by the SARS-CoV-2 virus, presents with a range of symptoms and outcomes, from asymptomatic to severe respiratory symptoms and death. Understanding the durability and breadth of neutralizing antibody (nAb) titres and its association with disease severity is important for future vaccine strategies. We performed a comprehensive screen of serum samples from hospitalized and outpatients from the Czech Republic.

Methods: We used a lentiviral pseudotype system to longitudinally assess neutralizing antibody responses in patients, who mostly had a history of infection, against the original Wuhan strain, variants of concern circulating at the time, Alpha and Delta, as well as Omicron BA2.75. Vaccinated patients received the main adenoviral (AstraZeneca and Janssen) and mRNA (Moderna and Pfizer) vaccines.

Results: Both outpatients and hospitalized patients generated robust neutralizing antibody titres, with some variation between vaccine regimens. mRNA based vaccines were usually associated with higher titres. Titres generally increased over time after boosters, across all vaccine platforms. Omicron BA2.75 was less susceptible to neutralization by serum, even though it maintained nAb titres over time. Wuhan PVs were more susceptible to neutralization by serum of healthy vaccinated individuals, followed by Alpha, Delta then BA2.75.

Conclusions: Our study shows the maintenance of nAb titres in patients with mild and more severe disease after a vaccination schedule across current and previous variants of concern. It is important to further elucidate differences in nAbs and other antibody functions and their association with disease outcomes.

P205

Neuropathology of SARS CoV2 Variants.

George Lagalle, Sarah Kempster, Neil Berry, Joanna hall, Claire Ham, Adrian Jenkins, Yann LeDuff, Matthew Hurley, Sarah Gilbert, Neil Almond, Debbie Ferguson

MHRA, South Mimms, United Kingdom

Abstract

As SARS CoV2 infections with increased capacity for upper respiratory tract replication have swept the world, the ability of viral variants to invade and impact brain function is of concern.

Syrian hamsters were infected intranasally with SARS-CoV2 Vic01 (Wuhan like) or variants beta, delta and omicron. Following termination 2, 10, 28 dpi, FFPE brain sections were analysed.

Immunohistochemical analysis did not reveal replicating virus within the brain following infection with any of the variants analysed.

Inflammatory responses (astrogliosis - GFAP, microgliosis – iba-1, blood brain barrier – ICAM1) were rapidly observed in all cases, peaking 10dpi.

Neuronal staining levels (dendrites - MAP2, mature neurons – NeuN) were reduced over this same timeframe.

Changes in staining patterns were also observed for oligodendrites (CNPase1), with elevated levels being present 2-10dpi.

In contrast, staining levels for myelin basic protein fell.

Reduced staining 2-10dpi was also observed for NMDA1, a neuronal receptor linked to synaptic plasticity, learning and memory functions.

Staining levels for BECN1, a key regulator of autophagy, increased 2-10dpi. Malfunctioning autophagy pathways have been implicated in development of neurodegenerative diseases however no evidence of Tau, alpha-synuclein or beta-amyloid were observed within this model or short timeframe assessed. Samples upto 112dpi are being analysed.

The impact on neuronal function at many levels and the ability of the brain to recover could explain the brain fog experienced by many following infection.

The observed impact on functions linked to neurodegenerative disease indicates SARS CoV2 may be another insult potentially pushing pathology towards longer term development of neurocognitive problems.

P206

Use of Air Sampling to Determine SARS-CoV-2 Variants within a Hospital

Stephen Bell ORCID iD[1], Shannon Venus[1], Dean Coey[1], John McGrath[1], Connor Bamford[1], Sara Hedderwick[2], Deirdre Gilpin[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Southern Health and Social Care Trust, Craigavon, United Kingdom

Abstract

Understanding the variants of SARS-CoV-2 and the location of infection outbreaks within a hospital environment could be pivotal in controlling the spread of health care associated SARS-CoV-2 infections, within the hospital and to the wider community. We investigated the use of UV-HEPA filters as a mechanism for cleaning air which can then be recirculated around the ward. To investigate if these filters can remove SARS-CoV-2 from the air we analysed a series of samples that were taken before and after the installation of the filters on a dedicated COVID-19 ward. We found that the SARS-CoV-2 gene copies per litre of air significantly reduced after the installation of the filters. Using Next Generation Sequencing, we detected multiple SARS-CoV-2 variants circulating in the ward. Indeed, these variants differed between bays located on the same ward on the same day. We detected spike protein mutations including K444T which have been shown to have reduced sensitivity to antibodies. Across the same time period that air samples were taken we sequenced wastewater samples from 25 sites across Northern Ireland for SARS-CoV-2. We found that the variants detected in the hospital were either not detected in wastewater or they preceded the date at which they were found in wastewater by up to one week. The findings in this study show that air sampling within hospitals could be an effective method for determining the variants that are hospital associated but also those that are leading to hospitalisations.

P207

SARS-CoV-2 Proteins and Their Effect on NF-κB Activation

Wejdan Albalawi

University of Liverpool, Liverpool, United Kingdom

Abstract

Viruses are rapidly evolving pathogens that adapt to host pressures that drive or dampen specific immune responses or modulate disease causing potential. Dysregulation of the immune response is one of the factors that contribute to the development and progression of SARS-CoV-2 infection. It was revealed that severity of inflammation is associated with impaired modulation of genes encoding proinflammatory factors by NF-kB. Therefore, over the last two years NF-kB signalling pathway has been extensively studied as a very important factor in the immune response in COVID-19. Our in vitro published studies demonstrate that HCV Env glycoprotein modulates HIV-1 LTR activity via activating host ER stress pathways known to inhibit NF-κB activity. Here, we have studied the effect of the SARS CoV2 structural proteins in modulating HIV-1 long terminal repeat (LTR) activation. Measuring the HIV-1 LTR activity in HEK293T and more physiologically relevant BEAS-2B human lung epithelial cell lines co-transfected with SARS CoV2 structural proteins and HIV-1 LTR promoter driving expression of a Luciferase report molecule demonstrated that the proteins down-modulate HIV-1 LTR activity. These findings suggest that SARS CoV2 proteins may modulate HIV-1 LTR activity via disrupting the NF-κB pathway. To explore the mechanism underling these effects, transcriptomic analysis in cells expressed SARS-CoV-2 structural proteins using ONT's MinION platform has been performed.

P208

ANALYSING A HYPERVARIABLE GENE IN FELINE LEUKEMIA VIRUS USING SANGER AND NEXT GENERATION SEQUENCING

Cristobal Castillo ORCID iD, Adam Blanchard, Rachael Tarlinton

University of Nottingham, Nottingham, United Kingdom

Abstract

Feline Leukaemia Virus (FeLV) is a gammaretrovirus of cats. FeLV is widespread among domestic cat populations and can produce a serious disease threat to domestic cats. The main consequences of FeLV infection are hematopoietic disorders and neoplasia. Domestic cats have both endogenous (enFeLV) (copies of virus in the cat genome) and exogenous (exFeLV) variants of FeLV, these frequently recombine producing variants with recombinant envelope genes and alternate receptor usage.

This study applied envelope gene PCR and NGS (Illumina and Nanopore) to compare and determine the envelope gene diversity and transmission dynamics of FeLV variants in Chilean domestic cats including sequences from FeLV-A, B and endogenous FeLV. FeLV-A sequences obtained from Sanger sequencing, are clustered in a Chilean group closer to sequences from the United States and Brazil.

Sequences obtained from NGS, showed a higher variability in the envelope gene, markedly in the C-Domain which may function as a second receptor-binding domain (Cano-Ortiz et al., 2022). Additionally, LTR sequences from enFeLV in Chilean cats showed diversity in mutation points and two main deletions in an indel point and in the poly(A) tract. The enFeLV Chilean sequences form their own phylogenetic clade located next to the previously described Group-I enFeLV which may indicate multiple introductions of enFeLV in domestic cats (Polani et al., 2010). The next step will be to apply Nanopore Sequencing to evaluate long fragments of FeLV-B to confirm the recombination points between FeLV-A and enFeLV that produce FeLV-B.

P209

A comparison of different variants of SARS-COV2; Severity of Omicron and delta-associated pathogenesis differs in a mouse model

Parul Sharma[1], Adam Kirby[1], Ellie Bentely[1], Ximeng Han[1], Anja Kipar[2,1], Daniele Mega[1], Rhiannon Johnson[1], James P. Stewart[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Zurich, Zurich, Switzerland

Abstract

Covid-19, caused by SARS-COV2 has affected worldwide human population and emergence of new variants of concern (VOC), challenged the treatment regimen as well as the vaccine development program. In this study, we aimed to compare the pathogenesis of different variants of SARS-COV-2, in context to omicron (B1.1.529) and other sub-lineages such as BA.2, BA.5, and Delta (B.1.672) which is highly pathogenic. We utilized 6-8 week old K-18 hACE2 mouse model to understand the pathogenesis of SARS-COV2

Mice were infected with 10^3 pfu of a different strain of SARS-COV-2 intranasal and monitored daily for weight loss or any possible respiratory symptoms. The oropharyngeal swabs were taken on 1, 3 and 5 days post-infection to identify the viral load by qPCR. On day 7 post-infection all mice were culled and lung and nasal turbinates' were harvested to quantify viral load by qPCR. Formalin fixed Lung and Brain tissue were analysed after H and E staining to see the virus antigen.

The data reveal that delta is the most pathogenic among them and infected mice lost more weight with a high viral load in swabs, lung, and nasal turbinates. In terms of omicron, BA.1, BA.2, and BA.5 show similar levels of viral load in different tissue. Although there was more viral load in the early days of infection in omicron compared to the delta. In conclusion, omicron lineage associated infection is less severe than delta and linked with recovery, unlike delta variant.

P210

The stimulation of leukocytes by the SARS-CoV-2 accessory protein ORF7a modulates increased binding to the endothelium.

James Hallwood ORCID iD, David Bates ORCID iD

University of Nottingham, Nottingham, United Kingdom

Abstract

Introduction

The SARS-CoV-2 virus has reshaped our understanding of pandemics in the 21st century. SARS-CoV-2 causes widespread inflammation across different organs and has recently been shown to contribute the endothelial dysfunction. ORF7a is speculated to facilitate leukocyte interactions due to its binding affinity to CD11/18 found on various leukocytes. We tested the hypothesis that stimulation by ORF7a would facilitate increased adhesion of monocytes to an endothelial cell layer.

Methods

The properties of recombinant ORF7a were tested in vitro using a monocyte-endothelial adhesion assay. HUVECs were seeded into 96-well plates, and primary monocytes were stimulated with concentrations of ORF7a, TNFa or an ORF7a/TNFa mix for 4 hours. Monocytes were tagged with calcein-AM and incubated with the HUVECs for a further 2 hours. Fluorescence was measured at 485/515nm excitation/emission.

Results

Monocyte adherence (measured by calcein fluorescence) increased by 395±11% when exposed to 10nM ORF7a for 4 hours (compared with cell only control, N=3). Monocytes exposed to a ORF7a/TNFa mix experienced a 314±21% increase in adherence (N=3).

Conclusions

These results demonstrate ORF7a's ability to activate monocytes and facilitate their binding to the endothelium. This could provide a mechanism through which SARS-CoV-2 can cause activation and inflammation independent of the spike protein.

P211

Investigating the antiviral activity of natural product derived compounds and synthetic xanthones against coronaviruses

Gemma Cooper[1], Bethanie Dean[1,2], Timothy J. Snape[1], Maitreyi Shivkumar ORCID iD[1]

[1]Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom. [2]Present address: Department of Chemistry, University of Warwick", Warwick, United Kingdom

Abstract

In the last 20 years three pandemic coronaviruses have emerged; with many more coronaviruses circulating in animal reservoirs, future spillover into humans is likely. The development of novel pan-coronavirus antivirals is vital to target new emerging coronaviruses and enhance future pandemic preparedness. Here, we investigated the antiviral activity of a panel of natural product compounds derived from *Swertia chirayita* for their anti-coronavirus activities.

A panel of compounds and synthetic xanthones based on *Swertia* bioactive compounds was assessed for antiviral activity against human coronavirus OC43 and 229E, and SARS-CoV-2 pseudoviruses. Infected cell lines were treated with the compounds and impact on infectivity assessed by quantifying viral titres. Alongside, cytotoxicity of the compounds was determined.

The initial screen identified six natural product compounds that display antiviral activity at non-cytotoxic concentrations. While the synthesised xanthones show a significant reduction in viral infectivity, the inhibition was not as potent as seen in the natural xanthone mangiferin. This suggests that the additional complexity of mangiferin and other naturally derived xanthones may contribute to their increased antiviral activities. Our preliminary data investigating the mechanism of action suggests that the hit compounds inhibit the virus at early stages in the viral lifecycle.

The six compounds identified are being carried forward for further investigations. With mechanism of action studies and viral target determination currently ongoing, this work is a step towards identifying novel pan-coronavirus antivirals.

P212

Investigation of the impact of NSP12 P323L substitution on SARS-CoV-2 infection

Muhannad Alruwaili[1,2], Stuart Armstrong[1], Tessa Prince[1], Andrew D. Davidson[3], David A. Matthews[3], Julian A. Hiscox[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Northern Border University, Arar, Saudi Arabia. [3]University of Bristol, Bristol, United Kingdom

Abstract

The mutational profile of SARS-CoV2 is variable across the viral genome and at a minor variant level. These mutations confer changes to transmissibility and pathogenicity. P-to-L amino acid substitution at the site 323 of SARS-CoV-2 polymerase (NSP12) is found in multiple variants after May 2020. However, the evolutionary importance of this mutation is poorly understood. LC-MS/MS was utilised to identify a shared interaction with the host between P323 and L323 as well as a unique interactome for L323. TRiC/CCT complex was identified as a binding partner of both NSP12P323 and NSP12L323 while STRN3 and PP2A were enriched in L323. The interaction of these proteins with P323 and L323 was validated by western blot analysis of the host proteins copurifying with NSP12. We report that inhibition of TRiC/CCT complex inhibited P323 and L323 replication in vitro in A549-ACE2 and Calu-3 cell lines. Furthermore, P323 and L323 exhibited reduced viral RNA synthesis in PP2A-depleted cells, although this effect was less prominent in L323. These findings may indicate that P323L is a host adapted substitution.

P213

Low affinity antibody prevents COVID herd immunity being an effective infection control strategy

Eleanor Parker ORCID iD[1,2], Myra McClure[1], Richard Tedder[1]

[1]Imperial College London, London, United Kingdom. [2]Synnovis, London, United Kingdom

Abstract

There has been much discussion on the general principle of "naturally-acquired" immunity from SARS-CoV-2 infection preventing reinfection, often referred to as Herd Immunity. Whilst recovered individuals have been shown to have high antibody titres, to date there has been no investigation of the avidity of this antibody. Low avidity antibody is unlikely to impact on the incidence of infection in the population, or indeed protect the individual.

A competitive enzyme linked immunoassay (Comp EIA), utilising a labelled neutralising monoclonal antibody binding to solid-phase receptor binding domain (RBD) to detect and characterise antibody to RBD, was developed to compare the performance of post-infection and post-immunisation responses.

We collected sera from recovery patients, taken up to 32 weeks from illness onset and from immunised persons, taken between 14 and 30 days after a second dose of vaccine and compared the inhibition displayed in the Comp EIA with the level of total anti-RBD detected in the Imperial hybrid double antigen binding assay. The level of inhibition of post infection antibody was significantly poorer than that of post vaccine antibody, despite both sets of samples displaying similar levels of anti-RBD. This indicated that post-vaccine antibody appears to have significantly greater avidity that post-recovery antibody.

This is the first time this disparity has been shown and suggests that 'natural immunity' is unlikely to impact on transmissibility of SARS-CoV-2. This highlights the importance of wide-spread immunisation for the control of SARS-CoV-2, rather than relying only on the concept of herd immunity protection, especially in health-care settings.

P214

Characterization Of Sars-Cov-2 Viral Variants Circulating In Brazil During Covid-19 Pandemic.

Isadora Correa, Gustavo da Silva, Romario de Souza, Pedro Telles, Anna Vianna, Marcela Cunha, Luciana da Costa

Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract

In Brazil, the complex dynamic of SARS-CoV-2 variants was characterized by the emergence of local VoCs such Zeta and Gamma besides the VoCs that circulated worldwide. These VoCs were related to increased infections reported in the country. We compare VOCs that circulated in Brazil throughout the pandemic upon replication kinetics, viral protein contents and the modulation of the mTOR pathway. Viral variants B.1.1.33, Zeta, Gamma, Delta, and Omicron were used to infect monkey Vero E6, Vero/hACE-2/hTMPRSS2 and the human pulmonary Calu-3 cells and the replication kinetics was evaluated. Our results indicate that although the Omicron VoC replicated to similar levels as the previous VoCs in Vero E6 and Vero/hACE-2/hTMPRSS2 cells, it had the lowest replication in Calu-3 cells up to 48 hours post infection. Delta VoC presented the highest replication rate in Calu-3 and induced the highest production of both S and N viral proteins. A direct competition assay confirmed the lower fitness of Omicron since the frequency of genomic RNA of this variant detected by NGS reached less than 10% when compared to the Gamma and Delta VoCs even when Omicron was used 9 times more for Calu-3 infection. SARS-CoV-2 variants differentially modulated mTOR phosphorylation over time with the absence of phosphorylation for B.1.1.33 variant, while the Omicron VoC did not alter mTOR phosphorylation levels when compared to uninfected cells. Our results demonstrated that SARS-CoV-2 VoCs have different replication capacities in lung cells and that the differential modulation of the mTOR pathway could be related to these differences.

P215

Assessment of the impact of SARS-CoV-2 variants on immune escape and species tropism in determining sustained virus transmission

Joseph Newman[1], Nazia Thakur[1], Ahmed Elreafey[1], Thomas Peacock[2], Wendy Barclay[2], Bryan Charleston[1], Dalan Bailey[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

The COVID-19 pandemic which continues to cause significant mortality and morbidity has been sustained by the evolution of new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These new variants exhibit changes to important viral properties including immune escape and species tropism.

We sought to measure the degree of immune escape exhibited by SARS-CoV-2 variants using sera from a cohort of vaccinees aged 50-89 collected longitudinally following each of their vaccine doses. Using pseudotype-neutralisation assays, we observed that early vulnerabilities in the breadth of response to antigenically diverse variants were overcome following multiple vaccine doses. Individuals with prior SARS-CoV-2 infection (Nucleoprotein ELISA positive) had markedly higher neutralising antibody titres against all variants tested, including against antigenically distinct Omicron sub-lineages.

Evolution of the spike protein away from neutralising antibodies has had an impact on the ability of variant spike proteins to bind to the angiotensin-converting enzyme 2 (ACE2) receptor. We quantified these impacts by measuring the ability of pseudo- and live viruses to enter cells expressing ACE2 receptors from different species, and by measuring the binding of recombinant spike and ACE2 proteins. We show that sequential virus variants have generally increased affinity for human ACE2 and have overcome restrictions to ACE2 receptor usage for some species which can be attributed to specific mutations. This differential tropism and potential for reverse zoonosis poses a risk to the ongoing maintenance and evolution of SARS-CoV-2 in animal reservoirs, which may pose a future risk to human health.

P216

Development and validation of serological assays to SARS-COV-2 for cats and establish seroprevalence in Pakistan

Qaiser Akram[1], Ahsan Naeem[2], Waqas Ahmad[3]

[1]University of Veterinary and Animal sciences, Lahore, Lahore, Pakistan. [2]UVAS, Lahore, Lahore, Pakistan. [3]UVAS, Lahore, Pakistan

Abstract

SARS-CoV-2 is an enveloped, positive-sense RNA virus that is known to interact with the angiotensin-converting enzyme-2 (ACE2) receptor of several species, including humans, cats, dogs, minks, ferrets, deer, and others. However, the similarities in the ACE2 receptors of coronaviruses have sparked the question of which animal species are susceptible to SARS-CoV-2, and capable of viral transmission. Reverse-zoonosis has been described in a few of these species, indicating that human-animal interactions can pose two-sided risks for SARS-CoV-2 infection. Therefore, it is critical to produce accurate diagnostic serological tests for animals to understand prevalence of the virus in the animal population. Cats have been shown to be highly susceptible to SARS-CoV-2 by experimental inoculation and multiple natural infection. Experimental infection of cats demonstrated that cats produce neutralizing antibodies against SARS-CoV-2 as early as 10 days post infection and gain protective immunity against re-infection 3 weeks after primary infection.Cats sampled at regular intervals over the course of 110 days post infection showed that both S-RBD antibodies and neutralizing antibodies peaked at 20 days post-exposure and decreased continuously until day 110. These results show that although SARS-Cov-2 antibodies were produced at a high level, the peak titer was reached already 3 weeks post infection which may imply that convalescent cats at least two weeks post-inoculation are vulnerable to re-infection. This pattern has also been reported in human SARS-CoV-2 patients, strengthening the claim that cats can be used as a model for human SARS-CoV-2

P217

COVID-19 Vaccine Acceptance in Nigeria: A Rapid Systematic Review and Meta analysis

Victory Nnaemeka ORCID iD[1,2], Nnena Okafor[1], Anthony Ike ORCID iD[1], Ruth Anikwe[1]

[1]University of Nigeria, Nsukka, Nsukka, Nigeria. [2]Imperial College London, London, United Kingdom

Abstract

Vaccine hesitancy is a complex phenomenon that interferes with the efficiency of any vaccination programme. This study aimed to collect information on Nigeria's COVID-19 vaccine uptake rates and determinants. Science Direct, PubMed, Google Scholar, African Journal Online, Springer, and Hinari were all systematically searched through and completed in May 2022. Quality assessments of the listed studies were performed using the eight-item Joanna Briggs Institute Critical Appraisal tools for cross-sectional studies. We undertook a meta-analysis to calculate pooled acceptance rates with 95% confidence intervals (CI). Forty-two studies in total satisfied the inclusion criteria and were reviewed. A total of 24,533 respondents were studied. The total sample size of states in the Northern, Western and Southern parts of Nigeria were 3,206, 4,527 and 5,059, respectively, while 11,741 is the cumulative sample size of the Nigeria-wide studies. The average COVID-19 vaccine acceptance rate among all pooled study groups is 52.4% (95% CI: 46.9-57.9%, I 2 = 100%), while the average COVID-19 vaccine hesitancy rates is 47.81% (95% CI: 42.2 – 53.4% I 2 = 100%). In the sub-group analyses, the Western region (58.90%, 95% CI: 47.12–70.27%) showed the highest vaccine acceptance rate. COVID-19 vaccine acceptance rate was highest in 2020, with a pooled rate of 59.56% (46.34, 57.32%, I 2 = 98.7%). The acceptance rate in 2021 was only 48.48 (40.78%, 56.22%) while by May 2022, it spiked to 52.04% (95% CI: 35.7%, 68.15 %). The design of interventions to encourage vaccine uptake must be local to mitigate negative perceptions and safety concerns.

Session Topic: Microbes and their metabolites underpinning host-microbe interactions

P218

PD-L1 negatively regulates antifungal immunity by inhibiting neutrophil release from bone marrow

Yu Yao, Rongrong Wang, Xinming Jia

Tongji University, Shanghai, China

Abstract

Programmed death ligand 1 (PD-L1) has been shown to be inducibly expressed on neutrophils to suppress host immunity during polymicrobial sepsis, virus and parasite infections. However, the role of PD-L1 on neutrophil-mediated antifungal immunity remains wholly unknown. Here, we show that the expression of PD-L1 on murine and human neutrophils was upregulated upon the engagement of C-type lectin receptor Dectin-1 with its ligand β-glucans, exposed on fungal pathogen Candida albicans yeast. Moreover, β-glucan stimulation induced PD-L1 translocation into nucleus to regulate the production of chemokines CXCL1 and CXCL2, which control neutrophil mobilization. Importantly, C. albicans infection-induced expression of PD-L1 leads to neutrophil accumulation in bone marrow, through mediating their autocrine secretion of CXCL1/2. Furthermore, neutrophil-specific deficiency of PD-L1 impaired CXCL1/2 secretion, which promoted neutrophil migration frombone marrow into the peripheral circulation, thereby conferring host resistance to C. albicans infection. Finally, either PD-L1 blockade or pharmacological inhibition of PD-L1 expression significantly increased neutrophil release from bone

marrow to enhance host antifungal immunity. Our data together indicate that activation of Dectin-1/PD-L1 cascade by β-glucans inhibits neutrophil release from bone marrow reserve, contributing to the negative regulation of antifungal innate immunity, which functions as a potent immunotherapeutic target against life-threatening fungi infections.

P219

Extracellular vesicles shaping the colorectal cancer microbiome

Rawan Maani, Sarah Forbes, Melissa Lacey

Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Background: the human gut microbial ecosystem (gut microbiota) plays a vital role in the regulation of various physiological processes, and alterations in the composition and function of the system (dysbiosis) are associated with the pathogenesis of colorectal cancer (CRC). Although the causative link between CRC and microbiota is widely investigated, the underlying microbiota-gut interactions are not well understood yet. It is evident that extracellular vesicles (EVs) derived from CRC cells have an impact on various oncogenesis processes, however, their impact on the surrounding microbiota is not clear. Therefore, we hypothesise that EVs could have an impact on the microbiota and contribute to dysbiosis. Methods: two CRC cell lines (SW480, SW620) were cultured in CELLine AD 1000 bioreactor flasks, EVs were isolated from culture media by size-exclusion chromatography and characterised by nanoparticle flow cytometry, western blotting, and transmission electron microscopy (TEM). The impact of the EVs on the bacterial MG1655 E. coli and 11G5 E. coli (CRC-associated strain) phenotypic characteristics (growth curve, biofilm formation) was assessed. Flow cytometry and TEM were performed to assess the interactions between EVs and E. coli strains. Results: NanoFCM analysis showed a high yield of EVs with characteristic size profile. TEM analysis indicated an interaction between the EVs and E. coli with clear surface binding, and EV treatment had an impact on bacterial phenotypic characteristics; an increase in E. coli growth and a decrease in the ability of the bacteria to form biofilm were shown. Overall, EVs appeared to be capable of mediating CRC-microbiome interactions.

P220

Cross-kingdom pathogen interactions: *Candida albicans* and *Pseudomonas aeruginosa* an *in vivo* infection assay in an insect model

Alejandra Esparza Mora, Lisa Kahl, Markus Ralser

Charité-Universitätmedizin Berlin, Berlin, Germany

Abstract

There are many examples of the interaction between prokaryotes and eukaryotes. One example is the polymicrobial infection by the opportunistic pathogenic yeast *Candida albicans* and the ubiquitous bacterium *Pseudomonas aeruginosa*. During such polymicrobial infections, interactions between the different microbial species can alter host responses and/or microbial virulence and pathogenesis, often complicating patient treatment and resolution of the infection. *In vitro*, the interaction between *C. albicans* and *P. aeruginosa* is characterised as being competitive and antagonistic. Their interaction *in vivo* with a host with an active immune system remains poorly understood. In this study, we examine the mechanisms of the interaction between *C. albicans* and *P. aeruginosa* using the *Galleria mellonella* insect model to show the effect of polymicrobial infection on virulence and antimicrobial resistance. We find that plymicrobial infection is more lethal to *G. mellonella* than the respective mono-infections. However, *P. aeruginosa* mono-infection can also cause high mortality of *G. mellonella* larvae. We examined the bacterial and fungal burden in *G. mellonella* and *C. albicans* burden was increased 24 hours after polymicrobial infection. Together these observations suggest that, *C. albicans-P. aeruginosa* cross talk *in vivo* can benefit both organisms to harm the host. Understanding the complex interplay between *C. albicans-P. aeruginosa* may lead to the development of novel therapeutic strategies for impeding polymicrobial disease.

P221

Microbial Bile Acid Conjugation as a New Mechanism of Host-Microbiome Crosstalk

Robert Quinn ORCID iD, Douglas Guzior

Michigan State University, East Lansing, USA

Abstract

Through 170 years of bile acid chemistry research, our knowledge of mammalian bile acid conjugation was limited to the amino acids glycine and taurine. We recently discovered that our gut microbiome also conjugates bile acids with a variety of other amino acids, greatly increasing the diversity of the bile acid pool. These microbially conjugated bile acids (MCBAs) are produced by a multitude of gut anaerobes with broad amino acid and bile acid substrate specificities. These molecules are prevalent in humans, particularly those with Crohn's disease, but their role in mammalian GI health is poorly understood. MCBAs can also act as signaling molecules through interaction with human gut receptors such as FXR. Recently, we discovered that the reverse action of the enzyme bile salt hydrolase (BSH) as a transferase is responsible for MCBA production. BSH enzymes from various gut bacteria have different amino acid conjugation profiles dictated by the structure of their active site. To better understand the role of host and microbial bile acid conjugation we have leveraged a knockout mouse model devoid of host-conjugated bile acids to better understand how MCBAs alter GI metabolism. Preliminary data from the mouse model shows that bile acid conjugation is important for early life development in mammals and MCBAs contribute to this. The discovery of MCBAs represents a sea change in our understanding of human bile acids and further evidence that our microbiome is intricately linked to our physiology by the production of small molecules.

P222

Identifying transient and stable bacteria-metabolite interactions from longitudinal multi-omics data

Dan Zhang[1], Benjamin Mullish[2], Jinfeng Wang[3], Grace Barker[4], Despoina Chrysostomou[4], Shengtao Gao[3], Lu Chen[5], Julie McDonald[6], Julian Marchesi[4], Lu Cheng[7]

[1]Key Laboratory of Birth Defects and Related Diseases of Women and Children of MOE, Department of Laboratory Medicine, State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, China., Chengdu, China. [2]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, St Mary's Hospital Campus, Imperial College London,, London, United Kingdom. [3]College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, China. [4]Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, St Mary's Hospital Campus, Imperial College London, London, United Kingdom. [5]Key Laboratory of Birth Defects and Related Diseases of Women and Children of MOE, Department of Laboratory Medicine, State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu, China. [6]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom. [7]Department of Computer Science, Aalto University, Espoo, Finland

Abstract

Background

Understanding the complex relationships between bacteria and metabolites in ecological systems are extremely important in studies of different microbiomes. Longitudinal multi-omics study is adopted to investigate interactions between bacteria and metabolites, by directly associating their longitudinal profiles. Since a bacteria/metabolite may involve in many different biological processes, the longitudinal profile is an average of different interactions. Therefore, direct association could only uncover the strongest interactions.

Results

Here we present a computational approach that can rebuild short- and long-term bacteria-metabolite interactions from longitudinal multi-omics datasets. For this task, we re-analyse data (both microbial sequencing and metabolomic analysis) from an in vitro model of Clostridioides difficile infection and faecal microbiota transplant, a disease state and mode of therapy in which perturbed microbiome-metabolome interactions (and their reversal) are well-established to be pertinent. By analysing such a dataset, we generated both a short-term and a long-term interaction network, which predicted many new interactions. Four new interactions were randomly selected to be validated. In batch culture experiments, we validated two of them: (1) Ruminococcus gnavus and Ruminococcus luti could generate 3-ketocholanic acid (2) Blautia obeum could consume succinate.

Conclusions

The deconvolution of the raw longitudinal signal into short- and long-term trends can help users to gain a deeper understanding of their data. This tool will be useful for high-throughput screening of microbe/metabolite/host interactions from a longitudinal multi-omics setting.

P223

Early-life gut bacterial diversity in Thoroughbred racehorses is associated with disease risk and athletic performance later in life.

Joy Leng ORCID iD[1], Carla Moller-Levett[1], Robert Mansergh[2], Ruth O'Flaherty[3], Rachael Cooke[1], Patrick Sells[4], Charlie Pinkham[5], Oliver Pynn[6], Richard Ellis[7], Falko Steinbach ORCID iD[1,7], Alexessander Couto Alves ORCID iD[1], Roberto La Ragione[1], Chris Proudman ORCID iD[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University College London, London, United Kingdom. [3]Sunbeam Veterinary Hospital, Cork, Ireland. [4]Orbital Veterinary Services, Cobham, United Kingdom. [5]Pinkham Equine Veterinary Services, Salisbury, United Kingdom. [6]Rossdales Equine Practice, Newmarket, United Kingdom. [7]Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

The equine gut microbiota has been shown to be linked to the health status of the horse. However, it is not yet clear how differences in the development of the equine gut microbiota could impact the health and performance of horses in later life. To investigate this, 52 foals, born at five different Thoroughbred studs based in the UK, were enrolled in this study and nine faecal samples were collected from each foal in the first year of life. Weekly health updates were obtained for the first three years of the foal's lives and subsequent racing performance was measured as earnings, placings and official ratings. Bacterial DNA was extracted from all faecal samples, submitted for 16S rRNA gene sequencing and the resulting sequencing data analysed using QIIME2. The relationship of bacterial diversity and family abundance with time to first health event and measures of racing performance were explored using cox and glmm regression models. Samples from the first month had low diversity with a higher relative abundance of Enterobacteriaceae and Fusobacteriaceae bacteria. However, by three months the bacterial diversity and composition of the bacterial communities was similar to that of adult horses. Higher bacterial diversity was associated with a reduction in the risk of respiratory disease and a higher official race rating later in life ($p < 0.05$ for all diversity measures used). This study demonstrates that low bacterial diversity in the equine microbiota at one month old impacts both health and racing performance of the horse later in life.

P224

Exploring the associations between the gut microbiota and breast cancer

Nancy MY Teng ORCID iD[1,2], Matthew J Dalby[1], Raymond Kiu[1], David Seki[3], Stephen D Robinson[1,4], Lindsay J Hall[1,3,5]

[1]Gut Microbes and Health, Quadram Institute Bioscience, Norwich, United Kingdom. [2]Faculty of Medicine and Health, University of East Anglia, Norwich, United Kingdom. [3]Intestinal Microbiome, School of Life Sicences, ZIEL, Institute for Food & Health Technical University of Munich, Freising, Germany. [4]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom. [5]Faculty of Health and Medicine, University of East Anglia, Norwich, United Kingdom

Abstract

Sequencing advancements are allowing researchers to probe into microbial community in association with diseases. Recently, breast cancer (BC) has shown to have a microbial signature associated with disease status and/or treatment outcomes.

To investigate the gut microbiota in BC we established the BEAM study, a longitudinal study following newly diagnosed BC-patients in Norfolk throughout their treatment. Initial analysis indicates a shift in microbiome profiles post-surgery, with *Faecalibacterium*, *Bacteroides* and *Blautia* genera dominating. Using cultureomics we established an isolate bank with 50 different species to use for assessing immunomodulatory effects on cancer cells.

Concurrent to BEAM, we analysed the oral and gut microbiota data from the KELLY study, a phase 2a clinical trial (NCT03222856) looking at the safety and efficacy of Pembrolizumab with eribulin in metastatic hormone receptor positive (HR+) BC patients. Overall P+E did not cause significant gut or oral microbiota perturbations, indicative of drug-related microbiota toxicity. Dominant gut genera included: *Bacteroides* and *Faecalibacterium*, with a common oral microbe *Prevotella*, also present. LefSE analysis suggested clinical benefit was driven, in part, by gut-associated *Bacteroides fragilis* and oral-associated *Streptococcus* with an abundance of ≥40%.

A BEAM isolate matched the metagenome-assembled genome of *B. fragilis* in the KELLY study, which was used to further probe into potential mechanistic pathways of *B. fragilis* on BC cells in vitro. Preliminary findings suggest potential avenues for downstream microbiota patient stratification before commencement of treatment. Further investigation is required in larger cohorts and any findings further validated using in vitro assays.

P225

Characterising the mandrill scent-gland microbiome and its potential role in olfactory communication

Daniel Lewis ORCID iD[1], Leena Kerr ORCID iD[2], Sharon Kessler ORCID iD[3], Barthélémy Ngoubangoye ORCID iD[4], Jo Setchell ORCID iD[1]

[1]Durham University, Durham, United Kingdom. [2]Heriot-Watt University, Edinburgh, United Kingdom. [3]University of Stirling, Stirling, United Kingdom. [4]Centre Interdisciplinaire de Recherche Médicale de Franceville (CIRMF), Franceville, Gabon

Abstract

Olfactory communication uses the release and detection of semiochemicals (chemical signals) which convey information about the releasing individual's state. How signals affect behavioural responses, such as avoidance, attraction, or individual recognition, has implications for survival and reproduction. Animals harbour a diverse array of microorganisms both on and in their bodies, many of which have co-evolved with the host to facilitate metabolic pathways that the host alone would be unable to process. Microbes inhabiting glands may digest secretions, producing volatile organic compounds which act as odour cues and contribute to scent; this is the fermentation hypothesis. Mandrills (*Mandrillus sphinx*) are one of the few African monkey species to possess scent-glands, in both males and females. This project will determine the composition of mandrill scent-gland bacterial communities and how these differ with host-specific traits such as age, sex and social rank, as well as with the amount of glandular secretions. We collected skin swab samples from the sternal-glands of mandrills living in a large semi-free ranging mandrill colony in Franceville, Gabon. From these samples, we will isolate genomic DNA, then use 16s rRNA amplicon sequencing and bioinformatic analyses with QIIME2 to determine the bacteria and archaea present. This first description of an African monkey's glandular microbiome using high-throughput Illumina sequencing methods will test the fermentation hypothesis, and allow greater inference into the potential for microbiota to mediate signals used in mate choice (sexual selection) and individual recognition (facilitating kin selection of related animals), and the co-evolutionary consequences of this symbiosis.

P226

Bespoke formulation of *F. prausnitzii* for future therapeutic use

Hansini Abeysekera, Linh P Ta, Sarah Corrigan, Richard Horniblow

University of Birmingham, Birmingham, United Kingdom

Abstract

Background:

Faecal Microbial Transplantation (FMT) is proving revolutionary in reversing colonic dysbiosis in many disease settings, including recurrent *Clostridium difficile* infection. However, issues surrounding FMT limit its clinical longevity. Consequently, there is a need to develop effective oral probiotic formulations to enable the introduction of bio-therapeutic bacterial strains into the intestinal microbiome.

Methods:

Faecalibacterium prausnitzii (*F. prausnitzii*), as a recognised anaerobic probiotic bacteria, was utilised as a candidate strain in these studies. O2 tolerability was evaluated using established CFU assessments along with LIVE/DEAD fluorescent measurements. The impact of bile-acids and gastrointestinal environment changes (using standardised INFOGEST protocols) was examined. The prebiotic nature of candidate formulation materials was also verified in growth studies.

Results:

F. prausnitzii was confirmed as a strict anaerobic bacteria, with even less than 1 hour exposure to oxygen significantly limiting subsequent growth. Moreover, the phase of *F. prausnitzii* growth dictated both response to oxygen sensitivity and the time of bacterial capture for formulation encapsulation. *F. prausnitzii* was significantly affected by exposure to bile-acids, which was reversed with the use of bile-acid sequestrants. Lastly, based on our culture data and pH responsiveness, the ideal site for gastrointestinal release was established.

Conclusion:

To successfully deliver *F. prausnitzii* (or other, similarly anaerobic probiotic bacteria), characterisation of *F. prausnitzii* viability through gastrointestinal transit is vital, to create bespoke, viability-enhancing oral formulations. Here, we examined the potential negative impacts faced by *F. prausnitzii* in any newly developed oral formulation. These results will infer our next steps in formulation design and development.

P228

Inter-regulatory interactions among pneumococcal Rggs

Aisha Baqasi, Hasan Yesilkaya

University of Leicester, Leicester, United Kingdom

Abstract

Streptococcus pneumoniae can communicate with each other through the quorum sensing systems (QSS). These systems enable coordination in response to various stimuli through the secretion and detection of signalling molecules called pheromones. One of the peptide-mediated QSS is a regulatory gene of glycosyltransferase (Rgg). Pneumococcus has six Rgg locus which controls a wide range of physiological conditions including bio-film formation, virulence, bacteriocin production and oxidative stress. However, inter-regulatory relations among different Rggs have not been studied in detail. This study investigated the regulatory interactions between Rgg144 and Rgg1518 using isogenic mutants and reporter strains. It was found that both Rgg144 and Rgg1518 play a role in mannose and galactose metabolism as the mutants lacking these systems attenuated in growth and both QSS was found to be induced on these sugars. In addition, we found that the full induction of each pathway required the presence of the other, indicating the inter-regulatory interactions.

P229

Isolation, Identification and Characterization of *Bifidobacterium* Isolated from Exclusively Breastfed Malawian Infants.

Angeziwa Chunga ORCID iD

Malawi Liverpool Wellcome Programme, Blantyre, Malawi. Kamuzu University of Health Sciences, Blantyre, Malawi

Abstract

Background: An infant gut microbiota dominated by *Bifidobacterium* is associated with improved early and later life. During early life, *Bifidobacterium* thrives on complex carbohydrates found in breast milk.

Methodology: Stool samples were collected from 30 healthy, exclusively breastfed infants in Blantyre, Malawi. 16S rRNA sequencing was done for microbiota profiling. Whole genome sequencing was done on *Bifidobacterium* isolated from the stool samples. The presence of Human Milk Oligosaccharide digesting genes was assessed by BLAST comparison of the Malawi genomes against 90 known HMO digesting genes.

Results: *Bifidobacterium* was the predominant gut microbiota member, with 73% as the highest relative abundance. Malawian exclusively breastfeeding children were colonised by *Bifidobacterium longum, breve, bifidum, and speudocatenulatum*. Ninety percent of the isolated *Bifidobacterium* longum belonged to the subspecies infantis. *Bifidobacterium longum* subspecies *infantis* had the highest number of HMO digesting genes compared with other species and subspecies. comparative phylogenomic analysis revealed that *Bifidobacterium* genomes from Malawi were phylogenetically diverse and distinct from global genomes.

Conclusion: Novel sub-clades of *Bifidobacterium longum* sub-species *infantis* dominated the gut microbiome of exclusively breastfed infants. The most successful sub-species encoded the most HMO-digesting enzymes

P230

Metabolic profiling of airway niches during *Streptococcus pneumoniae* carriage and disease identifies branch chain amino acids as signatures of bacterial colonisation

Angharad E Green[1], Sian Pottenger ORCID iD[1], Manal Monshi[1], Marie Phelan[2], Daniel R Neill[1]

[1]Clinical Infection Microbiology and Immunology, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]NMR Centre for Structural Biology, Institute of Integrative Biology & Technology, University of Liverpool, Liverpool, United Kingdom

Abstract

Background: *Streptococcus pneumoniae* shows remarkable phenotypic plasticity, responding rapidly to environmental change. Pneumococcus is primarily a nasopharyngeal commensal, but can cause severe infections following spread within the airways. Challenges and opportunities in resource acquisition within different airway niches may contribute to the commensal-pathogen switch when pneumococcus moves from the nasopharynx into the lungs.

Methods: We used NMR to characterise the metabolic landscape of healthy and infected mouse airways. Paired nasopharynx and lung samples from naïve animals identified differences in metabolite bioavailability between airway niches. Following identification of key metabolites that could play a role in pneumococcus adaptation, we assessed differences in the intracellular concentrations of these metabolites in bacterial cells and in the expression of genes relating to their metabolism and transport.

Results: Pneumococcal pneumonia was associated with rapid, dramatic shifts in the metabolic landscape of lungs. By contrast, pneumococcal nasopharyngeal carriage showed only modest changes in upper airway metabolite profiles. Multivariate dimensionality reduction methods distinguished between NMR spectra derived from the nasopharynx of mice infected with closely-related pneumococcal strains which differ in colonisation potential. Resulting models highlighted increased branch-chain amino acid (BCAA) bioavailability in the nasopharynx as a feature of infection with the strain with highest colonisation potential. The expression of BCAA metabolism and transport genes and intracellular concentrations of BCAAs were also increased.

Conclusions: Movement from upper to lower airway environments is associated with shifting challenges in metabolic resource allocation for pneumococci. Efficient biosynthesis, liberation or acquisition of BCAAs is a feature of adaptation to nasopharyngeal colonisation.

P231

Improved colonic delivery of next-generation probiotics using novel microcomposite formulations to enhance gastrointestinal protection and biotherapeutic effectiveness

Linh P. Ta, Sarah Corrigan, Hansini Abeysekera, Richard D. Horniblow

School of Biomedical Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom

Abstract

Background

Many new next-generation probiotics (NGPs) have emerged as potential novel biotherapeutics, yet these bacteria are highly sensitive to environmental changes. Such conditions, particularly with respect to oral-delivered NGPs include O_2, pH changes, contact with bile-salts and competition with resident microbes. High levels of viable bacteria must reach the colon, yet traditional formulation methods are not suitable. In this study, we create novel microcomposite formulations which address these issues and demonstrate their efficacy in a colonic bioreactor model.

Methods

Anaerobic bacterial microencapsulation was accomplished within a O_2-free chamber. The polymeric scaffold of the formulation was optimised to limit O_2-ingression using O_2-fluorescent nanoprobes as reporter for inner-particle O_2 concentrations. Encapsulation at stationary/exponential growth phases was examined through growth and viability studies. Acid tolerances were examined using simulated gastrointestinal fluids, while O2-tolerability was examined using traditional enumeration assays. Lastly, the engraftment and colonisation of NGPs was examined using a sophisticated intestinal model - MIMic (Model of the Intestinal MICrobiome).

Results

We have developed a microencapsulation system that significantly improves the viability of *Akkermansia muciniphila* (AM). Furthermore, the oxygen protection of AM within the microcomposites was achieved through incorporation of antioxidants and specific biopolymeric materials. The AM-containing microcomposites were able to deliver, release and enable AM to colonise the bioreactor colonic-microbiome.

Conclusion

This study presents a new formulation platform for anaerobic bacteria which sustains viability during oral administration and facilitates intestinal colonisation. Such technology can be now employed to encapsulate bacterial consortia to significantly alter microbial communities which could be associated with dysbiosis.

P232

Growth and carbon utilization for a genetically diverse collection of Enterotoxigenic *Escherichia coli* and transcriptional responses to bile, bicarbonate and mucin for strain E1779 (CS5+CS6-LT+STh)

Anne Bishop ORCID iD[1], Astrid Von Mentzer[1,2], Nicholas Thomson[1,3]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]University of Gothenburg, Gothenburg, Sweden. [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Enterotoxigenic *Escherichia coli* (ETEC) strains carry heat labile (LT) and/or heat stable (ST) toxins, which cause diarrhoea, and colonization factors (CFs) that facilitate gut adherence. ETEC causes over 80 million episodes of human diarrhoea globally per year. A wealth of genomic information reveals a great deal of ETEC genetic variation, while we know less about gene expression for genetically diverse strains, which has focused on one or two strains from CF types CS1+CS3, CFA/I or CS5+CS6.

We compared growth for nine genetically diverse ETECs with bile, mucin or bicarbonate and profiled their carbon utilization using Biolog phenotypic arrays. For E1779 (CS5+CS6-LT+ST) we compared gene expression responses to bile, mucin or bicarbonate by RNA-Seq.

From lineage 1, strain E925 (CS1+CS3+CS21-LT-STh) struggles to grow in M9-glucose+/-Oxoid#3-bile compared to E1739 (CS1+CS3-LT-STh) and is deficient in glycerol utilization, while from lineage 3 strain E36 (CFA/1+CS21-LT-STh) struggles to grow with bicarbonate compared to E2980 (CS7-LT). RNA-Seq with E1779 shows large shifts in gene expression in response to mucin or bicarbonate and select differential expression, biased towards predicted surface and secreted proteins, in response to bile.

Thus, we have revealed intra-lineage variation in ETEC growth and catalogued ETEC differential gene expression responses: to mucin for the first time; to bicarbonate for the first CS5+CS6 strain; to bile for E1779, confirming responses of other CS5+CS6 strains. These data underpin ongoing RNA-Seq comparing eight diverse ETECs in order to: reveal evidence of either conserved or varied evolutionary adaptations; determine whether potentially protective antigens are transcribed.

P233

Novel molecular mechanisms by commensal bacteria to inform immunomodulatory microbiome therapies

Selvin Solis[1], Elaina Maldonado[1], Jorge Gutierrez-Merino ORCID iD[1], Fernando Martinez-Estrada[1], Carlos Maluquer de Motes ORCID iD[1], Subhankar Mukhopadhyay[2]

[1]University of Surrey, Surrey, United Kingdom. [2]King's College London, London, United Kingdom

Abstract

The microbial diversity in human (and animal) microbiomes and their correlations with medical conditions have revolutionized the fields of Medicine and Biosciences. However, it is uncertain which host commensal microbes are the main drivers that restore/protect health from disease, and the molecules they possess to interact with the immune system. We have addressed this important question using a luciferase reporter infection model to reveal the molecules that commensal bacteria utilize to induce host-beneficial responses. Our investigations have shown that certain species of lactobacilli, which are popular as probiotics worldwide, trigger macrophages to produce high levels of type I interferon (IFN-I) cytokines, which are essential to confer protection against microbial infections and auto-immune disorders. For the first time, we have proved that this IFN-I activation is predominantly driven by cGAS, a molecule that activates the cytosolic sensor STING upon the recognition of bacterial DNA. Furthermore, we have observed that lactobacilli encode some surface proteins with the potential to interact with macrophages for subsequent phagocytosis via non-opsonic scavenger receptors. Therefore, we are focused on determining the role that these surface proteins play as a port of entry in macrophages and characterizing the IFN-I-mediated intracellular signaling initiated by cGAS. Elucidating these unknown mechanisms will be important to inform on how specific molecules of commensals modulate or stimulate host responses that, in unhealthy individuals, are exacerbated or inhibited. Overall, our studies will provide a better understanding on the molecular crosstalk between the microbiome and mammalian cells, paving the way for major therapeutic discoveries

P234

The Effects of Amino Acids on Bacterial Colibactin Expression

Sofia Sandalli, Jennifer Hallam, Gillian Douce, Andrew Roe

University of Glasgow, Glasgow, United Kingdom

Abstract

Some *Escherichia coli* strains of the B2 phylogroup synthesise a potent genotoxin, named colibactin, encoded for on their genomic *pks* island. In eukaryotic cells, colibactin induces DNA damage, chromosomal instability, and cell-cycle arrest, and it has been shown to trigger the development of colorectal cancer. Our results demonstrate the inhibitory effect of several amino acids on the *pks* island in colibactin-producing strains. All proteinogenic L-amino acids and corresponding D-enantiomers were tested for their ability to modulate *clbB* transcription through a *pclbB-GFP* reporter-assay in four different types of growth media. The most inhibitory amino acids were further validated by RT-qPCR. D-serine, amongst the most repressive amino acids, was selected for further experiments in two colibactin-producing strains, CFT073 and Nissle 1917. We observed that repression of colibactin by D-serine reduces the cytopathic responses normally observed during infection of HeLa cells with *pks+* strains. Levels of γ-H2AX (a marker of DNA double strand breaks) were reduced 2.75-fold in cells infected with D-serine treatment; and exposure of *pks+ E. coli* to D-serine during infection caused a reduction in cellular senescence observable at 72 hours post infection. The association between *pks+* commensal *E. coli* and colorectal cancer emphasises the need for colibactin-targeting therapeutics. We show that D-serine has the potential to prevent colibactin-associated disease and that several other amino acids may be considered for further investigation as therapeutic candidates.

P235

iEC1073: A genome-scale metabolic model to investigate metabolic changes facilitating host switching in *Photorhabdus*.

Elena Lucy Carter[1], Chrystala Constantinidou[1], Nicholas Waterfield[1], Mohammad Tauqeer Alam[2]

[1]University of Warwick, Coventry, United Kingdom. [2]United Arab Emirates University, Abu Dhabi, UAE

Abstract

Photorhabdus asymbiotica is a potent insect pathogen, forming a symbiotic relationship with soil-dwelling nematodes to facilitate its mutualistic and pathogenic life cycle. Unlike most other species belonging to the genus, *P. asymbiotica* can grow above 34°C and is an emerging human pathogen, causing clinical cases of skin and soft tissue infection termed Photorhabdosis. Transcriptomic analysis at temperatures simulating insect and mammalian infection have revealed temperature-dependent differences in the metabolic profile of the organism. To further investigate the mechanism of this metabolic switch due to temperature shift, the genome-scale metabolic model of *P. asymbiotica* (iEC1073) is reconstructed. The metabolic model was built and reconstructed using automated reconstruction tools and extensive manual curation. Phenotypic predictions of iEC1073, generated using flux balance analysis, were corroborated against published experimental data to validate the network, including gene essentiality and phenotype microarray data to verify the utilisation of various nutrient sources. Following validation, iEC1073 was integrated with published transcriptomics analysis of *P. asymbiotica* at 28°C and 37°C to create temperature-specific metabolic reconstructions, whereby flux through different metabolic pathways is representative of the expression of the gene associated with the reaction at the temperature of interest. Pathway enrichment analysis and comparison of reaction fluxes between the two temperature-specific models revealed that at 37°C, *P. asymbiotica* up-regulates purine and pyrimidine metabolism pathways alongside glycolysis and the TCA cycle. The up-regulation of these pathways is indicative of a stress response in the organism to the higher temperatures, contributing to increased DNA biosynthesis and energy production.

P236

Indole or Indon't: Does the Signalling Molecule Indole Produced by the Intestinal Microbiota Affect the Motility of *Salmonella*?

John L. Clark-Corrigall ORCID iD[1], Horeyah Abdalkrim[1], Bethany Gollan[1], Heather E. Allison ORCID iD[2], C. M. Anjam Khan ORCID iD[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Salmonella are important foodborne pathogens which cause a spectrum of disease in humans ranging from gastroenteritis to Typhoid fever. Motility is essential in physiology and contributes to competitive advantage enabling *Salmonella* to find a hospitable niche within the gut through chemotaxis. One of the vital roles provided by the intestinal microbiota is to repel invading pathogens from establishing a niche and causing disease, a phenomenon termed colonisation resistance. The small molecule indole, which is produced by members of the intestinal microbiota, has been shown to regulate basic physiology, virulence and biofilm formation in a number of pathogenic bacteria. The objective of this study was to determine whether indole can effect the motility of *Salmonella* and if so, elucidate how indole is sensed and the subsequent signalling cascade.

P237

Pathogenesis of otitis media: the role of epithelial cells' PAFr and NTHi's PCho in the invasion of middle ear epithelium

Isabel Parreira ORCID iD, Colin Bingle, Lynne Bingle

University of Sheffield, Sheffield, United Kingdom

Abstract

Otitis media (OM) is a middle ear (ME) infection prevalent in children aged 1 to 4 years old leading to hearing loss, cholesteatoma and meningitis, thus significantly hampering development. The mainstay of treatment is antibiotics, however, the emerging antibiotic resistance crisis calls for urgent alternative therapies. OM represents a substantial financial burden to healthcare systems and families.

Non-typeable Haemophilus influenzae (NTHi), an opportunistic pathogen of human respiratory tract, is commonly associated with respiratory infections and acute OM. Although NTHi's interactions with airway epithelial cells have been thoroughly investigated, little is known about its contribution to ME diseases.

This is the first study to investigate the interaction of NTHi with the cell surface receptor platelet-activating factor receptor (PAFr) through the phosphorylcholine (PCho) of NTHi lipooligosaccharide (LOS) and to relate this to the invasion of ME cells by NTHi.

The mechanisms driving NTHi invasion were investigated in adapted-gentamicin protection cell invasion assays using strains differentially expressing PCho (NTHi-375, Rd, H457, H446 and H491). In addition, the role of epithelial PAFr during bacterial invasion was assessed by blocking bacterial access to PAFr via competitive binding with the PAFr antagonist ABT-491.

Furthermore, this study used cytokine arrays to evaluate the differential expression of immunomodulatory mediators to NTHi-mediated infection in hMEEC-1 and primary fetal ME cells resolving the challenge posed when using cell lines, which notoriously present more limited responses to infection.

Our study will contribute to a better understanding of how NTHi evades the immune system, allowing the development of novel therapeutic strategies.

P238

GUT MICROBIOTA-DERIVED METABOLISM OF 5-FLUOROURACIL AFFECTS DRUG EFFICACY

Despoina Chrysostomou ORCID iD[1], Mark J David[1], Tanara V Peres[2], Filipe J Gomes Cabreiro[2,3], James M Kinross[1], Julian R Marchesi[1]

[1]Imperial College London, London, United Kingdom. [2]LMS, Imperial College London, London, United Kingdom. [3]University of Cologne, Cologne, Germany

Abstract

Background: Fluoropyrimidines are currently used as a first-line treatment for colorectal cancer (CRC). The impact of gut bacteria on 5-Fluorouracil (5FU) activity remains relatively unknown. The objective of this analysis was to delineate potential bacteria-drug interactions that modify 5FU.

Methods: We assessed the ability of 20 common bacterial species from the gut microbiome, to chemically modify 5FU. Following co-incubation of bacteria of interest with 5FU, ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) was employed to identify changes in drug concentration as well as products of bacteria-driven drug metabolism. Statistical analysis using bioinformatic pipelines in Rstudio was used to determine significant changes in drug concentration. We also employed a high-throughput screen, looking at hatching fraction of *Caenorhabditis elegans* (*C. elegans*) worm eggs, aiming to investigate the effect of 5FU bioproducts produced by microbe-derived metabolism of the prodrug on host cellular proliferation.

Results: *Escherichia coli* (BW25113), *Bifidobacterium longum, Citrobacter freundii* and *Enterococcus faecalis* significantly modify 5FU in vitro, an interaction examined by Wilcoxon spare rank test. Fraction of hatching following incubation of *C. elegans*' eggs with 5FU bioproducts was significantly reduced, showing that bacteria-drug interactions can activate 5FU.

Conclusion: This is the first report of 5FU metabolism by *Escherichia coli* (BW25113), *Bifidobacterium longum, Citrobacter freundii* and *Enterococcus faecalis* in vitro, results that have been further validated using *C. elegans* model. This study provides an insight into the potential of large and small intestinal bacteria in chemically modifying chemotherapeutic agents and therefore modulating their activity.

P239

The *Mycobacterium ulcerans* exotoxin mycolactone causes catastrophic loss of the endothelial glycocalyx and basement membrane: a new indirect mechanism driving tissue necrosis in Buruli ulcer

Belinda Hall ORCID iD[1], Louise Tzung-Harn Hsieh ORCID iD[1], Jane Newcome[1], Tom Mendum[1], Yagnesh Umrania[2], Michael Deery[2], Francisco Salguero[3], Rachel Simmonds ORCID iD[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]United Kingdom Health Security Agency, Salisbury, United Kingdom

Abstract

Buruli ulcer is a necrotising skin infection caused by *Mycobacterium ulcerans*. We recently provided clinical evidence that, in addition to the direct cytotoxicity caused by its diffusible exotoxin mycolactone, vascular dysfunction plays a role in disease aetiology. Although many bacteria use toxins to disrupt the endothelium and promote infection, the mechanisms driving the endothelial dysfunction in Buruli ulcer are unclear. We show mycolactone's inhibition of the Sec61 translocon induces changes in endothelial morphology, adhesion, migration, and permeability. Unbiased quantitative proteomics of human microvascular endothelial cell membrane fractions identified a profound effect on the glycocalyx, due to downregulation of both the core protein components and the Golgi enzymes required for synthesis of their glycosaminoglycan (GAG) chains. These Type II membrane proteins are highly sensitive to Sec61 inhibition and are rapidly depleted by mycolactone. Loss of the glycocalyx is likely to be of mechanistic importance, since knockdown of the GAG linker-building enzyme B3Galt6 phenocopied the permeability and phenotypic changes caused by mycolactone. The basement membrane is a second key endothelial target of mycolactone with many secreted basement membrane components depleted, including laminins α4 and α5. Remarkably, exogenous addition of laminin-511 reduced cell rounding, restored cell attachment and reversed the defective migration caused by mycolactone suggesting that this might present a viable therapeutic avenue in the future.

P240

The role of the fosfomycin resistance *fosB* gene in *Staphylococcus epidermidis*

Francesco Flandi[1], Valeria Alonzi[1], Alessandro Poletti[1], Freya Bowden[2], Ijeoma Okoliegbe[3], Francesca Bonvicini[1], Giovanna Angela Gentilomi[1], Karolin Hijazi[3], Marco Rinaldo Oggioni[1,2]

[1]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy. [2]Department of Genetics and Genome Biology, University of Leicester, Leicester, United Kingdom. [3]Institute of Dentistry, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Aberdeen, United Kingdom

Abstract

Fosfomycin has a broad spectrum of activity against Gram-positive and Gram-negative bacteria including *Staphylococcus epidermidis*. Fosfomycin is an inhibitor of the MurA enzyme. One mechanism that confers fosfomycin resistance is based on the BSH-dependent FosB S-transferase that adds bacillithiol to fosfomycin.

Sequencing data of *S. epidermidis* blood culture isolates showed that the *fosB* gene can be present on the core-genome and on accessory-genome elements, such as plasmids. Moreover, the *cg-fosB* gene was seen to be characterized in 67% of cases by a stop codon or a frameshift.

To define which is the role of *S. epidermidis fosB* in fosfomycin resistance, we performed MIC tests. This showed that, in absence of plasmids, the fosfomycin susceptibility is not dependent on the *cg-fosB* arrangement, as neither stop codons or frameshifts in the *cg-fosB* gene influenced the phenotype. Fosfomycin resistance appeared to be due only by the presence of plasmids or chromosomal elements carrying an additional *fosB* copy. Fosfomycin MIC values of carriage isolates from Italy were about four fold lower than those of blood culture isolates. Absence of resistance and high overall susceptibility is surprising due to the utilization of fosfomycin for urinary tract infections in the community in Italy.

Our data indicate that, in contrast to the *ag-fosB* gene, the *cg-fosB* paralog does not show any fosfomycin related phenotype in *S. epidermidis*. Combined with the data showing lower overall fosfomycin resistance in community, isolates indicates that likely other factors influence fosfomycin susceptibility in *S. epidermidis*.

P241

Investigating Gut & Oral Microbiome Development in Late and Moderate Preterm Infants

Sinéad Ahearn-Ford ORCID iD[1], Andreas Kakaroukas[2], Janet Berrington ORCID iD[2], Nicholas Embleton ORCID iD[2], Christopher Stewart ORCID iD[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]Newcastle Hospitals NHS Trust, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Abstract

Late and moderate preterm (LMPT) births represent 6-7% of all births in the UK and are associated with higher risks of morbidity in infancy and adulthood, contributing to rising pressure on healthcare systems. Abnormal microbial colonisation may be responsible for poorer outcomes, however, to date, there is a paucity of data regarding LMPT microbiome development.

During six visits over the first two years of life, 161 LMPT infants (born 32 to <37 weeks gestation) were followed and data was collected on nutrition, anthropometry, body composition, development, demographics and medical history. Longitudinal stool (n=372) and saliva (n=404) samples underwent DNA extraction and 16S rRNA gene sequencing to determine gut and oral microbiomes.

The development of LMPT gut and oral microbiomes up to 12 months was described. Gut and oral microbial profiles were significantly different at all timepoints, and this difference was more pronounced with increasing age. We report a number of factors, including gestational age, antibiotic use and length of hospital stay, that were significantly associated with microbiome composition. Feeding type was important in driving microbiota composition, with infants fed different diets exhibiting differences in specific bacterial taxa colonising the gut and oral microbiomes.

Given the role of the microbiome in health, understanding the factors associated with its development is essential to improving outcomes. Current clinical practices vary markedly for LMPT infants; this work provides much needed insight into this population and may help with updating guidelines to improve clinical care.

P242

Exploring the potential adjuvant role of bacterial extracellular vesicles derived from *Bifidobacterium pseudocatenulatum* strains

Anne Jordan ORCID iD[1], Lindsay Hall ORCID iD[1,2,3], Simon Carding ORCID iD[1,2]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [3]School of Life Sciences, ZIEL Institute for Food & Health, Technical University of Munich, Munich, Germany

Abstract

Differences in the composition of the intestinal microbiota emerge to play a key role in the variation of vaccine efficacy between infants from geographically distinct locations. In particular, high abundances of *Bifidobacterium* species and strains have been positively associated with improved vaccine-related immune responses. However, underlying mechanisms and potential adjuvant applications are still highly understudied. Based on the recent research of commensal bacterial extracellular vesicles (BEVs) as vaccine adjuvants and antigen-carriers, we explored the production and characteristics of BEVs derived from different immunomodulatory *Bifidobacterium pseudocatenulatum* strains.

Ideal BEV harvesting time points were determined through CFU assays and BEV purification using crossflow filtration was standardised for *B. pseudocatenulatum* strains. Subsequent BEV batches were evaluated using nanoparticle tracking analysis and TEM resulting in the production of a bank of replicable BEV isolates. *In vitro* studies using different cell lines indicate potential modulation of intestinal barrier responses in CaCo2 cells and stimulation of NF-kB pathways including the production of IL-8 in THP-1 monocytes which was confirmed by colleagues *in vivo*. Further potential immune stimulation by bifidobacterial BEVs is currently investigated in more detail using ELISAs, proteomics, and further *in vitro* and *ex vivo* characterisations.

To date, only a few publications investigated bifidobacterial BEVs and, to our knowledge, no in-depth characterisation has been performed on BEVs derived from *B. pseudocatenulatum* strains. This data may provide insight into potential immune stimulatory properties of *B. pseudocatenulatum* and their BEVs which could lead to a new generation of probiotic adjuvants potentially improving vaccine efficacy variations.

P243

Investigating interactions between *Pseudomonas aeruginosa* and *Streptococcus mutans* in a novel ventilator-associated pneumonia model

Nicole Gilardi, Freya Harrison, Dean Walsh

The University of Warwick, Coventry, United Kingdom

Abstract

Ventilator associated pneumonia (VAP) is a serious nosocomial infection with a 50% mortality rate in patients who are mechanically intubated using endotracheal tubes. Commensal oral organisms such as various *Streptococcus spp.* have been shown to be highly associated with biofilm formation in endotracheal tubes and so can end up occupying the same niche as hospital-acquired opportunistic pathogens such as *P.aeruginosa* in VAP patients. Previous research has suggested how the pathogenicity of *P.aeruginosa* can be altered based on interspecies interactions it forms in multispecies biofilms such as those found in VAP or in cystic fibrosis (CF). Previous studies investigating the interactions between *P.aeruginosa* and *Streptococci* have been conflicting, with *Streptococci* shown to either inhibit or promote *P.aeruginosa* biofilm formation depending on the study. However, most of this research uses CF isolates and/or is centred around the ecology of CF infections, therefore it remains unclear how these two microbes interact in the VAP environment. Using a novel VAP model, we observed and characterised how these two species interact in the VAP environment. Our model comprises sections of endotracheal tube and tailored growth medium that mimics the airway surface liquid in VAP patients. Through a series of optimization assays, followed by imaging of mixed species biofilm and virulence factor assays to determine the components that the biofilm growth is dependent on, we present innovative results that will contribute to the characterisation of the VAP microbe environment and its potential treatment.

P244

Differential proinflammatory cytokine gene expression and protein release from vascular endothelial cells infected with oral anaerobes

Antonia Cutts, Graham Stafford, Craig Murdoch

University of Sheffield, Sheffield, United Kingdom

Abstract

Periodontitis is a prevalent chronic inflammatory disease, where tissue damage is mainly caused by inappropriate inflammatory reactions to oral microbiota. Atherosclerosis is also associated with chronic inflammation, where increased expression and production of proinflammatory cytokines by vascular cells drives plaque formation and progression. During periodontitis, oral bacteria enter the bloodstream and interact with vascular cells. Here, we examine the cytokine response of endothelial cells to oral bacteria to determine if these interactions may play a role in plaque formation.

Human microvascular endothelial cells (HMEC-1) were incubated with oral bacteria (*Porphyromonas gingivalis* (Pg), *Fusobacterium nucleatum ssp nucleatum* (Fnn) & *polymorphum* (Fnp)) at increasing MOI (0.1 - 1000) for 4 hours, followed by conditioned medium collection and RNA isolation. Reverse transcription-qPCR was used to measure fold-change in cytokine gene expression, while ELISA was used to measure the change in protein secretion levels.

Fnp caused a significant ($p<0.05$) MOI-dependent fold-change increase in gene expression and protein release of CXCL8 and IL-6 compared to uninfected controls at MOI 100 and 1000. Similarly, Fnn caused a significant MOI-dependent increase ($p<0.05$) that reached a maximum at MOI 100 for fold-change gene expression and cytokine release, before declining at MOI 1000. In contrast, Pg did not cause any significance in gene expression or cytokine production.

The following data suggests that infection of endothelial cells with oral bacteria causes an increase in proinflammatory gene expression and subsequent protein release, which could directly contribute to the formation of an environment that favours atherosclerotic plaque development.

P245

Investigating SdrG mediated adhesion of *Staphylococcus epidermidis* to the surface of corneocytes in atopic dermatitis

Emma Simpson ORCID iD[1,2,3], Tim Overton ORCID iD[1], Joan Geoghegan ORCID iD[2], Mark Donovan[3], Francesca Zuttion[3]

[1]School of Chemical Engineering, University of Birmingham,, Birmingham, United Kingdom. [2]Institute of Microbiology and Infection, University of Birmingham,, Birmingham, United Kingdom. [3]L'Oréal Research and Innovation, Paris, France

Abstract

Atopic dermatitis (AD) is a chronic skin condition, characterised by the presence of areas of dry, red and itchy skin. Staphylococcal colonisation can exacerbate the condition with *Staphylococcus aureus* being associated with more severe AD and *Staphylococcus epidermidis* being associated with mild to moderate AD. The first step in colonisation of the skin is adhesion of bacteria to the skin's stratum corneum. The objective of this study is to uncover the bacterial and host factors that facilitate *S. epidermidis* adherence to the stratum corneum. Using in vitro solid phase adherence assays, we investigated if *S. epidermidis* adheres to the human stratum corneum proteins loricrin and corneodesmosin. These proteins were chosen as they were previously shown to be recognised as ligands by *S. aureus.* None of the *S. epidermidis* strains tested, including strains isolated from AD skin, bound to loricrin or corneodesmosin. We also investigated if the *S. epidermidis* cell wall-anchored protein SdrG promotes adherence to immobilized recombinant human loricrin and corneodesmosin when overexpressed in the surrogate host strain *Lactococcus lactis.* We found that *L. lactis* expressing SdrG did not adhere to the stratum corneum proteins loricrin or corneodesmosin but did adhere to a control protein (fibrinogen). This indicates that while the stratum corneum proteins loricrin and corneodesmosin are ligands for *S. aureus* they are unlikely to be involved in the interaction between *S. epidermidis* and the stratum corneum during skin colonisation.

P246

Antimicrobial activities in microbial-based cleaning products

Chidinma Lynda Akaihe ORCID iD[1], Christopher Knight[1], Aline Metris[2], Barrett Paul[2], Andrew J. McBain[1]

[1]The University of Manchester, Manchester, United Kingdom. [2]Unilever Safety and Environmental Assurance Centre (SEAC), Colworth Science Park, Sharnbrook, Bedfordshire, United Kingdom

Abstract

Background

Microbial-based cleaning products (probiotic cleaners) are believed to improve the hygiene of kitchen worktops, floors, and other surfaces through mechanisms that include antimicrobial activity of germinated *Bacillus* spores. We have microbially characterised a selection of probiotic cleaners and assessed the inhibitory activity of the cleaner vehicle and the associated microorganism(s).

Methods

Seven probiotic cleaners were analysed for the presence of bacteria/microbial spores. An agar well diffusion test was used to evaluate the antimicrobial effects of these products against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Each probiotic cleaner was centrifuged to get the supernatant bacteria-free product (BFP) and pelleted spores (if present). The antimicrobial activity of the BFP and the spores after germination were tested against *S. aureus, E. coli*, and *P. aeruginosa*.

Results

Five out of 7 MBCPs contained *Bacillus* spores. Four *Bacillus* isolates had direct antimicrobial effects in agar well diffusion tests against *S. aureus* only. None of the germinated spores from the products tested was able to produce an inhibitory substance against the growth of *E. coli* and *P. aeruginosa*. All the BFP tested inhibited the growth of *S. aureus*. BFP (6/7) inhibited the growth of *E. coli*, and BFP (3/7) inhibited the growth of *P. aeruginosa.*

Conclusion

The probiotic products contain a combination of two or more microbial species. MBCPs had antibacterial effects primarily attributable to antimicrobials in the product formulation. Further work will investigate the safety of MBCPs use with respect to changes to the Human microbiome.

P247

Bacteriophages in Human Milk: A large cohort study of preterm milk reveals a developing core community of phages associated with lipids

Wen Yew ORCID iD[1], Gregory Young[1], Andrew Nelson[1], William Cheung[1], Simon Bridge[1,2], Janet Berrington[2,3], Nicholas Embleton[2], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Newcastle upon Tyne Hospitals National Health Service Foundation Trust, Newcastle upon Tyne, United Kingdom

Abstract

Bacteriophages/phages are viruses that infect bacteria and archaea and are ubiquitous in nature alongside their cellular hosts. They are also found alongside beneficial bacteria, growth factors and immune proteins in human milk. In term milk studies, phages were found to be transmitted from mothers to infants during feeding and may prevent overgrowth of pathobionts in the gastrointestinal tract (GIT) of infants. We have found that certain phages are incorporated into milk fat fraction and it may act as a protective shield for phages during transmission to infant GIT. Here, we focused on the understudied phages of preterm milk and the lipid compositions. We used our previously published phage isolation protocol to isolate lytic and lysogenic phages from a total of 113 preterm milk samples expressed over the first 100 days of life, and used shotgun metagenomics and un-targeted lipidomics to characterise the isolated phage communities and lipid classes, respectively. Our data showed that phage richness and diversity increased significantly from colostrum to mature milk, with *Siphoviridae*, *Podoviridae* and *Myoviridae* predominantly presented and longitudinally dispersed in preterm milk. Saturated short chain triglycerides are more abundant in colostrum milk, whilst long chain mono- and poly-unsaturated fatty acids are more abundant in mature milk. In addition, we found a number of phages of putative infecting bacteria that are positively correlated with lipid classes, and this serves as baseline information for our on-going research on isolating viable phages from milk lipid and their contribution in modulating GIT microbiota of preterm infants.

P248

Identification of *Pseudomonas aeruginosa* as a live intracellular microorganism in *Acanthamoeba* isolated from a keratitis patient's domestic tap water

Binod Rayamajhee ORCID iD[1], Mark Willcox[1], Fiona L. Henriquez[2], Constantinos Petsoglou[3,4], Nicole Carnt[1]

[1]School of Optometry and Vision Science, Faculty of Medicine and Health, UNSW, Sydney, Australia. [2]School of Health and Life Sciences, University of the West of Scotland, Blantyre, South Lanarkshire, G72 0LH, Scotland, United Kingdom. [3]Sydney and Sydney Eye Hospital, Southeastern Sydney Local Health District, Sydney, Australia. [4]Save Sight Institute, University of Sydney, Sydney, Australia

Abstract

Acanthamoeba is a heterotrophic protist that can cause visually devastating *Acanthamoeba* keratitis (AK). *Acanthamoeba* feeds on different microbes, but some microbes can exploit it as host. This study investigated the prevalence of intracellular bacteria in *Acanthamoeba* species.

Household bathroom sinks were swabbed from nine AK patients residing in the greater Sydney region. Samples were cultured for *Acanthamoeba* growth followed by amoebal 18S rRNA PCR and sequencing of the *rns* gene for identification of genotypes. *Acanthamoeba* isolates were assessed for the presence of intracellular bacteria using molecular assays; PCR and fluorescence *in situ* hybridization (FISH) were performed. The viability of *Pseudomonas aeruginosa* in one strain was assessed by labeling with alkyne-functionalized D-alanine (alkDala). Identification of bacteria was confirmed by MALDI-TOF MS.

Among 17 processed domestic water samples, three were positive for *Acanthamoeba* spp. by culture, morphological analysis, and Sanger sequencing of 18S rRNA. Upon BLASTn search, all isolates were identified as *Acanthamoeba* spp. of genotype T4. One of the *Acanthamoeba* isolates harboured *P. aeruginosa* which was identified by PCR and confirmed by MALDI-TOF. These *P. aeruginosa* were seen throughout the *Acanthamoeba*'s cytoplasm by FISH and identified as metabolically active by alkDala staining. The *P. aeruginosa* strain was positive for the *exoU* gene as a potentially cytotoxic strain.

Acanthamoeba strains were genotype T4, the most common strain causing corneal infection. One of these strains contained viable *P. aeruginosa*. As *P. aeruginosa* is a known corneal pathogen, this may indicate the potential for these intracellular bacteria in *Acanthamoeba* to cause superinfections during keratitis.

P249

Probiotic *Lactiplantibacillus plantarum* KCFe63 Attenuates Hypercholesterolemia By Regulating The Intestinal Expression Of Neimann-Pick C1-Like 1 (NPC1L1) And ATP-Binding Cassette Transporter (ABCG5) In Wistar Rats

Abigail Fernandes[1], Santosh Jathar[1], Anoop Kumar Yadav[1], Kanchanlata Tungare[2], Pamela Jha[3], Renitta Jobby[1]

[1]Amity Institute of Biotechnology, Amity University Maharashtra, Mumbai, India. [2]School of Biotechnology and Bioinformatics, D. Y. Patil Deemed to be University, Navi Mumbai, India. [3]Sunandan Divatia School of Science, SVKM'S NMIMS University, Navi Mumbai, India

Abstract

Advancement in gut microbiome research has unveiled its connection to hypercholesterolemia, a prevalent cardiovascular risk factor. Probiotics and synbiotics have emerged as promising cholesterol-lowering candidates. Due to poor consumer compliance, low-fat diets are not efficacious, therefore functional food products that can reduce blood cholesterol need to be developed. Hence, this study explored the cholesterol-reducing potential of a non-dairy ready-to-reconstitute synbiotic product containing probiotic *Lactiplantibacillus plantarum* KCFe63 (MT982170) and chicory root powder. The cholesterol-lowering efficacy of the probiotic, prebiotic and synbiotic product was tested on a hypercholesterolemic Wistar rat model. Significant ($p<0.01$) reductions in the body weight of animals supplemented with the probiotic and synbiotic were observed. The four-week supplementation resulted in a significant reduction ($p<0.001$) of serum lipids compared to the statin-administered group. No significant changes in the fecal *Lactobacillus* count were observed. Histopathological analysis revealed mild inflammation and necrotic changes in the probiotic group compared to the other groups. Moreover, an improvement in hepatic steatosis was observed and there were no signs of mitosis, pleomorphism, or peripheral inflammation. Further investigation showed that probiotic and synbiotic supplementation downregulated intestinal expression of Neimann-Pick C1-Like 1 (NPC1L1) by 0.63 folds. In contrast, ATP binding cassette (ABCG5) cholesterol transporter expression levels were significantly higher ($p<0.05$) compared to the disease control group. Altogether, this study reveals the therapeutic potential of BSH-active KCFe63 by decreasing intestinal absorption of cholesterol and can be explored further as a natural candidate for alleviating hypercholesterolemia.

P250

Eating fats to stay alive: elucidating the mechanism of *Salmonella enterica* chronic infections

Jessica Rooke[1], Rochelle Da Costa[1], Richard Strugnell[2], Ian Henderson[1]

[1]The University of Queensland, Brisbane, Australia. [2]The University of Melbourne, Melbourne, Australia

Abstract

Salmonella enterica is a diverse pathogen that is well adapted to cause infections in various hosts. Up to 5% of *S. enterica* infections result in a phenomenon termed chronic carriage in both humans and animals. These chronic infections are often associated with gall bladder colonisation and enable bacteria to be shed through the infected hosts faeces and this facilitates the spread disease. In addition, these infections are often associated with biofilms on cholesterol gallstones and are resistant to routinely used antibiotics. While some known virulence proteins have been linked with gall bladder colonization and phospholipids identified as potential carbon sources in the host gall bladder, the mechanistic details connecting these two aspects remained unclear. We hypothesised that *S. enterica* effectors that contribute to chronic carriage would be highly conserved in *S. enterica* but not in other closely related Enterobacteriaceae. Using bioinformatics approaches, we identified 125 S. enterica specific genes that were absent in the genomes of *Escherichia col*i, *Citrobacter rodentium*, *Yersinia pestis* and *Klebsiella pneumoniae*. From this list of genes, we further characterised an outer membrane esterase. Loss of this esterase in Salmonella led to the inability to use lipids as a sole carbon source and decreased gall bladder colonization in murine models of infection. We demonstrate that this enzyme is essential for biofilm formation under lipid rich conditions and is a target for small molecule inhibitors. Our study provides evidence of a novel target to prevent, and or treat, chronic S. enterica infections.

P251

Autoinducer-2-based quorum sensing system of the meat spoilage microorganism, *Pseudomonas fragi*

Yasemin Küçükata ORCID iD, Hasan Yetim ORCID iD, Banu Metin ORCID iD

İstanbul Sabahattin Zaim University, İstanbul, Turkey

Abstract

Quorum Sensing (QS) is a system that bacteria use to sense their population density via signals called autoinducers. While autoinducer-1 (acylated homoserine lactones, AHL) is a QS signal molecule produced by Gram-negative bacteria, autoinducer-2 (AI-2) is seen in both Gram-positive and Gram-negative species. These signal molecules are produced and detected by spoilage-causing microorganisms to modulate various mechanisms, including biofilm formation, proteolytic activity, and motility. *Pseudomonas fragi* is a significant meat spoiler that causes deterioration under refrigerated conditions resulting in an unacceptable product associated with economic losses. *Pseudomonas fragi* does not produce AHL, but this species recognizes and responds to AI-2. The effect of the *P. fragi* AI-2 on meat spoilage has yet to be examined in detail. In this study, 104 *Pseudomonas* species were isolated from beef and minced meat samples collected from 12 local butchers. A phylogenetic analysis using rpoD sequences indicated that while most of the isolates (59%) were *P. bubulae*, a recently defined species close to *P. fragi*, and *P. fragi* (12%); the remaining ones included *P. deceptionensis*, *P. versuta*, *P. paraversuta*, *P. saxonica*, *P. lundensis*, and *P. weihenstephanensis*. There were also isolates forming separate clades indicative of possible new species, related to *P. fragi* and *P. bubulae*. In further studies, the AI-2 production of the isolates will be screened using the luminescence created by the biosensor *Vibrio harveyi* strain. Later, the spoilage-related properties of the isolates will be analyzed. The results will give information on the role of AI-2 production on meat spoilage by *P. fragi*.

P252

Investigating Gonococcal viability using Sub-cellular Fluctuation Imaging

Darryl Hill, Goergina Plant, Massimo Antognozzi

University of Bristol, Bristol, United Kingdom

Abstract

The novel microscopy technique Sub-Cellular Fluctuation Imaging (SCFI) has been developed at the University of Bristol for use as a rapid antibiotic susceptibility test (AST). We aim to investigate how this technique can be applied to the pathogenic organism Neisseria gonorrhoeae, due to its high level of antimicrobial resistance, following a successful proof of concept with Escherichia coli. The future implementation of this technique in clinical settings would eliminate the need for overnight culture of samples, decreasing the time taken to identify effective antimicrobials.

The evanescent field of a totally internally reflected laser measures internal nanoscale fluctuations of individual bacterial cells that are immobilised in a microfluidic channel. Analysis of these fluctuations indicates the metabolic state of each bacterium, therefore distinguishing it as either dead or alive. The technique can also distinguish between different metabolic states, and therefore, measurements of a bacterial population therefore result in a metabolic status for the entire sample.

Results presented here show that different metabolic states of N. gonorrhoeae can be distinguished, such that populations of dead, stationary and exponential phase bacteria can be identified. Fluctuations observed are significantly lower than those found with samples of E. coli. We hypothesise that this disparity is due to the morphology and the amount of biological material the laser travels through.

Results of the application of antimicrobials with E. coli and N. gonorrhoeae show that the SCFI technique can determine antibiotic killing in real time. SCFI can also distinguish resistant and susceptible strains.

P253

Single and sub-cellular metabolomics: the final frontier to understanding cellular heterogeneity and the interaction between *Mycobacterium tuberculosis* and its host

George Mayson, Jordan Pascoe, Abi Cook, Jane Newcombe, Melanie Bailey, Dany Beste

University of Surrey, Guildford, United Kingdom

Abstract

The ability to understand and measure cellular heterogeneity is considered an impediment to the successful treatment of infectious diseases such as tuberculosis, which is once again the leading cause of infectious death globally. Whilst methods for measuring genetic and transcriptomic heterogeneity are well established, metabolic heterogeneity is poorly studied in bacteria and their host cells due to the technical challenges associated with sampling and detection of metabolites at the single cell level. Here we exploit the Single Cellome™ System to sample from a macrophage model of tuberculosis, allowing the sampling of single infected macrophages and their sub-cellular compartments including phagosomes. We are developing mass spectrometry techniques to analyze these samples with the overall goal of measuring the metabolic heterogeneity of Mycobacterium tuberculosis host cell micro-environment. A detailed understanding of metabolic heterogeneity and how this drives the disease process will guide new potential treatment strategies so urgently needed to combat the growing threat of antibiotic resistant tuberculosis.

P254

Impact of Inulin on ameliorating neurodegeneration in a *Drosophila* model of Alzheimer's Disease

Zak Vincent, Lesley Hoyles, Shreyasi Chatterjee

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Currently, 50 million people in the world are affected by dementia, the most prevalent form of which is Alzheimer's Disease (AD). Two of the neuropathological hallmarks of AD are extracellular amyloid beta plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated and aggregated tau protein.

The composition of the gut microbiota is strongly influenced by diet and lifestyle, and might be contributing to our susceptibility to neurodegenerative diseases. Metabolites produced by gut bacteria are known to influence the integrity of the blood-brain barrier, and studies in mice have shown that faecal microbiota transplantation from old mice to young mice can impair spatial learning and memory. Human studies have demonstrated that the gut microbiota composition in AD patients is different from that of age-matched healthy controls. However, most published studies have focused on Ab pathology with very few reporting on the impact of the gut microbiota on tau protein.

Drosophila melanogaster is a model organism that has been utilised to investigate tau pathology in AD by overexpressing wild-type human tau protein. Our previous studies have shown that the overexpression of human tau in the *Drosophila* eye produces a characteristic "rough-eye" phenotype implying neurodegeneration. In our current investigations, we have shown that feeding larvae/flies the prebiotic Inulin can ameliorate the tau-induced neuronal loss in the *Drosophila* eye. We will now use tissue-specific drivers to characterise the gut microbiota of control and tau-expressing cohorts. This research will contribute towards understanding the importance of the microbiota-gut-brain-axis in the development of neurodegenerative diseases.

P255

Impact of bacterial quorum sensing on oral epithelial cells

Malee Nagi[1], Praveen Sharma[2], Iain Chapple[2], Josefine Hirschfeld[2], Sarah Kuehne[1]

[1]Oral Microbiology Group, School of Dentistry and Institute of Microbiology and Infection, College of Medical and Dental Sciences, The University of Birmingham, Birmingham, United Kingdom. [2]Periodontal Research Group, School of Dentistry, College of Medical and Dental Sciences, The University of Birmingham, Birmingham, United Kingdom

Abstract

Quorum sensing (QS), a form of bacterial cell-to-cell communication based on the interaction of small signalling molecules, is known to support the formation of bacterial multispecies communities (biofilms), also called dental plaque. The persistent presence of plaque is often associated with periodontitis (gum-disease).

Whilst many QS molecules like C8-acyl homoserine lactones (AHL) and autoinducer-2 have been described in the oral cavity, their direct impact on host cells remains poorly understood. This work aims to identify the host response to C8-AHL on oral epithelial cells, in the pursuit of understanding QS influence on host cells and its immune-activating potential. Oral epithelial cells play an important role in recruiting neutrophils to sites of microbial challenge by releasing interleukin (IL)-8.

Oral epithelial cells (H400) were grown at 37 °C in 5% CO2 in the presence of a range of C8-AHL concentrations (100, 10, 1 and 0.1 µM) for 18 hours. Following this, cell proliferation was analysed by cell counting, and the release of the proinflammatory cytokine IL-8 was measured by ELISA.

C8-AHL decreased cell counts in a concentration-dependent manner, however, this was not statistically significant. This result indicates that C8-HSL may reduce the proliferation of oral epithelial cells at higher concentrations than those tested here. IL-8 production was not influenced by the presence of C8-AHL at different concentrations, but was constitutively expressed, indicating that C8-AHL has no impact on neutrophil recruitment. Future experiments will investigate the release of further inflammatory mediators and assess possible mechanisms of the observed reduction in cell proliferation.

P256

Towards the development of a continuous culture system representative of the sphenoid sinus microbial communities in patients with pituitary adenomas

Mona Alqarni[1,2], Gavin Humphreys[1], Kanna Gnanalingham[3], Ruth Ledder[1], Federico Roncaroli[1]

[1]School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom. [2]Faculty of Medicine, King AbdulAziz University, Jeddah, Saudi Arabia. [3]Department of Neurosurgery, Salford Royal NHS Foundation Trust, Salford, United Kingdom

Abstract

Pituitary apoplexy (PA) is a life-threatening clinical syndrome developed following haemorrhage or infarction of a pre-existing pituitary adenoma. A recent study suggest a potential role of the microbiota in precipitating this condition. The primary aim of this project is to (i) better understand the microbiota of the sinuses in patients with non-functional pituitary adenomas (NFPA) and (ii) to use this data in the validation of a continuous culture model system representative of the sphenoid sinus. 29 sphenoid sinus mucosa specimens were obtained from subjects with NFPA. All samples were subjected to genomic DNA extraction and next generation amplicon sequencing of the V4 region of the 16S rRNA gene. Samples were analysed in QIIME, and profile data used to design a continuous culture Multiple Sorbarod Device (MSD) representative of the sphenoid sinus communities in patients with pituitary adenomas. Taxonomic analyses of the sequences suggested that the staphylococci were the most abundant genera across all samples. Model systems were able to support key genera identified in sinus samples with dynamic community stability achieved 3 days after bacterial inoculation. Compositional reproducibility was broadly observed across replicated model runs with regards to total aerobic and total anaerobic viable counts according to Levenes test. A validated model of the sinuses in this patient cohort could be used in the eventual pre-clinical testing of diagnostics and bacterio-therapies. The interaction of sinus pathogens in a validated model system will help to better understand the role of these bacteria in the aetiology of PA.

P257

Interactions of *p*-cresol derivatives with the blood–brain barrier highlight the complexity of microbiota–host communication pathways contributing to the gut–brain axis

Lesley Hoyles ORCID iD[1], Simon McArthur ORCID iD[2]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]Blizard Institute, London, United Kingdom

Abstract

p-Cresol is a by-product of microbial fermentation of tyrosine and phenylalanine in the large intestine. Gut-derived *p*-cresol undergoes extensive conjugation in both enterocytes and the liver, reaching the systemic circulation predominantly as *p*-cresol sulfate (pCS) with ten-fold lower levels of *p*-cresol glucuronide (pCG). In metabolically healthy individuals, pCS and pCG are efficiently cleared by the kidneys, whereas in patients with renal impairment they accumulate within the blood; pCS is thought to contribute to the impaired cognitive function frequently observed in these patients.

We examined the effects of physiologically relevant concentrations of pCS and pCG on the blood–brain barrier (BBB). *In vitro* and in mice, pCG prevented the BBB-permeabilising effects of endotoxin, acting by antagonising the LPS receptor TLR4. In contrast, pCS increased paracellular permeability and disrupted intercellular tight junctions *in vitro* and in mice. It elicited significant changes in the whole-brain transcriptome, suppressing neuronal activity, transcription and mitochondrial respiration pathways. pCS stimulated the epidermal growth factor receptor (EGFR), leading to mobilisation of matrix metalloproteinase (MMP)-2/9. *In vivo*, the BBB-damaging effects of pCS could be prevented by the EGFR antagonist erlotinib or the MMP2/9 inhibitor SB-3CT. Human hCMEC/D3 endothelial cells exposed to serum from haemodialysis patients, but not from healthy donors, showed an erlotinib-sensitive increase in paracellular permeability that closely correlated in size to the total serum pCS content.

Our data demonstrate the complexity of microbial metabolite–host communication pathways underlying the gut–brain axis, and identify means by which microbiome-associated metabolites can be targeted to improve brain function.

P258

Characterising the role of temperate bacteriophages in Pseudomonas aeruginosa engraftment and infection in chronic respiratory disease of Cystic Fibrosis (CF)

Mariklairi Kiourkou, Adnan Tariq, Andrew Nelson, Darren Smith

Northumbria University, Newcastle, United Kingdom

Abstract

Cystic fibrosis (CF) is a autosomal recessive genetic disorder and presents as mutations in the conductance regulator gene (cftr gene) resulting in the limitation in the movement of Chloride ions, leading to dehydration and the build-up of thick mucus in the lungs. Its incidence rate in the UK is 1 in 2500. Pseudomonas aeruginosa (Pa) is the most prevalent bacterial pathogen in chronic infection in CF patients associated with morbidity and mortality. The role of phages in early colonisation or engraftment, bacterial adaptation and evolution in Pa is somewhat overlooked. Temperate phages integrate their genomes into the Pa chromosome thus introducing cargo genes which may drive their evolution in the lung. We are focus here on paediatric CF samples because we have access to first isolates that may help understanding how Pa engrafts and alters over time in the CF lungs. A key aim is to begin to characterise temperate phages as markers of evolution by comparing phage carriage at the genomic level and how this relates to subversion of Pa in CF patients.

Some of the objectives include the isolation of phages from their clinical samples and subsequently assessing their infectivity against a range of Pa hosts.

Moreover, looking at the phage carriage in early, more than 60 paediatric samples and how these phages provide a selective advantage towards chronic infection. This will be achieved with genome sequencing of phages and Pa isolates and comparison of repository data and longitudinal data thus highlighting differences and similarities between phages.

P259

Characterising Prophage diversity and function in the preterm infant pathogen, Staphylococcus epidermidis.

Jasmine Jessop ORCID iD[1], Mohammed Adnan Tariq[1], Gregory Young[1], Nicholas D Embleton[2], Janet E Berrington[2], Christopher J. Stewart[3], Darren Smith[1], Andrew Nelson[1]

[1]Department of Applied Sciences, Northumbria University, Newcastle upon tyne, United Kingdom.
[2]Newcastle Neonatal Service, Newcastle Hospitals NHS Trust, Newcastle upon tyne, United Kingdom.
[3]Clinical and Translational Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon tyne, United Kingdom

Abstract

Staphylococcus epidermidis is a leading opportunistic pathogen due to its ability to form biofilms on indwelling medical devices. MLST analysis of S. epidermidis has identified strain type (ST) 2 and 5 as important in human infections and these carry a larger number of antimicrobial resistance genes. S. epidermidis is one of the most predominant bacteria found in human breast milk and has emerged as a leading pathogen in late onset sepsis in preterm infants, which leaves survivors with poor developmental outcomes. The project aim is to characterise S. epidermidis colonising early preterm infants and identify prophage regions present in the chromosome. Stool samples from very preterm infants (<32 weeks gestation) from the NICU in the RVI, Newcastle upon Tyne in the first week of life and were sequenced on the HiSeq X10 2x150bp chemistry. The data was quality trimmed and host signature removed using kneaddata and assembled using megahit. S. epidermidis was identified, with genome completion varying from 50.46%-99.81%. MetaBAT2 was utilised to bin the contigs and GTDB-Tk identified bacterial classification of the bins. MLST analysis enabled the identification of 6 different ST's, including ST2 and ST5. Prophage regions were predicted using PHASTER and analysed for AMR, virulence determinants and auxiliary genes. Our data demonstrates that S. epidermidis STs colonising the preterm infant gut harbour prophage regions. We hypothesise that prophage induction during antimicrobial stress in S. epidermidis will drive the evolution of this organism and alter the role it plays in the gut community which warrants further investigation.

P260

Role of Sa3int prophages in *Staphylococcus aureus* virulence and their possible implications in chronic rhinosinusitis

Roshan Nepal, Sarah Vreugde

The University of Adelaide, Adelaide, Australia

Abstract

Introduction:

Staphylococcus aureus colonizes 30% of the human population, but only a few cause severe infections. *S. aureus*' virulence varies and partly depends on the presence of prophages (viral DNA embedded in its genome) such as Sa3int prophage. Exotoxins and immune-modulatory molecules encoded by this prophage can further inhibit human innate immunity, increasing *S. aureus* pathogenicity.

Aim:

Investigate genomic plasticity of *S. aureus* and changes in extracellular proteins after acquisition of Sa3int prophage.

Methods:

We sequenced *S. aureus* isolated from the sinus cavities of a severe chronic rhinosinusitis patient using Oxford Nanopore Technology. *In-sillico* analysis revealed presence of a Sa3int prophage. Using mitomycin C, we induced the prophage, transduced it into a Sa3int-free isolate and confirmed by sequencing. We compared growth-kinetics, biofilm biomass and metabolic activity between parent and lysogen by establishing growth curves, crystal violet and resazurin assays. Exoproteins were identified and quantified using mass spectrophotometry.

Results:

Integration of Sa3int prophage transiently down-regulated the beta-hemolysin expression but did not alter the growth kinetics, adhesion and metabolic activity of biofilm. However, the acquisition of Sa3int prophage significantly increased biofilm biomass ($p=0.05$, t-test). Further, Sa3int prophage acquisition significantly changed the expression of secreted proteins with a significant up-regulation of 45 proteins and down-regulation of 23 proteins, mainly involved in immunomodulation.

Conclusion:

S. aureus carrying Sa3int prophage releases immune-modulatory toxins that help them escape innate immunity and cause chronic infection. These findings contribute to the development of novel mechanisms that render *S. aureus* susceptible to immune response by blocking prophage-associated defence mechanisms.

P261

Investigating skin microbial interactions and metabolite production on artificial sebum

Danita Kasi Patel ORCID iD, Drupad Trivedi ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

Sebum is a lipid-rich biofluid, secreted by sebaceous glands, which lubricates skin, and acts as a protective barrier against infection1. Volatile organic compounds (VOCs) present in sebum have previously been identified as potential biomarkers for Parkinson's disease (PD)2. *Malassezia*, lipophilic commensal fungi, proliferation and seborrheic dermatitis caused by skin microbiome dysbiosis, are directly associated with a significantly increased risk of PD development4. Microbial colonisation of sebaceous glands is dependent on sebum composition. Recent work has also shown composition of sebum changes in diseases such as PD, Alzheimer's, COVID-19 and tuberculosis. We hypothesised that changing sebum composition requires skin commensal microbes to adapt metabolically.

We investigated the growth ability of three skin-specific bacteria, Staphylococcus epidermis, hominis, and capatis, and three sebum-specific bacteria, Cutibacterium acnes, Corynebacterium ureicelerivorans, and suicordis on artificial sebum6 in single cultures and co-cultures for 72 hours. Skin-specific bacteria do not rely on sebum for their feed, but sebum-specific bacteria are known to exclusively use sebum as their energy source. Lipase activity of each culture was measured through the quantification of the release of p-nitrophenol at timepoints 0, 24, 48, and 72 hours. Additionally, microbial metabolite production was determined, with both endogenous and exogenous metabolites obtained from the microbial cultures at timepoints 0, 24, 48 and 72 hours analysed using gas chromatography-mass spectrometry (GC-MS). The data presented show in vitro adaptation to changing lipid feed and metabolic response of these bacteria. Microbial metabolic markers, in vivo, may potentially be useful substrates for disease diagnosis.

P262

Dissecting the role of bacteriophages on bacterial community composition and function in the human preterm infant gut

Theodora Tanou[1], Gregory R. Young[1], Wen C. Yew[1], Jasmine L. Jessop[1], Nicholas D. Embleton[2], Janet E. Berrington[2], Christopher J. Steward[3], Andrew Nelson[1], Darren L. Smith[1]

[1]Faculty of Health and Life Sciences, Northumbria University, Newcastle Upon Tyne, United Kingdom. [2]Newcastle Neonatal Service, Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom. [3]Clinical and Translational Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Late onset sepsis (LOS) and necrotising enterocolitis (NEC) are diseases affecting very preterm, low birthweight infants. Imbalances in bacterial communities of the infant gut have been associated with a greater risk of preterm infants developing NEC, but exact mechanisms remain poorly understood. Lysogenic and lytic bacteriophages from maternal breastmilk may play a role by interacting with bacteria within the gut, affecting bacterial gene expression, microbial composition and driving horizontal gene transfer, which may offer positive or negative outcomes for the infant.

To identify the impact of bacteriophages on bacterial communities, their transduced function, and impact on the preterm, we will investigate microbial communities within maternal breast milk and neonatal stool samples. Bacteriophages of interest will be isolated to provide an important and novel panel. Their infectivity will be characterised by creating mock preterm gut community types (PGCTs) using isolated bacteria from patient samples. This study focuses on a PGCT that is enriched with Klebsiella species, as they are commonly associated with NEC. Co-culture experiments will expose mock communities to the bacteriophage panel. Bioinformatic tools predicting prophage presence will inform identification of bacteriophages that correlate to LOS and NEC. These bacteriophages will be classified using DNA extraction, genome sequencing, and viral structure.

The characterisation of bacteriophages and their impact on the bacterial communities will provide a clearer picture of their role in the composition of the preterm infant gut. Gaining insight into bacteriophage-host interactions, within the gut microbiome, may inform future studies concerning the immunity and survival rates of preterm infants.

P263

Bacteriophage communities of the preterm infant gut vary across NICUs and in health & disease

Gregory Young ORCID iD[1], Nicholas Embleton[2], Janet Berrington[2], Christopher Stewart[2], Andrew Nelson[1], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Preterm infants are at greater risk of diseases such as necrotising enterocolitis (NEC) and late onset sepsis (LOS). Multiple studies have interrogated the microbial communities associated with neonatal intensive care units (NICUs) and preterm infant mucosal surfaces.

Using targeted bacterial sequencing and mass spectrometry we demonstrate significant differences ($R2 = 0.30$, $P < 0.05$), between microbial and metabolite compositions of preterm infant stools from NICUs across the UK. However, a large degree of variance between microbial and metabolic compositions remains unexplained.

Bacteriophages are viruses that infect bacteria and remain a comparatively under-explored component of gut microbiota acquisition and development. We compared bacteriophage communities in stools of 53 preterm infants (including 15 with NEC) from a single UK NICU with those of 25 preterm infants (including 9 with NEC) from a single US NICU. Viromes were characterised by metagenomic sequencing and demonstrated community similarity through several shared taxonomic features, including those infecting *Staphylococcus*, *Enterococcus*, *Escherichia*, and *Enterobacteria* spp of bacteria. Significant differences in overall viral richness were observed between NICUs ($P < 0.05$). Longitudinal patterns of viral richness were also different between NICUs with significant increases in inducible viral richness ($P = 0.04$) and significant decreases in free viral richness ($P = 0.02$) preceding NEC in UK NICUs. These patterns were not observed in US NICUs.

These results suggest inclusion of bacteriophages may represent a new frontier in clinical studies as biomarkers of health and disease states by adding greater resolving power than bacteria or metabolites alone.

P264

Understanding the role of bacteriophages in maternal milk on preterm infant microbiota development

Rui Andre Nunes dos Santos[1], Gregory R. Young[1], Wen C. Yew[1], Jasmine L. Jessop[1], Nicholas D. Embleton[2], Janet E. Berrington[2], Christopher J. Stewart[3], Andrew Nelson[1], Darren Smith[1]

[1]Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Newcastle Neonatal Service, Newcastle Hospitals NHS Trust, Newcastle upon Tyne, United Kingdom. [3]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Microbial colonisation and subsequent disequilibria within the preterm infant gut correlate to a higher risk of developing diseases such as necrotizing-enterocolitis (NEC) and late-on sepsis (LOS). Maternal milk (MM) has been associated with reducing the risk of preterm infants developing these diseases. This is partly due to the critical role of MM in development of the preterm infant gut microbiota and association with a developing immune system.

MM and stools from mother-infant pairs share bacterial strains but also a multitude of bacteriophages. The transmission of bacteriophages via breastfeeding modulates the gut microbiome by bacteriophage infection of bacterial hosts and may play a role in augmenting or stimulating environmental fitness of bacterial species through selecting a bacterial community structure or through acquisition of novel function.

The role of bacteriophages transmitted to the infant, via MM, remains poorly characterised. This study aims to explore the impact of bacteriophages isolated from MM on defined bacterial preterm gut community types (PGCTs). This study focuses on one PGCT associated with early life dominated by *Enterococcus faecalis*. Phages isolated from MM and stool will be used in infectivity assays against bespoke mock communities of bacteria isolated directly from this PGCT. Genomic and community-based analyses will promote understanding of prophage carriage within *Enterococcus faecalis* and other clinically relevant strains from this PGCT.

Identification of a novel panel of bacteriophages or bacterial lysogens with defined roles in the preterm infant gut may inform future studies to promote the establishment of a healthy gut microbiota in this high-risk cohort.

P265

Culturing the so-called unculturable rumen bacteria

Theano Stoikidou[1], Ziming Wu[1], Corey Roulston[1], Jnana Sree Pakalapati[1], Jack Jordan[1], Maria Buckland[1], Katie Lawther[1], James A Pickup[1], Fernanda Godoy Santos[1], Linda B Oyama[1], Conrad P Ferris[2], Sharon A Huws[1]

[1]School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, Belfast, United Kingdom. [2]Agri-Food and Bioscience Institute Hillsborough, Large Park, Hillsborough, United Kingdom

Abstract

The rumen represents the largest fermentative compartment in the ruminant stomach. It is a complex and dynamic ecosystem, comprising mainly anaerobic bacteria, protozoa, fungi, methanogenic archaea, and bacteriophages. Our current understanding of the rumen microbiome is impeded by poor culture collections and a lack of effort placed on culturing following an explosion in 'omic' technologies in recent years. This has led to an assumption that many microbes are unculturable, when it is recognised that with modest concentrated effort they could be cultured. In this study, we bring many of the missing bacteria from ruminal bacterial families into pure culture to enhance their current culture collection. Using the dilution to extinction method, ruminal fluid samples from sheep and cattle were plated onto three growth media namely Hobson's M2 medium, Bovine Heart Infusion (BHI) medium, and PC basal medium to isolate anaerobic bacteria. This resulted in isolation of over 120 ruminal bacteria, with observed differences in their profile with respect to the medium used highlighting the importance of media diversification during isolation. Additionally, 16S rRNA gene sequencing analysis reveals a potential new species of ruminal bacteria with only a 94.38% identity with the most closely related bacteria, Parabacteroides goldsteinii in the NCBI database. Further characterisation of this new species may provide insight into their role in the rumen microbiome and importance to the host. Overall, our results provide an enhanced understanding of the rumen microbiome, allowing for novel research on microbiome function/role in the health, productivity, and sustainability of ruminants.

P266

The effect of dietary bioactives on the gut microbiome diversity (DIME) – A pilot study

Duncan Ng[1], Federico Bernuzzi[1], Jennifer Ahn-Jarvis[1], Maja Omieljaniuk[1], George Saava[1], Paul Fingas[1], Falk Hildebrand[1,2], Maria Traka[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Earlham Institute, Norwich, United Kingdom

Abstract

The gut microbiota is crucial for the determination of host health. Its functions vary from producing essential nutrients for the host to preventing the colonisation of pathogens. Changes in the diet have been shown to modulate the gut microbiota. Phytochemicals (non-nutrient food components) found in fruit and vegetables can modulate metabolic processes and promote better health. However, the interaction between phytochemicals and gut microbiota is poorly understood.

To address this, we conducted a randomised 2x2 crossover study to estimate the effect of diets rich in dietary bioactives on the gut microbial diversity and markers of metabolic health in 20 healthy participants. Participants consumed a diet high in bioactive-rich food (containing polyphenols, Sulphur (S)-metabolites, and carotenoids) and a diet low in bioactive-rich food for 2 weeks, with a washout period of 4 weeks in between. Extensive food diaries are collected and analysed to ensure compliance with the diet. We did not observe a significant difference in the microbial composition with the dietary intervention. However, we did observe a significant difference in the relative abundance of *Phocaeicola vulgatus* after the high bioactive arm ($p = 0.048$). Interestingly, it was not observed in the low bioactive arm. Our results suggest members of the gut microbiota community can be influenced by dietary bioactives.

P267

Generating single cell suspension from adult mosquito tissues as tool for arbovirus research

Ioanna Kollia[1,2], Mathilde Laureti[1], Yin Cheung[1], Kevin Maringer[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom

Abstract

Aedes aegypti is a major mosquito vector of human arthropod-borne viral (arboviral) diseases, including chikungunya virus, dengue virus, yellow fever virus and Zika virus, all of which are emerging or re-emerging globally. For transmission between humans to occur, these arboviruses must infect and successfully replicate in their mosquito vector. Therefore, a better understanding of arbovirus-vector interactions is invaluable in helping us develop new strategies to control the transmission and emergence of arboviruses.

The mosquito midgut is an important initial site of arbovirus replication, the virus then disseminates to secondary tissues including the salivary glands. The goal of this project is to develop protocols for isolating single-cell suspensions from midguts, for use in flow cytometry and other molecular *ex vivo* studies. A range of digestive enzymes were tested alone and in combination, with different temperatures and timings, in order to determine which conditions lead to the release of single cells. Cell viability was also measured.

Mechanical disruption by vigorous pipetting and the combined enzymatic activity of collagenase, dispase and DNase enzymes, at 28°C in an overnight incubation led to the generation of single cell suspension, with a cell viability of approximately 100%. Cell transfectability was also tested and optimised to facilitate plasmid-based overexpression of fluorescent markers or other proteins.

Our protocols for generating tractable single-cell suspensions from midgut tissue will facilitate molecular studies into arbovirus-vector interactions in model cell systems more relevant to *in vivo* infection compared to existing cell culture tools.

P268

In vitro phagocytosis model to explore the role of macrophage selectivity in shaping the respiratory microbiome during COPD.

Karanjot K. Sandhu ORCID iD[1,2], Aaron Scott ORCID iD[2], Amanda L. Tatler ORCID iD[3], Kylie B. R. Belchamber ORCID iD[2], Michael J. Cox ORCID iD[1]

[1]Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom. [2]Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom. [3]Centre for Respiratory Research, School of Medicine, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

Abstract

During chronic obstructive pulmonary disease (COPD) there is an altered composition of the lower respiratory tract microbiome compared to healthy individuals. As professional phagocytes, macrophages are crucial in the clearance of microbial pathogens from the lungs during infection and disease. Evidence from previous phagocytosis models indicates that macrophages exhibit selectivity to uptake particular bacteria over others during COPD, however these models primarily used heat-killed bacteria. The aim of this project was to develop and improve an *in vitro* phagocytosis model using live bacteria, to investigate whether an altered microbiome composition may be attributed to macrophage selectivity. This assay compares uptake of live, clinically relevant isolates and commensal bacteria, to provide a more realistic and representative model of host-microbe interactions in the airways. Monocyte-derived macrophages (MDM) derived from whole blood samples were co-exposed with live *H. influenzae* or *S. pneumoniae* for 4 hours, to allow for determination of internal and external bacteria. Quantification of internalised bacteria was achieved by viable colony counts in macrophage lysate after 4 hours. In future, this assay will be used to challenge MDM from healthy individuals and COPD patients, with clinical isolates of pathogens and commensals extracted from COPD sputum samples to investigate both species and strain-level selectivity. Further development of this model will involve introduction of background communities of the respiratory microbiome to study their impact on MDM phagocytosis. Understanding the role of macrophage selectivity could provide insight into host and microbial therapeutic targets to reduce infection-induced COPD severity.

P269

The impact of iron and lactoferrin on the infant and adult gut microbiota

Jiyeon Jang, Simon Andrews, Gemma Walton, Marie Lewis, Soon Gweon

University of Reading, Reading, United Kingdom

Abstract

Iron deficiency is the most common micronutrient malnutrition globally. While many staple food items such as cereals and infant formula milk are fortified with iron, the absorption rate of dietary iron is low (~20%). A number of studies reported that a high dose of dietary iron increased pathogenesis especially in iron-deficient children's gut microbiota. And yet, iron supplements are widely used in most age groups. Lactoferrin is an iron-binding protein that is naturally rich in colostrum and potentially has a role in promoting breastmilk to be more bioavailable. Recently, it is also used as a supplement for adults, as it is claimed to be beneficial to immune system. In this project, we aimed to explore the effect of iron and lactoferrin in realistic doses on both infant and adult gut microbiota by in vitro models.

From a 48-hour fermentation in adults (n=4), iron induced less growth in the family of Lactobacillaceae (-3.1 L2FC*), while more growth was observed with lactoferrin (3.61 L2FC), compared to the control. On the other hand, a greater growth than the control was resulted with both iron and lactoferrin in infants (n=3; 1.56 and 2.60 L2FC respectively). Lactoferrin also promoted the microbial diversity in both adults and infants. While iron decreased the diversity in infants, a slightly increased diversity was resulted by iron in adults.

Continuous fermentation experiments (12 and 32 days for infants and adults) are on progress to find further effects in different regions of the large intestine for a longer time period.

P270

Direct Swab Analysis By Desorption Electrospray Ionisation – Mass Spectrometry (DESI-MS) For Preterm Birth Risk Stratification In Patients With Cervical Shortening

Katia Capuccini[1,2], Gonçalo D.S. Correia[1,2], Pamela Pruski[1], Holly Lewis[1], Lynne Sykes[1,2,3], Phillip R. Bennett[1,2], Zoltán Takáts[4,5], David A. MacIntyre[1,2]

[1]Institute of Reproductive and Developmental Biology, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, United Kingdom. [2]March of Dimes Prematurity Research Centre at Imperial College London, London, United Kingdom. [3]The Parasol Foundation Centre for Women's Health and Cancer Research, London, United Kingdom. [4]Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, United Kingdom. [5]National Phenome Centre, Imperial College London, London, United Kingdom

Abstract

Vaginal microbiome and host inflammatory response mediate preterm birth (PTB) risk, but the strongest PTB risk factor is a previous preterm delivery. Here, we aimed to determine if cervicovaginal metabolic profiling using Direct Swab Analysis by DESI-MS permits stratification of PTB risk phenotypes associated with cervical shortening and vaginal microbial composition.

Vaginal swabs collected longitudinally throughout pregnancy (n=1018 swabs) were analysed by DESI-MS using an LTQ-Orbitrap Discovery mass spectrometer and custom rotating swab holder. Metataxonomic profiling (V1-V2 hypervariable regions) was performed on a MiSeq platform. The ability of DESI-MS metabolic profiles to predict clinical outcomes and microbiota composition was explored using multivariate PLS-DA and random forest models with repeated K-fold cross-validation.

DESI-MS cervicovaginal metabolic profiles were weakly predictive of pregnancy history (previous PTB n=204, previous cervical treatment n=109; (AUCneg=0.58, AUCpos=0.61)) and subsequent treatment for cervical shortening (n=85 interventions, n=85 controls, (AUCneg=0.67, AUCpos=0.65)). Profiling of samples collected following intervention indicated capacity to distinguish women who subsequently shortened their cervix despite cerclage (n=14) from those who maintained cervical length (n=37) (AUCneg=0.65, AUCpos=0.68). The capacity of DESI-MS to predict microbiota composition in women with a cerclage in situ was strong at major species and genera level (AUCneg=0.92, AUCpos=0.93).

Our data indicate that early pregnancy cervicovaginal fluid metabolic profiles are not strongly reflective of PTB history risk phenotypes but offer potential for stratifying women who subsequently experience cervical shortening. DESI-MS swab profiling can still robustly predict vaginal microbiome composition after cerclage, which is an important mediator of maternal and neonatal health outcomes during pregnancy.

P271

Characterising the preterm gut microbiota, metabolome and circulating T lymphocytes: An integrative study

Lauren Beck ORCID iD[1], Thomas Sproat[2], Christopher Lamb ORCID iD[1,2], Janet Berrington ORCID iD[2,1], Nicholas Embleton ORCID iD[2,1], Christopher Stewart ORCID iD[1]

[1]Newcastle University, Newcastle Upon Tyne, United Kingdom. [2]Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom

Abstract

Infants born very preterm (<32 weeks of gestation) have immature immune systems and are subject to abnormal microbial colonisation, a combination which can increase the risk of disease. Characterising the complex relationship between temporal development of the gut microbiome (both structure and function) and systemic immunity alongside clinical information is therefore of significant importance. In this study, 266 longitudinal stool samples from 66 very preterm infants underwent 16S rRNA gene sequencing to analyse gut microbial structure. To further explore the functional status of these gut members, and immune profile in this population, a subset of these samples underwent metabolomics (n = 101), and a subset were matched for serum (n = 41) to explore T-cell sub-populations using cytometry by time of flight.

The strongest association was found between age and the gut microbiota ($P < 0.001$). There was no association between clinical information and metabolite or T-cell profiles, including no association with age ($P > 0.05$). We next sought to look for relationships between the three datasets, finding no robust correlation between gut bacteria, luminal metabolites, and circulating T cell sub-populations. This study provides an important insight into the potential network of relationships underlying preterm gut microbiome structure and function with the host immune system. We find very little correlation between the datasets, potentially owning to the dynamic nature of microbiome and immune development during the initial weeks of life in preterm infants. Further work with a higher number of matched samples is needed to confirm these findings.

P272

Exploring the neuromodulatory potential of an arsenal of fermented foods

Ramya Balasubramanian ORCID iD[1,2,3], John Leech ORCID iD[4], Samuel Mortensen ORCID iD[5,2], John Cryan ORCID iD[2,6], Paul Cotter ORCID iD[7,2]

[1]Teagasc Food Research Centre, Cork, Ireland. [2]APC Microbiome Ireland, University College Cork, Cork, Ireland. [3]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland. [4]Teagasc, Cork, Ireland. [5]Teagasc Food research center, Cork, Ireland. [6]Department of Anatomy and Neuroscience, University College of Cork, Cork, Ireland. [7]Teagasc, Moorepark, Cork, Ireland

Abstract

The gut microbiome has increasingly been implicated in the development and progression of various neurological disorders via modulation of the microbiome gut brain axis (MGBA). Fermented foods are a rich source of beneficial metabolites that are produced by a robust community of microorganisms and can therefore be used to target the MGBA. Herein we use computational techniques to identify a small arsenal of fermented foods that could be adopted in modulating the MGBA, thereby conferring therapeutic benefit to the host. We functionally annotated 150 fermented food metagenomes sourced from two geographical locations using HUMAnN3-BIOBAKERY pipeline. Food metagenomes were annotated with tools of neuromodulatory significance, specifically: I) OMIXER-RPM, II) TRYPPNET, III) vitamin B metabolism and IV) immunomodulatory peptides production- MAHMI database. We found that neuromodulatory potential of the fermented foods was driven by food substrate categories. Fermented sugar based samples possessed most of the genes pertaining to tryptophan metabolism and this is supported by incorporation an additional of 300 sugar based fermented foods. Substrate categories of dairy, bean and millet exhibited genes influencing levels of short chain fatty acids and neuroactive compounds. In doing so we comprehensively identified broad substrate categories of fermented foods that have the potential to modulate the underlying neurological state of the host.

P273

Immune response in house dust mite induced asthma in mice

Michele Zeinieh, Rana Samadfam

Charles River laboratories, Montreal, Canada

Abstract

Allergic asthma is a chronic inflammatory disorder characterized by airway hyperresponsiveness, tissue remodeling, and inflammatory infiltration resulting in airway obstruction. The house dust mite (HDM), Dermatophagoides pteronyssinus, is associated with asthma. This study investigated the effect of acute HDM exposure on mice lungs inflammation.

Balb/c were subcutaneously sensitized with HDM/Alum (10 µg) on Day 1 and a boost was given on Day 8. They were then exposed to a repeated intranasal challenge of HDM (10 µg) on Days 10-13, 17-20, and 24-27. The model induction was not associated with any significant clinical signs. Mice were euthanized at Days 21, 28 (peak of inflammation), and 35 (resolution). A 90% increase in total immune cell infiltration was observed in the bronchoalveolar fluid on Days 21 and 28 after the respective last challenges (Days 20 and 27). Interestingly, 80% of the total cell count was constituted of eosinophils suggesting their major role in this model. Indeed, asthma induces a Th2 induced IL5 response to recruit eosinophils. Eosinophils significantly decreased at Day 35 one week after the last challenge at Day 27. Neutrophils, macrophages, and lymphocytes although significantly less pronounced followed the same pattern. In addition to cell infiltrate, increases in proinflammatory cytokines in BAL (IL5, IL4, IL-6 and TNF) were observed on Day 28.

In conclusion, HDM model of respiratory inflammation reliably recapitulates the inflammatory response observed in asthma. Therefore, this model represents an interesting translational tool in drug development focusing on respiratory diseases.

P274

The population dynamic and ecological responses of diverse *Pseudomonas aeruginosa* strains to oxidative stress

Taoran Fu ORCID iD, Michael Brockhurst ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

It is believed that pathogens experience a variety of environmental influences when adjusting to a new in-vivo environment during infection. However, there is still no clear answer to what the key within-host environmental factors are that drive the evolution of pathogenic bacteria and how bacteria adapt to these key factors, such as novel nutrients and stressors. In this study, we experimentally determined the population dynamics of three *Pseudomonas aeruginosa* strains under oxidative stress (OS) in a defined medium mimicking cystic fibrosis sputum. Surprisingly, LESB58, a clinical isolate from cystic fibrosis chronic infection, is particularly susceptible to hydrogen peroxide compared to both PAO1, a commonly used lab strain, and PA14, a highly virulent strain. Between the last 2 strains, the more sensitive one, PA14, failed to adapt to OS in a 12-day serial transfer experiment. 89% (n=9) of PA14 lineages failed to adapt under OS while PAO1 maintained a high population density until the end of the evolution experiment. In addition, the extinction of PA14 lineages due to OS is rescued by reduced pH. Future studies will strive to better comprehend the interaction between population dynamics and ecological responses of *P. aeruginosa* strains to adapt to oxidative stress.

P275

Changing the Gastrointestinal Transit Time in Healthy Volunteers Affects Gut Health Through Changes in the Microbiome and Bile Acid Pool.

Evette Hillman ORCID iD[1,2], Danielle Carson[1], Sjoerd Rijpkema[1], Elizabeth Wellington[2], Ramesh Arasaradnam[2,3], Stephen Lewis[4,5]

[1]Medicines and Healthcare products Regulatory Agency, London, United Kingdom. [2]The University of Warwick, Coventry, United Kingdom. [3]University Hospital Coventry & Warwickshire, Coventry, United Kingdom. [4]University Hospitals Plymouth NHS Trust, Plymouth, United Kingdom. [5]University of Plymouth, Plymouth, United Kingdom

Abstract

Changes in gastrointestinal transit time have been associated with a number of diseases such as: Parkinson's disease where sufferers usually experience constipation, irritable bowel syndrome which can present as constipation and/or diarrhoea, and colorectal cancer associated with reduced motility. The composition of the gut microbiome and its activity are major factors in gut health and often affect gut transit time. Nevertheless, the impact of the gut transit time on the microbiome and bile acid metabolism is not fully understood.

Healthy volunteers were administered Loperamide an anti-diarrhoea medication, or Senna, a laxative for 6 days. Analysis of the gut microbiome and bile acid composition and markers of bile acid synthesis were analysed.

We observed that a reduction of the gut transit time led to an increase of both primary and secondary bile acids in the stool, and the hormone FGF19 that regulates *de novo* bile acid synthesis was elevated following the administration of Senna. We also show that slowing gut transit by administration of Loperamide increases species richness of the microbiome and a significant increase of the probiotic species *Bifidobacterium dentium*. By contrast, administration of Senna resulted in a 6-fold elevation of specific species associated with dysbiosis such as *Ruminococcus gnavus*, while *B. dentium* decreased 23-fold. Lastly, we found that gut transit times significantly correlated with the level of bile acids, key microbiota species, and bacterial bile acid transforming genes in stool samples.

These findings could help elucidate the role of gut transit time in different disorders.

P276

In-vitro Fermentation of Choline in a Human Colon Model Reveals High Variation in Donors' Capacity to Produce Trimethylamine

Barbora Nemeckova[1], Priscilla Day-Walsh[1], Jasmine Percival[1], Emad Shehata[1,2], Shikha Saha[1], George Savva[1], Wendy Hollands[1], Arjan Narbad[1], Paul Kroon[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Chemistry of Flavour and Aroma Dept, National Research Centre, Giza, Egypt

Abstract

Plasma trimethylamine N-oxide (TMAO) is associated with cardiovascular disease. TMAO can be derived from choline that is metabolised by the gut microbiota into trimethylamine (TMA), and then converted into TMAO in the liver. The aim is to understand the relationship of individual's capacity to produce TMA from choline by the gut microbiota and their plasma TMAO measurements.

In-vitro fermentation of faecal samples from 24 human donors was undertaken in a batch colon model to investigate the metabolism of 2 mM choline by the gut microbiota over 48 hours under anaerobic conditions, constant stirring at 37ºC and pH 6.6-7. Plasma TMAO was measured from blood samples donated on the same occasion as faeces used for colon models. Fermentation samples and plasma were analysed by LC-MS method using isotopically labelled internal standards of methylamine compounds to measure choline and TMA concentration.

The time it took to utilise choline *in-vitro* varied, with most participants metabolising choline between 24 and 36 hours. However, there was no significant difference in the rate of choline utilisation per hour between fast and slow metabolisers. There was a distinct group of participants who showed limited TMA production. Furthermore, the rate of choline utilisation measured from *in-vitro* experiments showed no correlation with mean plasma TMAO levels measured in the same subjects.

In-vitro fermentation of choline into TMA is highly variable and is not correlated with plasma TMAO levels. Investigating the *in-vitro* fermentation of other TMA precursors could aid our understanding of their correlation with TMAO values.

P277

What are bacteria doing in our lungs? : Effect of commensals vs pathogens on the lung epithelium

Anuradha Ekanayake ORCID iD[1], Angela Oates ORCID iD[1], Simon Hart ORCID iD[1,2], Laura Sadofsky ORCID iD[1]

[1]Centre for Biomedicine, Hull York Medical School, Hull, United Kingdom. [2]Hull University Teaching Hospitals NHS Trust, Hull, United Kingdom

Abstract

It is inarguably accepted that microorganisms and humans depend on each other. With the advancement of microbiome research, this has become clearer. Microbiome research has come a long way, especially gut microbiome research leading to the application of microbial therapy for human diseases. On the other hand, the respiratory microbiome is comparatively still in its infancy. Although the human respiratory microbiome is being studied, and a core microbiome is not yet compiled, some of the most common bacteria are well known while their role is being investigated.

We aim to investigate the relationship of these commonly identified bacteria as a commensal or a pathogen with human lung epithelium. For this purpose, we are maintaining human alveolar cell line A549, in an air-liquid interface (ALI). This helps cells differentiate in a manner that closely resembles the human alveolar epithelium. These maintained ALI would be subjected to transepithelial electrical resistance (TEER) measurements to monitor electrical resistance across the simulated epithelium with time to understand when it is most stable. We aim to introduce two bacterial species, Staphylococcus aureus and Pseudomonas aeruginosa to represent commensals and pathogens, respectively. This will be conducted as a mono-infection study as a preliminary step.

Changes to cell health (viability), adherence, and invasion of bacteria to the epithelial ALI will be measured. Cytokine levels are to be measured (Abcam cytokine antibody array) in addition, to understanding the differences or similarities of the molecular pathways that are activated/deactivated with each type of bacteria (Ingenuity pathway analysis, Qiagen).

P278

Identification of a novel pathway for trimethylamine *N*-oxide metabolism in *Klebsiella* spp.

Samuel Dawson ORCID iD, Jack C. Leo ORCID iD, Lesley Hoyles ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Trimethylamine *N*-oxide (TMAO) is an organic osmolyte found in fish and other foods. Elevated serum levels of TMAO have been linked to an increased risk of cardiovascular disease, while lower levels have been shown to have protective effects on the blood-brain barrier and metabolic health. In the human gut, bacteria metabolise TMAO to trimethylamine (TMA), which is then transported to the liver and converted back to TMAO before being excreted in urine. The protein TorA, predicted to be encoded by *Escherichia* and *Klebsiella* spp., was reported to drive TMAO to TMA metabolism in the human gut. Literature review determined that there were several pathways by which gut bacteria could convert TMAO to TMA. Our bioinformatic analyses of ~36,000 human-gut-associated bacteria showed that TorA was present in 14 of 9,898 of the *Klebsiella* genomes examined. Instead, the cytoplasmic molybdoenzyme BisC was found to be highly prevalent in *Klebsiella* spp. BisC has previously been shown to metabolise biotin sulfoxide and methionine sulfoxide. Due to the level of amino acid similarity (63.3 %) between BisC and TorZ, another molybdoenzyme that can metabolise TMAO, we hypothesised that BisC had TMAO reductase activity. The BisC enzyme from a caecal isolate of *K. pneumonia*e has been purified. Our results suggest that *K. pneumoniae* BisC can reduce TMAO under anaerobic conditions. Informing our laboratory work with bioinformatic analyses has demonstrated the importance of validating in silico predictions experimentally and has allowed us to develop a greater understanding of processes driving bacterial TMAO metabolism in the human gut.

P279

Bugs, Bad Smells and Pig Entrails: the Application of the Necrobiome as a Potential Tool for Estimating the Post-Mortem Interval.

Heather Angell ORCID iD, Christopher Rogers, Georgina Manning, Martin Khechara

University of Wolverhampton, Wolverhampton, United Kingdom

Abstract

Forensic taphonomy is a sub-discipline of forensic anthropology and has become a focal point for research in recent years. Research in this area is important to develop taphonomic models to assist with the estimation of the post-mortem interval (PMI) within the United Kingdom (UK). As the application of microbiology to forensic taphonomy continues to grow, necrobiomes have become a valuable potential tool for estimating the PMI especially when paired with further chemical analysis. An *in vitro* model has been established to isolate and differentiate bacteria which allows for the formation of struvite crystals through the detection of urease enzyme activity. Simultaneously, analysis of volatile organic compounds (VOCs) produced by organisms present in the gastrointestinal (GI) tract, particularly those identified from the necrobiome and allow for the formation of struvite crystals, was carried out through gas chromatography-mass spectrometry (GC-MS). VOCs were sampled *in vitro* from pure liquid and plate-based cultures to establish baseline profiles for each organism. Porcine analogues were used in an indoor and outdoor environment, *in vivo* and *ex vivo* measurements of VOCs were sampled during varying stages of decomposition and compared to the in vitro findings, leading to the identification of organisms present during different stages of decomposition and the presence of organisms which allow for the formation of struvite crystals. This research has the potential to contribute to a taphonomic model to help estimate the PMI within the UK.

P280

Effect of Mannan-rich fraction supplementation on commercial layer cecal microbiome during peak lay

Robert Leigh ORCID iD[1], Aoife Corrigan[2], Richard Murphy[2], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Kildare, Ireland. [2]Alltech, Dunboyne, Meath, Ireland

Abstract

Alternatives to prophylactic antibiotic use in agriculture is required to maintain healthy and high performing animals. Prebiotics can be used to augment the compositional cecal microbiota of agriculturally relevant animals. While the gut microbiota of broiler hens has been well established and successfully correlated to performance, relatively few studies have been completed on the composition or prebiotic supplementation in mature laying hens during peak egg production. This study focused on establishing the impact of mannan rich fraction (MRF), on the cecal microbiota of peak laying hens.

Taxonomic richness (using the Chao1 α-diversity index) was significantly increased for all ranks and taxonomic evenness (using the Simpson's evenness α-diversity index) was significantly decreased for all taxonomic ranks in MRF-supplemented birds ($P<0.005$). Use of principal coordinate (using Bray-Curtis dissimilarity matrices; β-diversity) and principal component analyses found significant variation between treatment groups. When assessed for compositional uniformity (a flock health indicator) using DBSCAN, microbiota in MRF-supplemented birds was more uniform (displaying significantly less outliers) than control birds at the species level. The food-borne pathogen, *Campylobacter jejuni,* was significantly decreased in MRF-supplemented birds. From a performance perspective, species associated with high body mass were significantly decreased while butyrate and propionate producers were significantly increased in MRF-supplemented birds.

Prebiotic use may be a key factor in limiting agri-food chain pathogen persistence and in promoting uniformity. This study established increased α- and β-diversity indices in peak layers (determinants of pathogen mitigation and performance), increased compositional uniformity, decreased pathogenic bioburden, and increased performance correlators in MRF-supplemented birds.

P281

Microbiome Metabolites Drive Adult Neural Stem Cell Activity *in vitro*

Clio Dritsa, Katja Muecklisch ORCID iD, Donal Wall ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Dysregulation of the gut microbiome has been implicated in metabolic and neurological disease via the gut-brain axis. Identifying microbiome-derived compounds mediating these effects remains an important topic of research. Previous research identified novel microbiome-derived metabolites produced by Lachnospiraceae, 3-M-4-TMAB and 4-TMAP, in both the gut and brain of mice. The metabolites structurally mimic carnitine, colocalize with carnitine and inhibit carnitine-mediated mitochondrial fatty acid oxidation (FAO) in white matter cells. FAO is implicated in regulating cellular proliferation and fate of neural stem cells (NSCs), and cultured NSCs permit *in vitro* modelling of nervous system development and diseases. Considering that defects in FAO are recognised as important risk factors in neurological diseases, it is critical to elucidate further the function of metabolites with the potential to inhibit FAO and brain function. This project investigates the effect of these metabolites and FAO inhibitors on NSCs *in vitro*. NSCs were expanded into proliferating and quiescent states using appropriate growth factors and nutrient-rich media. Confocal microscopy allowed quantification of proliferation with cell cycle marker Ki67. Data analysis did not reveal significant differences between treatments within activity states, but significant differences were noted post-treatment between proliferating and quiescent conditions. In future experiments, 3-M-4-TMAB and 4-TMAP will be administered to germ-free and specific pathogen-free mice to determine their effects on the brain *in vivo* using advanced imaging techniques such as mass spectrometry imaging.

P282

Monitoring of the gut microbiome in the course of selected cancers in mice

Bogumiła Szponar, Aleksandra Czajkowska, Magdalena Mielczarek, Joanna Wietrzyk

Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Abstract

The composition and metabolism of the intestinal microbiome are strongly related to the diet. An intake of dietary fiber results in a production of short-chain fatty acids (SCFA), contributing to the anti-inflammatory reaction by participation in maintaining a balance between the suppression of inflammatory mediators (TNFα, IL1β, IL6) and the induction of anti-inflammatory cytokine (IL10).

The SCFA levels were determined in stool of mice with colorectal cancer and a control group to assess microbiota homeostasis disorders related to a cancer progression, as well as an influence of high-fiber diet on the concentration of SCFA in feces and cancer progression. Analytical methods (HPLC with UV/Vis) and NGS sequencing were applied.

A diet enriched with cellulose had a positive effect on an intestinal abundance of *Akkermansia muciniphila* in cancer-affected animals. *Lactobacillus* abundance decreased when diet enriched by 20% cellulose was used. Animals consuming cellulose-rich diet reveal a lower *Firmicutes:Bacteroidetes* ratio comparing to group on standard diet.

A diet with potato starch caused an increase of *Bifidobacterium* and *Faecalibaculum*, and a decline in abundance of *Blautia*, *Peptocdoccus*, *Ruminococcus* UCG-010 and *Anaeroplasma*. The diet was the main modulator of the gut microbiome, moreover, a diet rich in potato starch significantly changed the SCFAs profile and increased their fecal concentration.

Session Topic: Genetics and genomics forum

P283

The evolution and international spread of extensively drug-resistant *Shigella sonnei*

Lewis Mason ORCID iD[1,2], David Greig[3], Lauren Cowley[4], Sally Partridge[5,6,7,8], Elena Martinez[7,9], Grace Blackwell[7,9], Charlotte Chong[2], P. Malaka de Silva[2], Rebecca Bengtsson[2], Jenny Draper[7,9], Andrew Ginn[7,8,9], Indy Sandaradura[6,7,9], Eby Sim[5,10,9], Jonathan Iredell[5,10,7,8], Vitali Sintchenko[5,6,7,8,11,12], Danielle Ingle[13], Benjamin Howden[13], Sophie Lefèvre[14], Elisabeth Njampeko[14], François-Xavier Weill[14], Pieter-Jan Ceyssens[15], Claire Jenkins[3], Kate Baker[1,2]

[1]NIHR HPRU in Gastrointestinal Infections at University of Liverpool, Liverpool, United Kingdom. [2]Department of Clinical Infection, Microbiology, and Immunology; Institute for Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [3]CJ/DG Gastro and Food Safety (One Health) Division, UK Health Security Agency, London, United Kingdom. [4]Milner Centre for Evolution, University of Bath, Bath, United Kingdom, Bath, United Kingdom. [5]Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, Sydney, Australia. [6]Western Sydney Local Health District, Sydney, Australia. [7]Faculty of Medicine and Health, University of Sydney, Sydney, Australia. [8]Sydney Infectious Diseases Institute, University of Sydney, Sydney, Australia. [9]New South Wales Health Pathology, New South Wales, Australia. [10]Western Sydney Local Health District, New South Wales, Australia. [11]New South Wales Health Pathology, New Sotuh Wales, Australia. [12]Centre for Infectious Diseases and Microbiology – Public Health, Institute for Clinical Pathology and Microbiology Research, Westmead Hospital, Sydney, Australia. [13]Department of Microbiology and Immunology, The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia. [14]Institut Pasteur, Université Paris Cité, Unité des Bactéries pathogènes entériques, Centre National de Référence des Escherichia coli, Shigella et Salmonella, Paris, France. [15]Division of Human Bacterial Diseases, Sciensano, Brussels, Belgium

Abstract

Shigella sonnei causes shigellosis, a severe gastrointestinal illness that is sexually transmissible among men who have sex with men (MSM). Multidrug resistance in *S. sonnei* is common and can include resistance to the World Health Organisation recommended treatment options, azithromycin, and ciprofloxacin. Recently, an MSM-associated outbreak of extended-spectrum β-lactamase producing, extensively drug-resistant *S. sonnei* was reported in the United Kingdom. Here, we aimed to identify the genetic basis, natural history, and international dissemination of the outbreak strain. Our genomic epidemiological analyses of 3,304 isolates from the United Kingdom, Australia, Belgium, France, and the United States of America revealed an internationally connected outbreak with a common, low fitness-cost resistance plasmid, previously observed in travel associated sublineages of *S. flexneri.* Our results highlight the persistent threat of horizontally transmitted antimicrobial resistance and the value of continuing to work towards early and open international sharing of genomic surveillance data.

P284

The Benefits of Global Genomic analysis of sporadic Salmonellosis – A case study with Salmonella Cotham associated with Salmonellosis in Children

henna irshad

UK Health Security Agency, London, United Kingdom

Abstract

Routine genomic sequencing for Salmonella has been in place at UKHSA since 2014, single nucleotide polymorphisms (SNP) or core genome multilocus sequencing typing (cgMLST) are used to detect genetically related strains for outbreak investigations. Certain serovars, such as S. Cotham, can cause invasive diseases and pose a clinical risk especially if young children are infected. Sporadic case transmissions can be difficult to trace due to a lack of local data. The aim of this study was to understand the possible source of sporadic S. Cotham cases in England using genomic sequencing in the context of global data.

An outbreak caused by the rare Salmonella serotype Cotham was first detected in 2012 in the United States. Research found that S. Cotham was linked to bearded dragons. There was a high percentage of infants which were infected with this very rare serotype.

S. Cotham isolates referred to the Salmonella reference laboratory between 2004-2021 were analysed in this study. Demographic data and travel information was analysed to assess trends and cgMLST of isolates was performed via Enterobase and mapped with globally available strains to understand the genetic diversity.

Further investigation could be done to see how many people were infected by Cotham. The limitation that we have with gastrointestinal infections is that the symptoms are self-limiting, which means that the illness is not reported accurately.

S. Cotham has the potential to lead to life threating infections. Therefore, the investigation of S. Cotham is important to help implement potential public health measures.

P285

Design and Use of Synthetic Recombination Vectors to Study the Activity of Φ24BInt

Mohammed Mohaisen ORCID iD[1], Heather Allison[2], Alan McCarthy[2]

[1]University of Anbar, Ramadi, Iraq. [2]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

Abstract

Synthetic biology takes a ground-up approach to the genetic engineering of cellular systems capable of building novel biomolecule components, networks, and pathways, while using these approaches to rewire and reprogram biological systems. Here we used a synthetic biological approach to create a recombination system for the identification of the essential nucleotides in attB sites that are required by Φ24B Int to promote DNA recombination. We have designed a vector that harbours both the attB and attP sites facing in opposite directions. These sites lie within a synthetically produced fragment of DNA that is 5271 bp long. The GFP reporter is placed within the vector so that it is expressed following a recombination event. Several vectors were designed in order to detect fluorescence from the recombination product in the in situ recombination assay. Depending on the orientation of the two att sites, the result was either a recombination producing a single recombined plasmid that enabled GFP expression, or recombination producing two circular DNA molecules without fluorescence detection. Recombination events could only occur once in the absence of a recombination directionality factor, which was not supplied in these experiments.

P286

A comparison of two *Acinetobacter* spp. with contrasting drug susceptibilities

Elizabeth M. Darby ORCID iD, Jessica M. A. Blair

University of Birmingham, Edgbaston, United Kingdom

Abstract

A. lwoffii and *A. baumannii* are the leading causes of *Acinetobacter*-derived bacteraemia in England and Wales, 33% and 18% respectively (PHE, 2022). Whilst both species are similar in the infections that they cause, they have markedly different antibiotic susceptibility profiles (PHE, 2022). *A. lwoffii* is almost pan-drug sensitive, whereas *A. baumannii* is frequently multi-drug and even pan-drug resistant, leading to life-threatening infections that are challenging to treat. It is currently unknown why these two species, that inhabit similar niches and cause similar infections, have such different responses to antibiotic treatment. Here, 4,809 *A. baumannii* and 38 *A. lwoffii* whole genome sequences were interrogated to investigate why *A. lwoffii* is more susceptible than *A. baumannii*. *A. lwoffii* encoded far fewer antibiotic resistance and efflux pump associated genes than *A. baumannii* explaining its greater antibiotic susceptibility. However, *A. lwoffii* encoded more DNA defense systems, indicating that *A. lwoffii* is more stringent about the DNA it maintains within its genome. Our results also show that *A. lwoffii* has a lower mutational frequency than *A. baumannii* and that *A. lwoffii* does not readily acquire mobile drug resistance genes. Understanding if *A. lwoffii* might develop drug resistance and the mechanisms of this will ensure appropriate treatment of *A. lwoffii* infections and shed light on how multi-drug resistant pathogens, such as *A. baumannii*, may have developed resistance.

P287

The (pit)fall and rise of the *Acinetobacter* empire: the Acinetobase

Adam Valcek ORCID iD[1,2], Chantal Philippe[3], Clémence Whiteway[1,2], Etienne Robino[4,2], Kristina Nesporova[1,2], Mona Bové[5], Tom Coenye[5], Tim De Pooter[6,7], Wouter De Coster[8,9], Mojca Strazisar[6,7], Johanna Kenyon[10], James Collier[11], Alexander Botzki[11], Charles Van der Henst[1,2]

[1]Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, Brussels, Belgium. [2]Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium. [3]Research Unit in the Biology of Microorganisms (URBM), NARILIS, Namur, Belgium. [4]Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, Brussles, Belgium. [5]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium. [6]Neuromics Support Facility, VIB Center for Molecular Neurology, Antwerp, Belgium. [7]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium. [8]Applied and Translational Neurogenomics Group, VIB Center for Molecular Neurology, Antwerp, Belgium. [9]Applied and Translational Neurogenomics Group, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium. [10]School of Biomedical Sciences, and Infection and Immunity Program, Institute of Health and Biomedical Innovation (IHBI), Faculty of Health, Queensland University of Technology, Brisbane, Australia. [11]VIB Bioinformatics Core, Ghent, Belgium

Abstract

Acinetobacter baumannii is a Gram-negative opportunistic pathogen, displaying resistance (intrinsic and acquired) to antibiotics, desiccation, and disinfectants. Since *A. baumannii* is being explored in epidemiological studies, but also regarding its persistence and virulence, we examined the limits of the plasticity and heterogeneity of *A. baumannii* strains. We have assembled the complete genomes of modern clinical isolates and broadly used strains of *A. baumannii*. Subsequently, we have evaluated genotypes (sequence types, capsule locus types, outer core lipooligosaccharide locus types, antimicrobial resistance genes and virulence genes) and the phenotypes (hemolytic and protease activity, capsule production, capsule thickness, macrocolony morphology, natural competence, and in vivo virulence). The whole-genome analysis revealed a high heterogeneity in capsule locus types and sequence types. The modern clinical isolates and the broadly used strains differed in colony morphology, cellular density, capsule production, natural transformability, and virulence. These differences between the currently circulating strains of *A. baumannii* and the broadly used strains could lead to strain-specific results if only a limited subset of non-modern established strains are used. As an answer to the high heterogeneity amongst *A. baumannii* strains, we propose a first comprehensive database called Acinetobase (https://acinetobase.vib.be/), which includes the bacterial strains and the associated phenotypic and genetic data. We have established the first database providing complex information such as genomic, phenotypic data and the strains of *Acinetobacter*. This new implementable repository, freely accessible to the entire community, allows selecting the best bacterial isolate(s) related to any biological question, using an efficient and fast exchange platform.

P288

Neisseria subflava strain KU1003-01 outcompetes *Neisseria gonorrhoeae* using a contact dependant mechanism.

Alan Calder ORCID iD, Lori Snyder ORCID iD

Kingston University, Kingston Upon Thames, United Kingdom

Abstract

Neisseria subflava is commensal and associated with human oropharyngeal colonisation, this species can however also colonise the human genitourinary tract. These same mucosal niches are sites for infection by the pathogen *Neisseria gonorrhoeae*. Competitive processes are known to occur at mucosal sites and commensals are known for their ability to interfere with a pathogen's potential for host colonisation. The aim of this study was to investigate whether *N. subflava* strain KU1003-01 outcompetes *N. gonorrhoeae* strain NCCP11945 in co-culture and whether the Type VI Secretion System plays a role in the competition.

The Type VI Secretion System in *N. subflava* strain KU1003-01 is composed of 13 core genes, present as two gene clusters. A bioinformatic analysis was carried out to identify *tssM*, a membrane protein gene essential for secretion system function. Using the kanamycin resistance gene (KAN) amplified from a EZ-Tn5 <KAN-2> kit, Overlap Extension PCR was used to generate a construct for *tssM* knockout. The final construct consisted of KAN flanked by sequences homologous to the KU1003-01 genome. The sequence upstream of KAN included the native *tssM* promoter, the sequence downstream included a neisserial DNA Uptake Sequence (DUS). Following transformation, *tssM* knockout was confirmed by Illumina whole genome sequencing.

Using the *tssM* knockout and wild type *N. subflava*, a significant difference ($P < 0.001$) was seen in recoverable *N. gonorrhoeae* CFU following co-culture assays. The findings indicate that *N. subflava* strain KU1003-01 outcompetes *N. gonorrhoeae* strain NCCP11945 and suggests the Type VI Secretion System plays a role in the competition.

P289

A recipe for success: Shotgun metagenomics for the skin microbiome

Iliana Serghiou ORCID iD[1,2,3], Matthew Dalby[1], Raymond Kiu[1], Rhiannon Evans[1], Dave Baker[1], Barry Murphy[3], Lindsay Hall[1], Mark Webber[1]

[1]QIB, Norwich, United Kingdom. [2]UEA, Norwich, United Kingdom. [3]Unilever, Bedford, United Kingdom

Abstract

The human skin microbiome is a complex ecosystem organised into communities, which have major contributions to health. Methods to study these community structures have been developed but have been largely limited to low-throughput quantification and short amplicon sequencing. Shotgun metagenomic sequencing has emerged as a preferred method for microbiome studies. However, the relatively low bacterial bioburden of skin makes obtaining enough DNA for shotgun metagenomic sequencing challenging. No commercialised kit protocol, which produces sufficient DNA from skin (including longer fragments) suitable for shotgun metagenomic sequencing, currently exists. Here we describe an optimised high-throughput DNA extraction, shotgun metagenomic sequencing, and analysis pipeline, which performed well on skin swabs from adults and babies. The pipeline established can effectively characterise the skin microbiota from bacterial skin swab samples with a cost end throughput suitable for larger longitudinal sets of samples. Application of this method to samples from mothers and babies will allow greater insights into community compositions and functional capacities of the skin microbiome.

P290

Conditional evolution of biocide tolerance in two nosocomial Gram-positive pathogens

Maria Solsona ORCID iD[1,2], Heather Felgate ORCID iD[1,2], Harsha Siani ORCID iD[3], Cynthia B. Whitchurch ORCID iD[1,4], Mark A. Webber ORCID iD[1,2]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [3]GAMA Healthcare Ltd., Hemel Hempstead, United Kingdom. [4]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

Abstract

Biocides are widely used to control and prevent healthcare-associated infections. The emergence of bacterial resistance to biocides and cross-resistance to antibiotics is a major concern. Understanding how nosocomial pathogens respond to biocidal agents is key to improve infection prevention and control.

A biofilm evolution model was used to study the evolutionary changes that occur in *Staphylococcus aureus* and *Enterococcus faecalis* with biocide exposure. Biofilm and planktonic lineages were exposed to sub-lethal concentrations of Chlorhexidine digluconate (CHG) and Octenidine dihydrochloride (OCT) in parallel for ≈250 generations.

Both pathogens were able to adapt significantly above the MIC of both biocides with planktonic lineages able to survive higher concentrations of CHG and OCT before growth was inhibited. Lower biofilm formation was seen in *E. faecalis* mutants after being stressed with CHG than with OCT. Similarly, for *S. aureus*, CHG exposure was consistently linked with low biofilm biomass. Evolved isolates had no major fitness deficit and low level changes to susceptibility to various other antimicrobials were observed after biocide exposure.

Sequencing of mutants identified various mutations including in genes associated with phospholipid synthesis. SNPs in *S. aureus* fatty acid kinase (*fakA*) and in *E. faecalis dak2* – a *fakA* homologue – were repeatedly isolated from independent lineages strongly suggesting a novel role in biocide tolerance.

This data shows important pathogens can adapt tolerance to two common biocides but that this has collateral impacts on biofilm formation, colony morphology and fitness. Genotyping revealed changes in phospholipid synthesis, which is consistent with the mechanism of action of both biocides.

P291

Characterising Genome Rearrangements within *Salmonella* Paratyphi A isolates associated with the Bacterial Carrier State

Alice Nisbet[1], Emma Waters[1], Marie Chattaway[2], Gemma Langridge[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]UKHSA, London, United Kingdom

Abstract

Enteric fever is the inclusive term for infections caused by *Salmonella enterica* serovar Typhi and serovars Paratyphi A, B and C: the typhoidal salmonellae. Humans can harbour typhoidal *Salmonella* asymptomatically and sporadically excrete bacteria, termed the carrier state. While the *S.* Typhi carrier state has been scrutinised, that of *S.* Paratyphi A has not, despite this pathogen accounting for >36% of enteric fever cases in endemic regions.

Large-scale chromosomal rearrangements have been shown to alter gene expression in clinically important bacteria, including in *S.* Typhi where they occur during chronic infections. In *Salmonella*, rearrangements typically occur around the seven ribosomal RNA operons, where genome fragments exhibit altered relative positions and/or orientations. These genome structures (GSs) are identified using long-read sequencing, which spans across highly repetitive regions into flanking DNA.

This project examined differences in GS patterns between *S.* Paratyphi A isolates responsible for acute disease, and those associated with convalescent, temporary and chronic carriage states (defined by the time since initial isolation from the carrier). Isolates were collected by UKHSA between 2004 and 2013, alongside corresponding short-read sequencing data. To characterise GS types, DNA was extracted from a subset of acute and carrier isolates using the RevoluGen PuriSpin Fire Monkey Kit. DNA underwent multiplexed, long-read sequencing on the Oxford Nanopore MinION, and GS analysis was performed using socru. Genetic relationships between isolates were also assessed.

Understanding genomic differences between *S.* Paratyphi A isolates provoking acute infection and bacterial carriage provides insights into the role GS plays in human carriage.

P292

Dissecting genetic determinants of meningococcal disease and carriage traits using high throughput phenotypic testing and Genome-wide association studies

Robeena Farzand[1], Mercy Kimani[1], Abdullahi Jama[1], Evangelos Mourkas[2], William Monteith[2], Megan De Ste Croix[1], Neelam Dave[1], Neil Oldfield[3], Jay Lucidarme[4], David Turner[3], Ray Borrow[4], Luisa Martinez-Pomares[3], Samuel Sheppard[2], Christopher Bayliss[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom. [4]UK Health Security Agency, Manchester, United Kingdom

Abstract

Meningitis is an infection of the protective membranes surrounding the brain and spinal cord. It is estimated to affect ~1.2 million people annually, with a death toll of ~ 135,000 people worldwide. Current policy in the UK is to generate whole genome sequences (WGS) of all invasive isolates. Large collections of meningococcal WGS have also been generated through carriage studies. Mining of these resources to identify genetic determinants of disease and carriage phenotypes using high throughput phenotypic testing and Genome-wide association studies (GWAS) and phase variation (PV) analyses will facilitate understanding of how these organisms spread and cause disease.

For high throughput phenotypic testing, we have adapted six assays, including adhesion to A549 cells, growth in minimal and enriched media, biofilm formation and sensitivity to serum. Assays have been performed on ~300 MenW cc11 and ~200 MenY cc23 isolates with equal proportions of disease and carriage isolates.

Statistical analyses with MenW isolates detected significant differences between disease and carriage isolates in multiple assays and between two closely-related MenW disease sub-lineages in the serum sensitivity assay. GWAS was performed utilising an elastic net model and the phenotypic data, phylogenetic trees and WGS. This model detected 55 significant hits and associations of specific variants with high/low values for specific phenotypes. PV analyses also detected associations between specific loci and phenotypic variation.

In summary we have established high-throughput assays for rapid phenotypic testing of multiple bacterial isolates and applied statistical analysis and GWAS/PV analyses to identify genetic determinants of disease-associated phenotype variation.

P293

Genomic diversity of *Campylobacter jejuni* strains within stool samples of gastroenteritis patients

Bilal Djeghout[1], Samuel Bloomfield[1], Steven Rudder[1], Ngozi Elumogo[2], Alison Mather[1], John Wain[1], Nicol Janecko[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Norfolk and Norwich University Hospital, Norwich, United Kingdom

Abstract

Campylobacter jejuni is a widespread pathogen with a complex ecology that necessitates adequate methods to define the genetic population diversity within a single specimen. This study was conducted on 92 genomes of *C. jejuni* strains isolated from stool of four gastroenteritis patients. Standard microbiological isolation followed by genome analysis using pangenomics, phylogenetics, and specific gene detection tools to determine sequence type (ST), resistance determinants and virulence.

Three patients were infected with a single *C. jejuni* ST: ST-21, ST-61, ST-2066 while one patient was infected with two distinct strains, ST-51 and ST-354. Each ST's pangenome contained 1406-1491 core genes and 231-264 accessory genes. From the same patient, isolates of the same ST shared 12-43 core non-recombinant SNPs and 0-20 frameshifts with each other. Statistically, neither the mutation nor the accessory genes were linked to a particular functional class. All STs included at least one resistance determinant, with ST-354 containing three. The number of virulence-associated genes varied amongst the isolates, ranging from 110-160 genes. All isolates contained genes for cytolethal distending toxin B gene (*cdtB*), Campylobacter adhesion to fibronectin F gene (*cadF*) , and the heat survival protein conferring gene (*htrB*) .

Our findings show that even within the same ST, the *C. jejuni* population within a single patient's stool was genetically diverse. Understanding the diversity of the *C. jejuni* population within a patient can inform future analytical approaches for public health investigations.

P294

Tempo and Mode in the Molecular Evolution of Viruses: A Taxonomy of Quantitative Differences in Parameters of Evolutionary Variations across Viruses.

Hanouf Alhamdan

Lancaster University, Lancaster, United Kingdom

Abstract

Tempo and Mode in Molecular Evolution of Viruses

Background

We aim to create a taxonomy of quantitative differences in parameters of evolutionary variation across viruses, in terms of Tempo (substitution rate) and Mode (selection pressure).

Methods

All viral Genbank sequences, containing coding sequences and collection dates, were parsed using biopython, and aligned to their reference sequences using Needle. Multiple sequence alignment was performed using MAFFT. Non-coding regions of genomes were removed.

Recombination analysis was performed using Simplot. Clock like behaviour was measured using Tempest. Substitution rate was measured using BEAST. Selection analysis was performed using SLR.

Results

The above methods produced 393 high quality alignments of less than 50kb with 10 to 50 sequences and at least 5 years of collection date range.

Selection Analysis 30% of alignments didn't show any selected sites and for the remainder, 1237 positively selected sites were present.

Molecular clock of the 60% of alignments that showed no recombination, 63% showed correlation coefficient > 0.5 in TempEst, indicating clocklike behaviour.

Substitution rate for those alignments with R > 0.5, substitution rates ranges from 1.45 E-3 to 1.55 E-7.

Conclusion

Segmented viruses behave differently in evolutionary parameters mentioned in results.

Recombinants signals appearance and substitution rates varied among various taxonomy levels.

density of selection sites/total sites within genomes showed bimodal distribution.

P295

ASSOCIATION BETWEEN PHASE VARIATION STATES AND MENINGOCOCCAL DISEASE AND CARRIAGE USING HIGH THROUGHPUT PHENOTYPIC TESTING

Mercy Kimai[1], Robeena Farzand[1], Evangelos Mourkas[2], Abdullahi Jama[1], William Monteith[3], Neelam Dave[1], Neil Oldfield[4], Jay Lucidarme[5], David Turner[4], Ray Borrow[5], Luisa Martinez-Pomares[4], Samuel Sheppard[2], Christopher Bayliss[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]University of Bath, Bath, United Kingdom. [4]University of Nottingham, Nottingham, United Kingdom. [5]UK Health Security Agency, Manchester, United Kingdom

Abstract

Neisseria meningitidis, an exclusive human pathogen, asymptomatically colonises the upper respiratory tract. However, it can invade and multiply systemically causing invasive meningococcal disease (IMD). Meningococcal virulence is driven by phenotypic differences that might result from genetic variation (allelic, accessory or phase variation) between lineages, sub-clones or arises during IMD.

IMD due to a novel MenW:cc11 strain expanded rapidly from 2009 with the emergence of a sub-strain in 2013 that was predominant by 2015. Phase variation (PV) is one of the process by which Neisseria meningitidis control expression of several genes involved in host adaptation. We have determined PV states for several outer membrane proteins (OMPs) and other PV genes on ~300 isolates to extend knowledge of the impact of PV on meningococcal disease and carriage traits. These isolates include equal proportions of disease isolates of the two MenW:cc11 sub-variants (termed the 'original'; and '2013' variants) and a mixed set of carriage isolates.

We will discuss the progress in establishing links between specific phase variable genes and the relative importance of PV as compared to other types of genetic variation in determining differences in phenotypes. Currently, our PV analyses have shown associations between some loci and phenotypic variation. We will also discuss the relevance of our findings for understanding how the MenW:cc11 lineage spreads within populations, causes disease and for the emergence of the 2013 sub-variant.

P296

Evolutionary Pressures Acting on Large High GC% Myxobacterial Genomes

Lewis Fisher, David Whitworth ORCID iD

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Myxobacteria are ubiquitous soil bacteria that are famous for social predation, secondary metabolite production, and having unusually large genomes of high GC% content. Bacterial genome evolution is thought to be driven by two major evolutionary dynamics - the 'Red Queen' dynamic, which rewards innovation and genome expansion, and the 'Black Queen' dynamic, which promotes specialism and genome streamlining. In theory, genome size is a balance between gene gain and gene loss, with larger genomes being indicative of Red Queen dynamics, and smaller genomes indicating the dominance of Black Queen dynamics.

We are investigating relationships between four interdependent factors (genome size, GC%, growth rate, prey range), which are likely to affect, and be affected by, Red and Black Queen dynamics. Via a series of experimental perturbations, we are attempting to engineer changes in the four factors defined above, with the goal of assessing their impacts on the other three factors. For instance, asking whether changing a myxobacterial genome's GC% content positively or negatively impacts the organism's growth rate, and whether a myxobacterium engineered to grow faster, exhibits increased genome streamlining? Currently, genes from the *mut* and *uvr* families are being targeted for genetic disruption, to (we expect) increase mutation frequency and mutational bias towards AT, and consequently change the genomic GC% content. Changes in GC% will be investigated by genome re-sequencing and strains with altered GC% will also be tested for changes in genome size, growth rate and prey range.

P297

Developing simulations of stochastic and persistent environmental selection barriers acting upon phase variable genes in the food borne pathogen *Campylobacter jejuni*

Jonathan Holmes[1], Christopher Bayliss[1], Michael V Tretyakov[2], Christopher Fallaize[2]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Phase variation is an important generator of phenotypic diversity across a range of bacterial species. Stochastic changes in gene expression, which can be mediated by the presence of hypermutable tracts, occurs during cell division and drives population wide phenotypic diversity. *Campylobacter jejuni*, a food borne pathogen and causative agent of gastroenteritis, contains a range of phase variable cell surface factors which may influence host colonisation and spread. Our aim is to model phenotypic diversity introduced through phase variation on bacterial populations and to determine whether this diversity has a role in adaptation of *Campylobacter jejuni* during infection and carriage. We aim to model this phenomenon by producing mathematical system models to understand the role of selection and non-selective bottleneck events on population structure and prevalence of particular PV genotypes. Through the use of an in silico model design, phase variation can be simulated under various complex scenarios. Using high volume and variety in silico data we have characterised the impact of selection and bottleneck events allowing the prediction of the environmental forces acting on an observed population. We have used evolving neural networks and machine learning on simulated data to untangle the complex stochastic events associated with non-selective bottlenecks in parallel with variable selection and mutation. Applying these methods to previously studied in vitro and in vivo examples highlights the validity of these models to real environmental stimuli acting upon observed bacterial populations and can allow predictions on how phase variation drives phenotypic diversity during infections.

P298

Understanding genome dynamics of *Streptomyces clavuligerus* during industrial fermentations

James Croxford ORCID iD[1], Benjamin Huckle[2], Iain Hunter[1], Paul Hoskisson ORCID iD[1]

[1]Strathclyde institute of pharmacy and biomedical sciences, Glasgow, United Kingdom. [2]GSK, Worthing, United Kingdom

Abstract

Endemic antimicrobial resistance has driven the requirement for the development of new antibiotics and a comprehensive understanding of the production of antimicrobials and their producing strains. Clavulanic acid (CA) is a β lactamase inhibitor administered alongside β lactam drugs to increase their efficacy and is synthesised industrially by *Streptomyces clavuligerus*. Decades of strain improvement through random mutagenesis have allowed the development of strains of *S. clavuligerus* that return higher yields of CA, often at the cost of genetic stability. Extensive genome sequencing efforts have shown that these strains have a dynamic genome organisation that may undergo genome rearrangements during the fermentation process, significantly affecting the production of CA. This work addresses critical problems regarding genomic dynamics of *S. clavuligerus* by investigating industrial strains during the fermentation processes, developing methods to isolate and track individual strains during the industrial process using techniques such as barcode sequencing (bar-seq). The work also links the genotype to various phenotypic traits of *S. clavuligerus*, including CA production, sporulation and nutrient uptake with a view of addressing key questions regarding the production of CA, with a view to using genetic intervention to stabilise the genome, thus returning higher and more consistent yields of CA.

P299

Large-scale genomic analysis reveals an association between the gonococcal β-lactamase plasmid, TEM alleles and gonococcal lineages

Tabea Elsener, Ana Cehovin, Christoph Tang

Sir William Dunn School of Pathology, Oxford University, Oxford, United Kingdom

Abstract

Neisseria gonorrhoeae (the gonococcus) is a significant health threat due to its high global disease burden and increasing antimicrobial resistance (AMR). The gonococcal β-lactamase plasmid p*bla* encodes a TEM β-lactamase that requires only one or two amino acid changes to become an extended-spectrum β-lactamase (ESBL) which would render the last resort treatment for gonorrhoea ineffective. p*bla* is a mobilizable plasmid that needs the gonococcal conjugative plasmid pConj to spread among bacteria.

Several variants of p*bla* exist, and little is known about their spread in gonococcal populations. We analysed available sequences of p*bla* and established a typing scheme based on distinctive patterns of gene presence/absence in the plasmid. Implementing the typing scheme, we assessed the distribution of p*bla* variants across 17,959 gonococcal isolates and identified three major variants circulating in gonococcal populations. We found that these variants are associated with different TEM alleles and show a distinct pattern in the gonococcal population. Our large-scale analysis revealed a specific association between p*bla* and variants of pConj, suggesting a co-dependency of the two plasmids for the spread of AMR.

Our p*bla* typing scheme allows robust analysis of the plasmid in databases of whole genome sequences and should enable prediction of whether a plasmid can be mobilized by pConj. Understanding the variation and distribution of p*bla* is essential for our ability to monitor and predict the spread of AMR in *N. gonorrhoeae*.

P300

Epigenetic Gene Regulation by the EcoKI Restriction Modification System in *Escherichia coli*

Yasmeen Althari, Marco Oggioni

university of leicester, Leicester, United Kingdom

Abstract

The EcoKI enzyme complex methylates the target sequence of (AACN6GTGC) across the *Escherichia coli* genome. Among 581 EcoKI target sequences in the *E. coli* genome, only eleven sites are positioned in or near a promoter region. We hypothesise that the EcoKI type I restriction-modification system (RMS) methylation of these sites could modulate gene expression via epigenetic changes.

In addition to known function of EcoKI as a host defence mechanism, we tested the system's ability to impact gene regulation upon methylation of target sequences. Synthetic DNA for three promoters were designed with the wild-type (wt) and altered EcoKI target site, inserted in a *lux* reporter plasmid and then into host strains with different EcoKI activities (intact system, *hsdM* –, or *hsdR* –). Luminescence measured as a proxy for gene expression showed that *argR* and *prpR* wt promoters were upregulated in a methylation-positive host. On the contrary, the *rnc* promoter was upregulated in the methylation-negative host. Comparing the expression between wt and modified EcoKI– promoters in a methylation-positive host indicated that *argR* and *prpR* promoters had higher expression from wt promoter. For the *rnc*, the expression was higher from the modified promoter. Cloning both wt and modified promoters in a methylation-negative host produced no significant difference in gene expression implying that the observed differences in expression were methylation dependant. This work documents a clear epigenetic effect of a non-phase variable type I RMS implying that these changes could occur in many bacterial genera as type I RMS are ubiquitous enzyme systems.

P301

Selected loss of type VI secretion systems in successful multi-drug resistant *Escherichia coli* clones

Elizabeth Cummins ORCID iD, Robert Moran ORCID iD, Ann Snaith ORCID iD, Rebecca Hall ORCID iD, Alan McNally ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

The repeated emergence of pandemic multi-drug resistant (MDR) *Escherichia coli* clones is a threat to public health globally. In recent work, drug resistant *E. coli* were shown to be capable of displacing commensal *E. coli* in the human gut. We hypothesise that there are three factors potentially responsible for the apparent competitive advantage of drug resistant *E. coli* clones: production of lytic phage or bacteriocins; enhanced metabolic capabilities; or the presence of a type VI secretion system (T6SS). We employed genomic and experimental approaches to investigate. First, we searched for T6SS apparatus genes across a curated dataset of over 20,000 genomes representing the full phylogenetic diversity of *E. coli*. This revealed large, non-phylogenetic variation in the presence of T6SS genes. No association was found between T6SS gene presence and MDR clones. However, multiple MDR clones have lost essential T6SS genes. We've shown that the pandemic MDR clone ST131-H30Rx lost the essential T6SS gene *tssM* when it was interrupted by insertion of ISEc12. To investigate the importance of this, a three-way competition assay was designed to assess the contact-killing capabilities of three strains - ST131-H30Rx, its drug-susceptible TssM-producing ancestor, and laboratory strain K-12 MG1655. Our results confirmed the competitive advantage of ST131-H30Rx in the absence of antibiotics, but its success was not due to the presence of phage, secreted proteins or contact-dependent killing. Our findings suggest that metabolic advantages therefore may be a key component in the success of MDR *E. coli* clone ST131-H30Rx.

P302

Investigating *Streptomyces clavuligerus* Linear Replicons for Improved Clavulanic Acid Production

Elmira Mohit ORCID iD[1], Paul Herron[2], Steve Kendrew[3], Benjamin Huckle[3]

[1]University of Strathclyde, GLASGOW, United Kingdom. [2]University of Strathclyde, Glasgow, United Kingdom. [3]GlaxoSmithKline, Worthing, United Kingdom

Abstract

Streptomyces clavuligerus (Sclav) produces clavulanic acid and is composed of four giant linear plasmids (GLPs) and its chromosome. Various genes essential for the maintenance of linear replicons, such as *tap* and *tpg* which encode telomeric terminal proteins, are found on three out of four GLPs. We will investigate plasmid-chromosome interactions to determine the role of *tap-tpg* and aim to cure GLPs for decreased metabolic burden and increased clavulanic acid production. Previous work demonstrated a circularised chromosome and loss of plasmid after cutting the largest GLP, pSCL4, potentially due to the absence of *tap-tpg*. To determine the role of *tap-tpg* in chromosomal and plasmid linearity, we tested their inactivation using CRISPR-dcas9 multiplexing, targeting *tap-tpg*$_4$ on pSCL4, *tap-tpg*$_3$ on pSCL3 and *tap-tpg*$_2$ on pSCL2. *Sclav* colonies were screened for the loss of replicon telomeres. Illumina sequencing showed loss of plasmids and telomeres in multiplexed mutant strains. Future work will focus on determining GLP copy numbers in the mutants and elucidating the mechanisms of telomere replication in these multi-replicon organisms to eventually create a plasmid-free strain.

P303

Staphylococcus haemolyticus – friend or foe?

Heather Felgate ORCID iD[1], Lisa Lamberte ORCID iD[2], Dheeraj Sethi[1,3], Gemma Langridge ORCID iD[1], John Wain ORCID iD[1,4], Paul Clarke ORCID iD[4,3], Ad Fluit ORCID iD[5], Willem van Schaik ORCID iD[2], Matt O'Shea[2], Lindsey Hall ORCID iD[1,6], Jorunn Pauline Cavanagh ORCID iD[7], Mark Webber ORCID iD[1,4]

[1]Quadram Institute, Norwich, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom. [3]Norfolk and Norwich University Hospital, Norwich, United Kingdom. [4]University of East Anglia, Norwich, United Kingdom. [5]University Medical Center Utrecht, Utrecht, Netherlands. [6]Technical University of Munich, Munich, Germany. [7]The Arctic University, Tromsoe, Norway

Abstract

Introduction: *Staphylococcus haemolyticus* is a common skin commensal, along with many other non-*aureus* Staphylococci (NAS). NAS are important in the skin microbiome especially in neonates, priming the innate immune system and inhibiting pathogens. *S. haemolyticus* is however also a major cause of Late Onset Sepsis (LOS) especially in very low-birth-weight infants. As well as increased hospital stays, LOS can result in ischaemia, necrotising enterocolitis, meningitis, cerebral palsy and other neurodevelopmental impairment. There are many studies showing *S. haemolyticus* is associated with disease, but the wider phylogeny of the organism remains unclear.

Methods: Using Illumina and MinION platforms, we have sequenced >800 *S. haemolyticus* from across Europe isolated between 1975-2022, combined with all available NCBI *S. haemolyticus* genomes to create a phylogenetic tree based on core gene alignment.

Results: We have created the largest phylogenetic tree on *S. haemolyticus* to date which shows significant variation in the species. Near-identical isolates were isolated from hospitals in different European countries with a conserved core genome of 1816 genes. Genes significantly associated with neonatal bloodstream infection included *copAB* and cinA involved in copper homeostasis. The presence of *mecA* and the IS*256* transposase was widespread demonstrating how linked resistance to beta-lactams and aminoglycosides has spread on a mobile element. Genes associated with teichoic acid and capsule biosynthesis were also significantly over-represented in isolates taken from blood.

Conclusions: This research identifies the diversity of genotypes of *S. haemolyticus* associated with nosocomial infection and helps understand the evolution of the species over time and in different locations.

P304

GENOMIC ARCHITECTURE AND VARIATION IN THE MULTI-COPY GENES OF MENINGOCOCCAL SEROGROUP Y AND W STRAINS

Abdullahi Jama[1], Neelam Dave[1], Robeena Farzand[1], Mercy Kimani[1], Luke Green[2], Christopher Bayliss[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Sheffield, Sheffield, United Kingdom

Abstract

Neisseria meningitidis (Nm) occasionally causes invasive infections but is normally detected in the nasopharynx of asymptomatic carriers. Invasive meningococcal disease (IMD) presents as meningitis and/or septicaemia. Multiple serogroups and lineages cause IMD; MenB. MenY and MenW predominate in the UK. Meningococcal genomes encode multiple copies of several surface-located proteins or antigens. Many of these genetic loci are also associated with large repeat arrays that are thought to exhibit high levels of variation. Using whole genome sequence and PCR-based analyses, the genomic organisation of the opa, pilC, maf and lipopolysaccharide-associated lgt genes were examined in isolates of the MenY cc23/cc174 and MenW cc11 clonal complexes.

Four loci encoding Opa proteins were present in the same locations in all genomes and frequently exhibited duplication and recombinatorial mosaic alleles. Conversely variation in repeat arrays flanking opa genes was infrequent. Two conserved loci encoded phase-variable (PV) pilC genes that were divergent in a central gene region. Multiple lgt genes were present in all of these clonal complexes but only lgtG was PV in the cc11 lineage whereas one or more lgt genes contained polyC or polyG tracts in other lineages. Three loci encoding Maf proteins were identified in three maf genomic islands (MGI) in the genome of cc23 and cc174 and exhibited variability in sequence and content of these due to recombination and deletions.

Genomic variability in these multiple copy genes demonstrates the plasticity and adaptive potential of surface molecules in meningococcal lineages. These traits that may facilitate spread, host persistence and invasiveness.

P305

Identification and analysis of iron uptake systems in a large database of *C. jejuni* and *C. coli* isolates

Molly Webster, Julian Ketley

University of Leicester, Leicester, United Kingdom

Abstract

Campylobacter jejuni and *C. coli* are Gram-negative, helical, microaerobes, considered to be the leading bacterial cause of human gastroenteritis. However, they can live commensally in many animals, notably chickens. The acquisition of the crucial nutrient, iron, which is limited in host niches, is key for successful intestinal colonisation across a range of hosts. A database of 18,784 *C. jejuni* and *C. coli* isolates were examined for the presence and allelic variation of known iron uptake systems and their transporters. This study has shown that the haem (*chuA*), Fe^{2+} (*feoB*), and rhodotorulic acid (*p19*) systems are 100% conserved across a large collection of isolates with the main variation occurring in the enterobactin and lactoferrin iron utilisation systems. There was a high level of allelic variation within the cfrB enterobactin receptor and multiple protein variants were identified, functional and non-functional. Although *cfrB* was 100% present, a functional CfrB variant was only identified in 36.6% of isolates, with many of these lacking the CfrB cognate esterase, cj1376. This resulted in only 18.9% of isolates possessing a fully functional CfrB enterobactin uptake system, contrasting to the 83.4% of isolates that possess a functional CfrA based enterobactin iron uptake system. Analysis of the source from which isolates were obtained and the distribution of enterobactin iron uptake genes they possess indicates a potential advantageous aspect of having certain combinations of these genes which merits further investigation.

P306

Investigating the linker histone in the yeast Saccharomyces cerevisiae

Reham Alnajjar, Alastair Fleming

Microbiology, Dublin, Ireland

Abstract

The genomic DNA in eukaryotic nuclei is packaged as chromatin.The fundamental chromatin subunit is the nucleosome, which consists of two each of the histone proteins,H2A,H2B,H3andH4, around which about146DNA base pairs are wrapped.This structure can be compacted further to form higher order chromatin structures.In higher eukaryotes,the linker histoneH1 is known to play a pivotal role in the higher order compaction of chromatin.Previous work has identified that Saccharomyces cerevisiae stationary-phase(SP)cultures comprise two cell populations.During yeast SP,the younger daughter cells form a quiescent(Q)cell population whilst the older mother cells form a non-quiescent population(NQ)of cells that undergo apoptosis and necrosis.It is the quiescent cell population that will remain viable duringSP and it is these cells that will re-enter the cell cycle upon nutrient addition to the media.In Saccharomyces cerevisiae,however,the identification of histoneH1 is controversial Although the Hho1 protein is proposed to be the yeast histoneH1,it has a structure distinct from the canonical histone H1 and its role in the yeast is poorly characterized Recently,another candidate which may function as yeastH1 has been identified, Hmo1p.In this project,I will investigate the roles of Hho1p and Hmo1p in yeast cells.The aim is to identify which protein is the most likely candidate to be the yeast linker histone H1.I will test the hypothesis that the role of Hho1p and/or Hmo1p will be in the quiescent population of cells formed during yeast stationary phase where it will function to compact chromatin and repress global gene transcription

P307

Diverse Carbohydrate-Active enZyme (CAZyme) repertoires associated with host preferences of *Pectobacteriaceae*

Emma Hobbs ORCID iD[1], Tracey Gloster ORCID iD[1], Leighton Pritchard ORCID iD[2]

[1]University of St Andrews, St Andrews, United Kingdom. [2]University of Strathclyde, Glasgow, United Kingdom

Abstract

Like all micro-organisms, plant pathogens depend on environmentally-derived nutrients and energy sources, such as complex carbohydrates. Niche-adapted microbes are expected to adapt to exploit environment-specific resources by developing appropriate metabolic and enzymatic capabilities for processing them. For instance, host-restricted plant pathogens might be expected to develop Carbohydrate-Active enZyme (CAZyme) repertoires targeting the liberation of simple sugars from complex molecules specific to their hosts. In this way, delineation of a newly-sequenced pathogen's CAZyme complement might help predict its host range, or potential for host jumps.

Using software developed within our group we predict, catalogue and analyse the CAZyme complements of phytopathogen genomes from the Family *Pectobacteriaceae*, including *Pectobacterium* and *Dickeya* spp. (soft-rot pathogens), and *Brenneria* and *Lonsdalea* spp. (pathogens of woody plants). We identify diverse and distinctive CAZyme repertoires of these Enterobacterial plant pathogens, including coevolving sets of CAZymes that distinguish between taxa and that are potentially associated with host range. In particular, we identify CAZymes that appear to be specific to pathogens of woody hosts. These may be useful novel candidates for engineering towards more efficient industrial processes and achieving net zero targets, such as breakdown of recalcitrant woody lignocellulosic material in the production of biofuels.

P308

Diversity of Coriobacteriia associated with the mammalian gut microbiota

Onnicha Leelastwattanagul ORCID iD, Lesley Hoyles ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Bacteria belonging to the class Coriobacteriia, within the phylum Actinobacteria, are prevalent within the gut microbiota of a range of animals but their diversity is poorly understood. Culturomics studies and recovery of metagenome-assembled genomes (MAGs) from datasets has led to the identification of over 200 new species of Coriobacteriia. However, the taxonomy of this class is complicated, because 16S rRNA gene-based analyses are not sensitive and no single study has included all known species associated with the taxon. Consequently, some species are misclassified, a problem compounded by inaccurate reporting of species descriptions in the literature. The aim of this study was to accurately define the taxonomy of the class Coriobacteriia, particularly in relation to the families *Atopobiaceae*, *Coriobacteriaceae* and *Eggerthellaceae*. We collected 258 genomes (95 isolates and 160 MAGs) and, where available, associated 16S rRNA gene sequences of representative Coriobacteriia from NCBI and GTDB. Only 152 genomes were of high quality (>90 % completeness, <5 % contamination). Phylogenetic and functional (eggNOG-mapper) analyses were undertaken. Phylogenetic trees based on 16S rRNA gene sequences or whole-genome sequences showed the novel families RUG844 and QAMH01 belong to the family *Eggerthellaceae*, which the novel family UMGS124 clusters within the family *Coriobacteriaceae*. Principal component analysis of COG profiles also confirmed the affiliation of RUG844 and QAMH01, and UMGS124 with *Eggerthellaceae* and *Coriobacteriaceae*, respectively. In conclusion, greater care needs to be taken when incorporating novel species derived from culturomic studies or MAGs into existing taxonomic frameworks.

P309

GENOMIC EPIDEMIOLOGY OF RECURRENT CLOSTRIDIOIDES DIFFICILE INFECTION IN WESTERN AUSTRALIA

Daniel Knight ORCID iD, Thomas Riley ORCID iD

School of Biomedical Sciences, University of Western Australia, Perth, Australia

Abstract

Background: Recurrent cases of Clostridioides difficile infection (rCDI) remain one of the most common and serious challenges faced in the management of CDI. The accurate distinction between a relapse (caused by infection with the same strain) and reinfection (caused by a new strain) has implications for infection control and prevention, and patient therapy.

Methods: Here we used whole-genome sequencing to investigate the fine-scale epidemiology of 94 C. difficile isolates from 38 patients with rCDI in Western Australia.

Results: The C. difficile strain population comprised 13 sequence types (STs) led by ST2 (36.1%), ST8 (19.1%) and ST34 (11.7%). Among 38 patients clinically classified as having rCDI, core genome SNP (cgSNP) typing revealed 27 (71%) with primary and recurring isolates differing by ≤2 cgSNPs, suggesting a relapse of infection with the same initial strain, and 8 with isolates differing by ≥3 cgSNPs, suggesting reinfection. Almost half of patients with WGS confirmed CDI relapse suffered a recurrent episode that occurred outside the 8-week (56-day) boundary of the formal definition of rCDI. Other key findings included (i) potential C. difficile transmission events between epidemiologically unrelated patients; (ii) persistence of a single C. difficile clone in a patient for more than 2 years; (iii) clustering of C. difficile isolates from rCDI cases and environmental sources; and (iii) within-host strain diversity characterised by loss/gain of moxifloxacin resistance.

Conclusions: Genomics can better differentiate relapse from reinfection than conventional typing approaches. Current definitions based on the timing of recurrence to distinguish between relapse and reinfection require reconsideration.

P310

GENOMIC EPIDEMIOLOGY OF PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS PATIENTS IN WESTERN AUSTRALIA

Daniel Knight ORCID iD, Thomas Riley ORCID iD

School of Biomedical Sciences, University of Western Australia, Perth, Australia

Abstract

Background: Pseudomonas aeruginosa (PSA) is the most prevalent respiratory pathogen and a significant cause of morbidity in patients with cystic fibrosis (CF). Multi-resistant PSA is common in CF and cross-infection between patients with CF has been well documented.

Aim: This study aims to establish the first program of genomic and phenotypic surveillance for PSA in patients with CF in Western Australia (WA), providing clinically useful information to assist in timely infection prevention and control implementation, and improve patient outcomes.

Methods: The first dataset of 142 PSA isolates were collected from the sputum of 71 patients (two each) with CF in Sir Charles Gairdner Hospital (SCGH, Perth, WA) between January 2017 and May 2018. Strains were characterised for (i) evolutionary relatedness by multilocus sequence typing and whole-genome split kmer analysis, and (ii) AMR by in vitro susceptibility testing and in silico genotyping.

Results: The PSA strain population comprised 63 sequence types (STs) with ST775 (AUS-2, 17.6%) and ST649 (AUS-1, 6.3%) most prevalent. Overall, 70% of patients harbored two strains of the same ST. Whole-genome analysis of AUS-1 and AUS-2 subsets found no evidence of direct patient-to-patient transmission. Overall, 75% of PSA isolates were multiple antimicrobial-resistant and >750 AMR loci (31 types, 8 classes) were distributed across the strain population.

Conclusions: Genomic typing permits fine-scale analysis of pathogen transmission dynamics and AMR, providing insights into the effectiveness of existing infection prevention and control policies within healthcare. Our results show no evidence of direct PSA cross-infection amongst patients attending the SCGH CF centre.

P311

ONE HEALTH-FOCUSED MOLECULAR AND GENOMIC EPIDEMIOLOGY OF CLOSTRIDIOIDES DIFFICILE ISOLATED FROM DOMESTICATED HORSES IN WESTERN AUSTRALIA.

Daniel Knight ORCID iD[1], Thomas Riley ORCID iD[1], Natasha Hain-Saunders[2]

[1]School of Biomedical Sciences, University of Western Australia, Perth, Australia. [2]Harry Butler Institute, Murdoch University, Perth, Australia

Abstract

Background: Clostridiodes (Clostridium) difficile is a major cause of gastrointestinal disease in humans and animals, with considerable morbidity and mortality in horses. Increased antimicrobial resistance and evidence of zoonotic links suggests a novel One Health paradigm for C. difficile infection (CDI).

Aim: To better understand the epidemiology of C. difficile in Western Australian horses and the relationship between horse, human and environmental strains.

Methods: Faecal samples from 494 Western Australian domestic horses were cultured for C. difficile and isolates characterised by PCR ribotyping. Whole-genome sequencing and split k-mer analysis was performed on a subset of 45 ribotype (RT) 012 equine, human and environmental strains. A closed reference genome for an RT012 equine strain was generated by illumina and Oxford Nanopore Technology sequencing hybrid assembly.

Results: C. difficile was isolated from 27.9% of samples (n=133). Both novel (42.8%) and previously described (57.1%) toxigenic RTs were identified, including RTs 012 (n=14), 014/020 (n=10), 087 (n=7), 002 (n=5) and 023 (n=3), all prominent in human CDI. Whole-genome analysis identified a phylogenetic cluster of 10 closely related RT 012 strains of equine, human and environmental origin (separated by 0 to 62 SNPs; average 23) indicating recent shared ancestry. Possible evidence of clonal inter-species transmission or common source exposure was identified for a subgroup of three horse and one human isolates.

Conclusions: This study provides insight into the relationships between horse, human and environmental isolates of C. difficile and highlights the need for a One Health approach to C. difficile surveillance and antimicrobial resistance investigations.

P312

Revisiting LES prophages genomes inside *P. aeruginosa* PAO1 under single and co-infection

Enrique González-Tortuero ORCID iD[1], Revathy Krishnamurthi ORCID iD[2], Ian Goodhead ORCID iD[1], Jo Fothergill ORCID iD[2], Heather Allison ORCID iD[2], Chloe James ORCID iD[1]

[1]University of Salford, Salford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Pseudomonas aeruginosa Liverpool Epidemic Strain (LES) is a significant cause of mortality and morbidity in cystic fibrosis patients. Their increased fitness and survival in infection models are related to the presence of five active prophages, which are not well characterized due to the high percentage (~76.5%) of their hypothetical genes. Additionally, the combined influence of the co-habiting LES prophages on the success of their bacterial host is not well studied. Here, we created single-, double- and triple-lysogen variants of the well-characterised *P. aeruginosa* strain PAO1 after infection of combinations of the LES prophages Φ2, Φ3 and Φ4. High-quality genomic sequences of the different *P. aeruginosa* PAO1 lysogens and the LES prophages Φ2, Φ3 and Φ4 were retrieved. All this information will be relevant for RNAseq experiments to map the differential expression gene profiles of the LES prophages and predict the metabolic and physiological changes induced by these phages under inducing and non-inducing conditions. Moreover, these findings will be key for the role of temperate phages and for improving our understanding of host-phage coevolution.

P313

Exploring the distribution of mobile DNA in Avian Pathogenic E. coli (APEC) and its impact on population structure

Charlotte Birdsall, Roberto La Ragione, Jai Mehat

University of Surrey, Guildford, United Kingdom

Abstract

Background: Avian pathogenic E. coli (APEC) is an important extra-intestinal pathogen of birds, causing major economic losses to the poultry sector, and posing a substantial threat to global food security. APEC and human extra-intestinal pathogenic E. coli (ExPEC) are genetically similar. Therefore, understanding the determinants and evolutionary constraints that facilitate the emergence of pathogenicity in APEC is of paramount importance for both pathotypes. Our laboratory has previously demonstrated that APEC is comprised of multiple, independent genetic lineages. The aim of this study was to investigate the distribution of mobile DNA, such as plasmids and integrons in APEC and assess their impact on shaping APEC population structure.

Methods: 387 APEC isolates, recovered from chickens at post mortem examination, were sequenced and screened for the presence of AMR and APEC-specific virulence-associated genes using Abricate. Rfplasmid was employed to classify contigs as either plasmidic or chromosomal.

Results: The avian-associated phylogroup G was determined to encode a high burden of plasmid-encoded integrons relative to other phylogroups. These integrons confer resistance to 3 different classes of antimicrobials: sulfonamides, aminoglycosides and trimethoprim. Phylogroup B2 was predicted to be comprised of two broad MDR lineages: one of which encoded AMR genes on plasmids and the other on the chromosome.

Conclusion: The findings indicate significant genomic structural variations between APEC phylogroups, from a perspective of how virulence and AMR genes are encoded, which may influence transmissibility. Future studies will aim to investigate the diversity and transmissibility of plasmids between phylogroups and their influence on APEC fitness.

P314

Using a novel "artificial fitness gradient" to unravel the effects of population-wide fitness variation on the cost of antibiotic resistance.

Jack Duxbury Barber ORCID iD, Tom Vogwill ORCID iD

University of Aberdeen, Aberdeen, United Kingdom

Abstract

Understanding microbial evolutionary dynamics is key to anticipating and countering the emergence of antimicrobial resistance (AMR). However, the disconnect between *in vivo* observations of the fitness cost bacteria pay to maintain resistance in the absence of antibiotics and the cost observed during *in vitro* evolutionary experiments remains a longstanding challenge in AMR research. One potential explanatory factor for this discrepancy is the wide variation in fitness within "real-world" bacterial populations which could produce a corresponding variation in the cost of antibiotic resistance and the total lack of variation in fitness within clonal lab populations of bacteria. We aim to investigate the effect of fitness variation within bacterial populations on the cost of antibiotic resistance; to do this, we will create an "artificial fitness gradient" using classical experimental evolution techniques. By isolating populations of *E. coli* at regular time-points over a period of adaptation to unfavourable growth conditions, multiple sub-populations with variations in fitness will be generated. Each of these sub-populations will be made antibiotic-resistant, and the fitness cost of antibiotic resistance for the different sub-populations will then be measured. We anticipate that fitter sub-populations will pay a higher cost to evolve and maintain antibiotic resistance, and less-fit sub-populations will pay a comparatively lower cost. Any such variation across the artificial fitness gradient will confirm the hypothesis that variations in fitness within bacterial populations affect the cost of antibiotic resistance and will help to unravel why *in vitro* experimental evolution work fails to capture the true fitness cost of antibiotic resistance.

P315

In silico quantitative assessment of insertion sequences in *Acinetobacter baumannii* in comparison to other ESKAPE pathogens and *Escherichia coli*

Kristina Nesporova[1,2,3], Clémence Whiteway[1,2], Adam Valcek[1,2], Charles Van der Henst[1,2]

[1]Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, Brussels, Belgium.
[2]Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Brussels, Belgium, Brussels, Belgium.
[3]Current affiliation: Microbial Systems Cell Biology Group, Department of Biology, KU Leuven, Leuven, Belgium

Abstract

Background: *Acinetobacter baumannii* is an opportunistic pathogen prioritized for further research. There are well-described examples of how insertion sequences (ISs) play a role in the transfer of antibiotic resistance and in the overexpression of genes encoding resistance in *A. baumannii*. However, the ISs are still not well studied generally and quantitatively in *A. baumannii*.

Materials/methods: We performed analysis using the ISfinder database on the set of 413 *A. baumannii* complete genomes (47 from our collection and 366 from NCBI). Additionally, we did the same analysis for *Escherichia coli* and remaining ESKAPE pathogens (254-1933 complete genomes/species).

Results: We detected 103 different ISs in *A. baumannii* from which 67 were found in *A. baumannii* only. On average, we found 7.65 different ISs per genome and 33.18 ISs per genome counting all copies which placed *A. baumannii* as fourth from seven included bacterial species regarding ISs quantity after *Enterococcus faecium*, *E. coli* and *Klebsiella pneumoniae*. A total of 16 ISs were found to be present in more than 10% of *A. baumannii* genomes while only nine of them belonged to the IS*Aba*-type while five of them were detected in all five Gram-negative species.

Conclusions: Surprisingly and contradicting previous studies, the prevalence of ISs in *A. baumannii* is not high compared to *E. coli* and *K. pneumoniae*. On the other hand, the collection of different ISs found in *A. baumannii* is broader than expected and deserves a deeper investigation.

P316

High throughput annotation of a year worth of published Vibriophages genomes: a meta-analysis

Antoine Culot

Rime Bioinformatics, Palaiseau, France

Abstract

Vibrio spp. are responsible for several major fish diseases, leading to heavy loss in aquaculture farms. They represent a promising target for phage therapy. In-depth study of phages genomes is a key step to assess their safety before therapeutic use. However, phage genomes have different structures than their bacterial hosts, such as a higher coding density (CD) and smaller, overlapping genes.

179 published Vibriophage genomes were analyzed using a high throughput pipeline designed to screen for therapy compatible phages in large collections. To ensure repeatable results, multiple heuristics and databases were used for structural and functional annotations.

The published annotations yielded 24% of functionally annotated genes on average, and an 89% average coding density. After the new analysis, +6% new genes were detected on average, and 49% of the genes had functional annotation. The newly detected genes were small (215 bp on average; average overall gene size was 590 bp), and +9% overlapping genes were detected. Published mean intergenic region size (IRS) was 93 bp on average and decreased to 75 bp after the new study. Eventually, the average CD was +2.14% higher (92%) after our analysis.

Those results prove that the average quality of phage genome annotations can be strongly improved using our high throughput pipeline. However, 49% of functionally annotated genes is not enough to prove the absence of harmful genes (such as antibiotic resistance or virulence) in a genome. This pipeline is best suited to screen a large collection, before a deeper annotation.

P317

Diversity and Prevalence of Type VI Secretion System Effectors in Clinical *Pseudomonas aeruginosa* Isolates

Luca Robinson ORCID iD[1], Alice Collins ORCID iD[1], Ronan Murphy ORCID iD[1], Jane Davies ORCID iD[1,2], Luke Allsopp ORCID iD[1]

[1]National Heart and Lung Institute, Imperial College London, London, United Kingdom. [2]Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, Sydney Street, London, United Kingdom

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that commonly infects people with Cystic Fibrosis. The type VI secretion system (T6SS) is a molecular nanomachine that translocates effectors into target cells or the extracellular environment enabling intermicrobial interaction. *P. aeruginosa* encodes three T6SS clusters (H1-, H2- and H3-T6SS) and several orphan islands. Genetic diversity of T6SS-secreted effectors has been noted in reference *P. aeruginosa* strains but has yet to be explored in clinical isolates. Thus, we performed an in-depth bioinformatic comparative genomic analysis of T6SS effector genes in 52 high-quality clinical *P. aeruginosa* genomes from Cystic Fibrosis patients and housed in the Personalised Approach to *P. aeruginosa* strain repository. Several characterised *vgrG* and *PAAR* islands were missing from numerous isolates and disruption of T6SS genomic loci through mobile genetic element insertions was observed. We identified the orphan *vgrG7* island in strain PAK and five clinical isolates that contains a gene encoding a putative Tle2 lipase effector. We also identified genes encoding eight new putative T6SS effectors with the following putative functions: cytidine deaminase, lipase, metallopeptidase, NADase, and pyocin. Finally, we suggest the existence of core and accessory effectors through prevalence analysis of identified T6SS effectors in 532 *P. aeruginosa* genomes. Our comprehensive *in silico* study of the *P. aeruginosa* T6SS exposes a level of genetic diversity at T6SS loci not seen to date within *P. aeruginosa*, particularly in isolates from people with Cystic Fibrosis and provides a path for future experimental characterisation.

P318

Widespread divergent transcription arising from bidirectional promoters in prokaryotes

Emily Warman, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

Promoters are DNA sequences that stimulate the initiation of RNA synthesis. Bacterial promoters typically contain an AT-rich -10 promoter element, alongside additional sequences such as the -35 element. The arrangement of these elements dictates in which orientation RNA polymerase will bind to DNA. Thus, it has long been assumed that promoters in bacteria are directional.

We initially identified several promoters in *E. coli* where the direction of transcription was unclear. Many of these promoters were able to drive transcription in both directions; we define these as bidirectional promoters. A genome-wide analysis revealed that promoters in *E. coli* are frequently bidirectional. Mechanistically, this occurs because the -10 element has inherent symmetry and often overlaps on opposite DNA strands. Reciprocal stimulation between divergent transcription start sites also contributes. Evidence of bidirectional promoters is observed in multiple diverse prokaryotic species, including examples from archaea. This suggests that bidirectionality is a common feature of prokaryotic promoters, which has important implications for understanding gene regulation.

P319

The FIB replicon of plasmid F

Georgina Lloyd, Elton Stephens, Christopher Thomas

University of Birmingham, Birmingham, United Kingdom

Abstract

Plasmid F, archetype of the largest plasmid group in clinical Enterobacteriaceae, is one of the best studied examples of a multi-replicon plasmid. Displacing such plasmids may reduce antibiotic resistance in commensal bacteria of humans and animals. For F-like plasmids our plasmid-displacement strategy, blocking replication and neutralising addiction, is generally successful but with conjugative IncP-1 plasmid RK2 as vector for our "anti-F cassette", displacement of F'prolac was inefficient unless vector copy-number was raised 1.5- to 2-fold. By raising the copy number of different parts of the cassette we showed that it is the anti-FIB segment that needs potentiation. Then, by deleting the FIB rep, we showed that rep in the anti-F cassette is responsible for activating the FIB replicon that is partially defective in F due to the combination of a sub-optimal rep ribosome-binding-site and the weak CTG start codon. With single replicon plasmids, all three F replicon types (FIA from F; FIB and FII from pO157) were displaced efficiently by the complete anti-F cassette without potentiation. However, displacement of an F-FIB single replicon plasmid with its RBS mutated to be like the pO157 RBS still needs potentiation, suggesting that sequence divergence between F and pO157 has weakened incompatibility. Critically, deletion of the FIB rep gene from the anti-F cassette still improved displacement efficiency against F-FIB. These results show that small divergences can significantly influence the interactions between related low copy number plasmids, especially when Rep has both positive and negative influence on replication.

P320

Detecting in life influenza viral titres in mice by real time qPCR

Yenenesh Tekletsion[1], Sandy Kimber[2], Tina Rea-Davies[2], Matt Miller[2], Claire Richards[2], Caitlin Jukes[2], Louise Brackenbury[2]

[1]Charles River Discovery,, Bristol, United Kingdom. [2]Charles River Discovery, Bristol, United Kingdom

Abstract

Background

Sensitive methods to detect viral shedding 'in-life' are key to assessing the efficacy of novel antivirals and immunomodulators over time, whilst minimizing animal usage. To track influenza A/PR8/34 (H1N1) infection in mice, a qPCR method was established to detect viral load from oral and anal swabs.

Methods

Real time RT-PCR for influenza A virus detection was optimised using primers and probes designed against the M1 segment. A standard curve was generated from 10-fold serially diluted gBlock gene fragments. The lower range of sensitivity was 10 viral copies/mL (95% confidence interval). Following intra-nasal infection of BALB/c mice with 25xTCID50, oral and anal swabs were taken daily from day 0 to 6 to monitor viral shedding. Data was compared to standard lung viral titre assessment at termination using the Reed and Muench method by a TCID50 assay.

Results

Bodyweights, clinical scores, and survival data are presented together with a comparison of in life viral titres by qPCR in comparison to lung TCID50 viral titres.

Conclusion

By using qPCR in place of traditional lung viral titre assessment, group sizes can be decreased to refine animal usage. The non-invasive sampling technique also improves animal welfare. Here we demonstrate that by using qPCR, we show enhanced sensitivity and a more detailed kinetic analysis over time when compared to standard endpoint TCID50 assessment.

P321

Comparative description of incidence demographics and genetic composition of CC1, CC2, CC8 and CC9 *L. monocytogenes* clones.

Emily Fotopoulou

LONDON, LONDON, United Kingdom

Abstract

Listeria monocytogenes is a foodborne pathogen causing systemic listeriosis in the elderly, immunocompromised, and pregnant women and babies. At the UK Health Security Agency (UKHSA) genome sequencing is used for typing *L. monocytogenes* isolates from clinical cases, food, and food-production environments. The UKHSA surveillance database integrates genomes with associated metadata.

There are four lineages within *L. monocytogenes*, with lineages I and II being the most abundant in humans. The majority of isolates within lineage I belong to CC1 or CC2, whereas lineage II is commonly represented by CC9 and CC8. Data analysis, phylogenies and gene mapping were utilised for an in-depth comparison of the population structure and genetic composition of CC9 (n=360), CC2 (n=339), CC1 (n=261) and CC8 (n=223).

Human isolates are over-represented in CC1 (CC1=68%, CC8=31%, CC2=21%, CC9=15%), whereas CC8 and CC9 have the highest proportion of food isolates (CC9=66%, CC8=62%, CC2=42%, CC1=22%). Age-sex distribution revealed higher infection in females (CC1=59%, CC2=56%, CC9=51%, CC8=50%) and in the 70-90 years old age group in all CCs. Lineage I comprises a higher proportion of isolates that have LIPI-I and LIPI-3 pathogenicity islands (LIPI-1 CC1=90%, CC8=98%; LIPI-3, CC1=40%, CC8=90%), compared to lineage II CC9 (LIPI-1 0%, LIPI-3 20%). CC8 exhibited the highest proportion of virulence genes. Further investigation is ongoing to characterise and provide context on these virulence profiles.

Integrating genomic and epidemiological data enables us to understand how the repertoire of virulence genes within each clonal complex contributes to the clinical outcomes associated with vulnerable patients in different risk groups.

P322

An Experimental Model System to Explore the Evolutionary Consequences of CRISPR-Cas Acquisition to a Naïve Host

Josie Elliott[1], Bridget Watson[2], Edze Westra[2], Tiffany Taylor[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Exeter, Penryn, United Kingdom

Abstract

CRISPR-Cas defence systems are akin to an adaptive immune system, allowing the acquisition of resistance against invading foreign DNA, including phages. While CRISPR-Cas systems facilitate heritable acquired immunity, they also interact with the inflow of beneficial horizontally acquired genes and incur metabolic growth costs. Thus, although widespread, CRISPR-Cas systems are not ubiquitous across bacteria, and the ecological and evolutionary drivers that govern their prevalence and maintenance are currently unknown. Phylogenetic evidence shows that bacterial anti-viral defence systems are frequently exchanged via horizontal gene transfer (HGT). A high rate of HGT provides a potential mechanism to maintain CRISPR-Cas systems at low levels within populations, dispersing the fitness costs of maintaining CRISPR-Cas systems between hosts. However, the adaptive forces that select for the maintenance of, and adaptation to, horizontally acquired CRISPR-Cas systems remains understudied. We have modelled an HGT event by creating a synthetic, modular, minimal version of the CRISPR-Cas system from the opportunistic pathogen Pseudomonas aeruginosa and integrating it into the genome of the common soil bacterium Pseudomonas fluorescens, which lacks CRISPR-Cas. We use an experimental evolution approach to explore how the acquisition of a CRISPR-Cas system affects the co-evolutionary dynamics between P. fluorescens (SBW25) and its phage Φ2 – a well-studied model for rapid, reciprocal, antagonist arms race dynamics. This work offers a model system to test the long term, dynamic evolutionary consequences of CRISPR-Cas HGT events into naïve hosts, potentially allowing us to predict what abiotic and biotic selection factors are required for successful acquisition of new anti-viral systems.

P323

Characterising the evolutionary relationship, domain organisation, and structural similarity of *Pseudomonas* RpoN-dependent enhancer-binding proteins

Alan Rice, Tiffany Taylor

Milner Centre for Evolution, Department of Life Sciences, University of Bath, Bath, United Kingdom

Abstract

Success across a broad niche range relies on cellular processes interacting with the external environment, such as motility, virulence, biofilm formation, and nitrogen assimilation. Pseudomonas, a genus of gram-negative bacteria, has found such success, occupying a range of niches from human pathogen to growth-promoting diazotroph. Gene regulatory networks (GRNs) control gene expression allowing it to be fine-tuned in response to environmental conditions. This control facilitates the adaptation of a genus such as Pseudomonas to a wide range of habitats. In particular, sigma factor 54 and its associated network of RpoN-dependent enhancer-binding proteins (RpoN-EBPs) are critical to the transcription of genes involved in these external functions. In the Taylor lab, we have a tractable experimental system in P. fluorescens, where we explore the drivers of GRN rewiring and adaptation between RpoN-EBPs. In gene deletion experiments, functional redundancy is observed between RpoN-EBP family members. However, one of the obstacles in extrapolating experimental outcomes of loss-of-function mutations from one species to another is variation in RpoN-EBPs across Pseudomonas species. Here, we comprehensively characterise the evolutionary relationship, domain organisation, and structural similarity of RpoN-EBP members in Pseudomonas. Our results highlight the shared traits of this protein family and evaluate the extent of variation present. This work provides a foundation for future work on regulation, function, redundancy potential, and variation in GRN architecture between RpoN-EBPs in Pseudomonas species.

P324

The Population Structure, Antimicrobial Resistance, and Virulence Potential of *Klebsiella variicola* Isolated from a Household in Tanzania

Sally Felle ORCID iD, Alison MacFadyen, Katarina Oravcova

University of Glasgow, Glasgow, United Kingdom

Abstract

Klebsiella variicola has been found in a large array of environments. It is an emerging, opportunistic pathogen in humans and animals. Studies show *K. variicola* can cause bloodstream infections with a higher mortality then infections with *Klebsiella pneumoniae*. Antimicrobial resistance (AMR) in *K. variicola* is also a growing problem. However, little is known about its molecular epidemiology in Tanzania. This study aims to address this gap using whole genome sequence analysis.

Bacterial isolates were obtained from the environment within the Maasai community in Tanzania. Whole genome sequencing revealed 18 isolates to be *K. variicola*, obtained from dogs and milk tank swabs (MTS). Bioinformatic analysis was used to identify population structure, AMR genes, virulence genes and predicted plasmids.

A *K. variicola* specific MLST scheme identified 8 of the isolates as having a novel MLST. The phylogenetic relationship of the isolates was determined using a core genome alignment. The isolates formed three clades. Within the two dog associated clades were isolates obtained from MTS, suggesting overlap between the environments. All of the isolates had multiple virulence and AMR genes. Most isolates had multiple plasmid replicons, with putative plasmids being identified. When compared to global samples, the isolates from this study were closely related to isolates found in clinical environments.

The findings from this study indicate that the *K. variicola* isolates obtained from the environment of this household have the capacity to cause infections. Highlighting the importance of continued monitoring of *K. variicola* within the environment, as well as clinical settings.

P325

SNP variant analysis of multiple *Helicobacter pylori* subpopulations reveals different evolutionary histories.

Ebony Cave, Dr Sandra Beleza

University of Leicester, Leicester, United Kingdom

Abstract

Helicobacter pylori is a gut bacterium that has coevolved with humans for over 50,000 years and presently infects approximately half of the global population. Infection is associated with an increased risk of gastric diseases, including cancer. The population of Cabo Verde is unique because the people have combined African and European ancestry. Previous population genetic analysis identified four admixed subpopulations of *H. pylori*. These were mainly of African ancestry and European ancestry, along with the emergence of two clonal groups with predominantly European ancestry. This study aims to provide insights into each subpopulation's evolutionary dynamics, such as investigating their demographic history and selection modes. Genomes of 538 *H. pylori* isolates, from 178 asymptomatic Cabo Verdean hosts, were aligned to the European reference genome 26695 to identify SNP variants. Analysis with Tajima's D indicates different long-term evolutionary dynamics for African and European lineages versus the clonal lineages. The latter show signs of recent population expansion and positive/negative selection. Fixation indexes show that the two clonal groups are more divergent than any other pair of population groups, and sliding-window analyses reveal genetic regions of high and low divergence. With this, we have established that populations of *H. pylori* with different ancestries in Cabo Verde do indeed have different evolutionary histories. We conclude that this bacterial population structure should be a key consideration when running genotype-phenotype association analyses in gastric disease in this population.

P326

In vitro evolution of *Enterococcus faecium* in the presence of vancomycin

Freya Allen, Ross McInnes, Willem van Schaik

University of Birmingham, Birmingham, United Kingdom

Abstract

Enterococcus faecium is an important nosocomial pathogen which can cause a variety of infections. Treatment options for these infections can be greatly limited due to the ability of *E. faecium* to acquire resistance to the last line therapeutic, vancomycin, through horizontal gene transfer of a resistance transposon. To identify other mechanisms by which *E. faecium* can tolerate vancomycin, an *in vitro* evolution experiment was conducted. The vancomycin-susceptible *E. faecium* strain E1162 was grown in the presence of a range of vancomycin concentrations above and below the minimum inhibitory concentration (MIC) and incubated at 37 °C for 24 h. Growth was assessed and the culture that grew at the highest concentration of vancomycin was inoculated into fresh media with increasing concentrations of vancomycin. This was repeated each day for 11 d and an increase in tolerance was observed as *E. faecium* was able to grow in 1 µg/ml vancomycin in comparison to 0.5 µg/ml at the start of the experiment. Morphological changes were observed in the evolved strain in comparison to the parent strain and genome sequencing of evolved strains revealed a single nucleotide polymorphism in WalK (a kinase in a two-component system [WalKR] involved in the regulation of peptidoglycan biosynthesis) and YycI (a protein predicted to be involved in regulating WalK activity). As mutations in WalKR have been associated with increased tolerance to antibiotics such as daptomycin and vancomycin in *Staphylococcus aureus*, we postulate that exposure to sub-MIC vancomycin may cause increased tolerance to last-resort antibiotics in *E. faecium*.

P327

Involvement of the global regulator, CRP, in the expression of virulence determinants in the early stages of enteroaggregative *Escherichia coli* infection.

Munirah Alhammadi[1,2], James Haycocks[1], Sophie Titman[1], Niamh Hawes[1], Douglas Browning[3], David Grainger[1], Stephen Busby[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom. [2]Biology Department, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. [3]College of Health & Life Sciences, Aston University, Birmingham, United Kingdom

Abstract

Enteroaggregative Escherichia coli (EAEC) is a pathovar that likely evolved from commensal *E. coli* by acquisition of virulence genes. To understand how EAEC initiates infection, it is important to consider its behaviour in nutritionally challenging conditions. One of the best studied transcription regulators involved in nutritional stress responses in *E. coli* is the cyclic AMP receptor protein (CRP), a global transcription factor that regulates over 200 transcription units. One important EAEC virulence determinant is a mucinase, encoded by the *pic* gene, encoded on the EAEC chromosome, and previously, we showed that the expression of this gene depends on CRP. To identify other CRP-regulated virulence genes, we have used chromatin immunoprecipitation, together with high-throughput sequencing (ChIP-seq), to locate the full complement of DNA sites for CRP in EAEC strain 042. Our results identify more than 300 CRP targets in the EAEC 042 strain genome, with over 30 targets unique to EAEC. At some of these targets, CRP plays a regulatory role, whereas, at others, CRP appears to play a role in chromosome organisation. Our data argue that many bacterial pathogens use CRP to cope with challenging conditions and to assure the appropriate production of virulence factors in the early stages of infection.

P328

MULTI-DRUG RESISTANT ESCHERICHIA COLI DERIVED FROM PIGS AND POULTRY IN TANZANIA

Ruth Maganga ORCID iD[1], Conjester Mtemisika[2], Stephen Mshana[3], Willem van Schaik[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Catholic University of Health and Allied Sciences, Mwanza, Tanzania, United Republic of. [3]University of Birmingham, Mwanza, Tanzania, United Republic of

Abstract

Antimicrobial resistance is a threat to human and animal health. Inadequate veterinary services and the ubiquity of antibiotics in Tanzania may cause indiscriminate use of antibiotics among Tanzanian poultry and pig farmers, imposing selective pressure on gastrointestinal commensals and pathogens like E. coli. As a bio-indicator of antimicrobial resistance, Escherichia coli plays an important ecological role within resistant bacteria populations. Using this characteristic of E. coli, the present study investigates the prevalence of antimicrobial resistance and cross-species transmission of the bacteria in Tanzanian pigs and poultry. A total of 117 strains of E. coli were cultivated on Luria-Bertani media before being sequenced by illuminate sequencing. Of the 117 sequenced, 39 strains originated from broilers, 36 from layers, and 42 from pigs. About 46 resistance profiles were identified based on preliminary results. Tetracycline (9.89%), beta-lactamase (blaTEM-1) (6.67%), aminoglycoside (aph(6)-Id) (6.55%), and streptomycin resistance (aadA1) (6.55%) were among the most frequently detected resistance genes in E. coli isolates, whereas the remaining resistance genes had frequencies of less than 5%. Among the ST types, ST1196 had a dominant prevalence of 12.82%, followed by ST155 at 7.69%, ST101 at 5.98%, ST48 at 4.27%, and ST 711 at 3.42%. The remaining ST types all had frequency rates under 3%. The majority of isolates (40.87%) are members of phylogenetic group B1, followed by groups A (39.13%., D (5.22%), F (5.22%), G (3.48%), E (2.61%), and C (0.87%). The study confirms the presence of resistant E. coli strains amongst pigs and poultry in Tanzania.

P329

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P330

Understanding the role of genetic background in determining the evolutionary outcome of gene regulatory network rewiring events

Mitchell Reynolds, Tiffany Taylor

University of Bath, Bath, United Kingdom

Abstract

Previous work in the Taylor lab, using motility rescue as a model, has shown rapid and repeatable rewiring between the nitrogen and flagellar networks in *Pseudomonas fluorescens*. In the absence of the flagellar master regulator (FleQ), NtrC (response regulator associated with nitrogen uptake) is co-opted to resurrect motility. My research aims to explore the role of global regulatory network architecture, and the wider genome, in determining gene regulatory rewiring pathways. Initial work explores whether flagellar motility rescue can be repeatably achieved in other gram-negative bacteria, and the rewiring routes capable of this. We aim to delete the flagellar master regulator across several different bacteria: *Pseudomonas aeruginosa, Caulobacter crescentus, Vibrio cholerae and Escherichia coli*, which represent a diverse arrangement of flagellar regulatory networks. Replicate independent lines will be placed under strong selection for motility, and motile isolates sampled, and genome sequenced, to identify rewiring routes. Initial results from *P. aeruginosa* show flagellar motility rapidly evolved, similar to *P. fluorescens*. Isolates evolved slow motility after as early as 42 hours, with fast mutants emerging after as early as 66 hours. Evolved motile mutants will be genome sequenced to identify the rewiring routes used to regain motility. This work will contribute to a larger aim, which is to identify how differences in gene regulatory architecture determines the predictability of evolved rewiring pathways across different bacteria using motility rescue as a model system.

P331

Investigating Bacteriophage Dynamics in Intensive Care Patients using Novel Genomics Methods

Siu Fung Ho ORCID iD[1], Ann Snaith[1], Anna Casey[2], Alan McNally[1], Tony Whitehouse[1], Willem van Schaik[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]NIHR Surgical Reconstruction and Microbiology Research Centre, Birmingham, United Kingdom

Abstract

Critically ill patients are physiologically unstable and recent research suggests that the gut microbiome may play a critical role in the health of patients during their intensive care (ICU) stays. This is evidenced by a marked drop in microbial diversity, with pathogenic species, such as *Enterococcus faecium* and *Escherichia coli* dominating the gut (>80 % of metagenome). This is strongly associated with intravenous administration of meropenem. Bacteriophages are natural parasites of bacteria and thus can shape the composition, regulate the metabolism, evolution, and adaptability of the bacteriome in the gut. We therefore hypothesised that the bacteriophages that infect the dominating pathogen will also bloom in abundance, and that they may contribute to their hosts resistance to antibiotics via transduction. We extracted virus-like particles from stools samples of critically ill patients across their stay in the ICU and sequenced them using the Nanopore platform. These long reads were then assembled and filtered to retain viral operational taxonomic units (vOTUs). We also extracted whole community metagenomic DNA and shotgun sequenced them using Illumina technology to get contextual information on the bacteriome. The bacterial host and batceriophage data was then integrated with patient metadata to expose patterns of transmission and horizontal gene transfer. This study has shown the importance of the viral fraction of the gut microbiome in critically ill patients, as both a helper and a hinderance to their hosts.

P332

Vibriowatch: high quality genomes and metadata for *Vibrio cholerae*, with robust bioinformatics capability for virulence, antimicrobial resistance, finding related isolates, and identifying lineages

Avril Coghlan ORCID iD[1], Matthew J. Dorman ORCID iD[1], Sina Beier ORCID iD[1], Florent Lassalle ORCID iD[1], Corin Yeats ORCID iD[2], David M. Aanensen ORCID iD[2], Nicholas R. Thomson ORCID iD[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Centre for Genomic Pathogen Surveillance, Oxford, United Kingdom

Abstract

Since 1961 there has been a global cholera pandemic caused by *Vibrio cholerae*, involving many high-profile outbreaks and epidemics with substantial disease burden and mortality, including those in Haiti and Yemen. With the Haiti epidemic, it became clear that genome sequencing is useful for studying the *V. cholerae* responsible for an outbreak. It was then proposed it would be useful to have a "high quality genome database" for *V. cholerae*, with "both sequences and rich metadata", and that it was also important to "develop robust […] bioinformatics capability to rapidly generate and receive genomics-based data that can be turned into actionable public health knowledge" [Hendriksen *et al* 2011 PMID:21862630]. To achieve this, we have developed Vibriowatch, containing >5,000 published *V. cholerae* genomes, and manually-curated metadata from the literature, including strain names; place, date and source of isolation; and phenotypic data for biotype, serogroup, serotype and antimicrobial resistance. Vibriowatch's home is the Pathogen.watch website, where users can upload reads or assembled genomes for genotyping and comparative analysis of MLST, cgMLST, virulence, and antimicrobial resistance genes. Public and user genomes can be clustered via cgMLST allele similarity, and core SNP-based neighbour-joining trees built for collections of selected genomes. Using the PopPUNK software, Vibriowatch will assign isolates to lineages (e.g. the "epidemic causing" 7PET lineage). Such precise lineage definitions are important for monitoring, reporting and connecting cholera outbreaks globally. Vibriowatch is aimed for use by clinicians and public health workers studying cholera outbreaks, and for biologists investigating *V. cholerae* evolution.

P333

Urbanisation and human proximity contributes to the spread of antimicrobial resistance in wild bird species

Evangelos Mourkas ORCID iD[1,2], Jose Valdebenito ORCID iD[3], Tamás Székely ORCID iD[4], Ben Pascoe ORCID iD[5,1,2], Samuel Sheppard ORCID iD[1,2]

[1]University of Oxford, Oxford, United Kingdom. [2]Ineos Oxford Institute for Antimicrobial Resistance, Oxford, United Kingdom. [3]Universidad Austral de Chile, Valdivia, Chile. [4]University of Bath, Bath, United Kingdom. [5]Big Data Institute, Oxford, United Kingdom

Abstract

Campylobacter jejuni is a multi-host bacterial pathogen responsible for human diarrheal diseases and is frequently isolated from wild birds. While urbanization intensifies worldwide, more wild birds become adapted to urban environments, potentially increasing the risk of zoonotic pathogen transmission. The impact this is having on the microbiome of wild bird populations has not been characterised.

We combine microbial genomics techniques with an understanding of ecological and life-history traits to interrogate a large collection of *Campylobacter* genomes sampled from wild bird species. Our collection of more than 700 *C. jejuni* genomes, from 30 different wild bird species, were screened for the presence of antimicrobial resistance (AMR) genetic determinants to better understand the spread of antimicrobial resistance and potential for emergence of novel zoonotic pathogens. Spatial overlap analysis was used to measure bird proximity to urban habitats and phylogenetic generalized least squares models were used to detect associations between lineages, AMR, bird life-history traits and ecological variables.

C. jejuni populations were associated with either one (specialist) or multiple (generalist) wild bird species. Corvidae and Turdidae bird families demonstrated the greatest niche overlap with human habitation and harboured multiple *C. jejuni* lineages, that were often AMR. A positive correlation between proximity to urbanization and both lineage diversity and carriage of AMR determinants was noted for all bird species.

Understanding the epidemiological dynamics of zoonotic transmission and network of human-animal interactions will better inform us on the spread of antimicrobial resistance and help guide effective surveillance and control measures.

P334

Temperate phages of cystic fibrosis and non-cystic fibrosis bronchiectasis drive physiological changes in *Pseudomonas aeruginosa*.

Mohammad Tariq ORCID iD[1], Liberty Duignan[2], Audrey Perry[1], John Perry[3], Anthony De Soyza[3,4], Darren Smith[1]

[1]Northumbria university, Newcastle upon Tyne, United Kingdom. [2]Liverpool university, Liverpool, United Kingdom. [3]Newcastle upon Tyne NHS trust, Newcastle upon Tyne, United Kingdom. [4]Newcastle university, Newcastle upon Tyne, United Kingdom

Abstract

Pseudomonas aeruginosa (Pa) is an opportunistic respiratory pathogen of Bronchiectasis (BR) and Cystic Fibrosis (CF). Epithelial dysregulation associated with inflammation and scarring (CF, BR) and altered ion transfer through mutation of the CF conductance regulator gene (*cftr*) leads to a thick mucus environment linked to lowered lung function and poor clinical outcomes. Temperate bacteriophages integrate into the genomes of Pa and can carry genes that aid adaptation and selection in the lung. We aim to characterise and compare the genomes of these temperate phages that can mobilise from the bacteria to disseminate these genetic traits and function through their host range.

Twenty lysogens of PAO1 were created using phages induced from early and late CF and BR Pa isolates. Lysogeny was confirmed by sequencing and aligning to the naïve PAO1 host.

We show that temperate phages alter PAO1 susceptibility to subinhibitory concentrations of ceftazidime, colistin, meropenem and piperacillin. The lysogens were shown to alter Galleria mellonella survival in an infection model reducing virulence. Finally, lysogens of PAO1 have an influence on the metabolomic profiles. Comparing over 3K metabolites, 100 metabolites and 28 pathways were shown to be significantly different (p value <0.05). Some of the pathways were linked to pantothenate and CoA biosynthesis, valine, leucine and isoleucine biosynthesis, one carbon pool by folate and β-alanine metabolism.

This study illustrates, using PAO1 as a host we can infer the effects of adaptation, phage subversion and begin to look for the gene responsible for change in bacterial fitness, virulence, and AMR susceptibility.

P335

ChemGAPP; A Package for Chemical Genomics Analysis and Phenotypic Profiling.

Hannah Doherty ORCID iD[1], Manuel Banzhaf ORCID iD[1], Danesh Moradigaravand ORCID iD[2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Abstract

High-throughput chemical genomic screens produce vastly informative datasets, providing valuable insights into unknown gene function on a genome-wide level. Despite this, the extensive experimental data produced makes quality control and analysis difficult and time-consuming. Currently, there is no chemical genomics dedicated analytic package publicly available. Therefore, we produced ChemGAPP, a comprehensive analytic package to bridge this gap. ChemGAPP allows integration of various steps in a streamlined and user-friendly format. This includes rigorous quality control measures, handling noise, and accurately estimating fitness values under a variety of treatments. ChemGAPP provides three sub-packages for various types of chemical-genomic screens, varying in purpose, scope, and size: ChemGAPP Big, ChemGAPP Small and ChemGAPP GI. ChemGAPP Big, is specifically employed to handle large-scale high-throughput screens. Tested against the KEIO single mutant library in *Escherichia coli*, ChemGAPP Big revealed robust and reliable fitness scores, which displayed biologically relevant phenotypes. ChemGAPP Small, designed for the analysis of small-scale screens, demonstrated significant changes in *E. coli* phenotypes when tested on a small scale. ChemGAPP GI, analyses genetic interaction screen data and was benchmarked against three sets of genes with and without known positive and negative epistasis and successfully reproduced each interaction type. Together, the ChemGAPP sub-packages will enable the wider scientific community to use chemical genomics approaches to reveal significant, biologically relevant insights into gene function, drug mechanisms of action and antibiotic resistance mechanisms.

P336

Using next generation sequencing approaches to define the population biology of the neglected cystic fibrosis lung pathogen *Burkholderia multivorans*

Kasia Parfitt ORCID iD[1], Angharad Green ORCID iD[2], Thomas Connor ORCID iD[1], Daniel Neill ORCID iD[2], Eshwar Mahenthiralingam ORCID iD[1]

[1]Cardiff University, Cardiff, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Burkholderia multivorans is the most frequently isolated *Burkholderia cepacia* complex species recovered from cystic fibrosis lung infection. However, its pathogenesis and species population biology remain elusive. Understanding adaptational factors of *B. multivorans* to the CF lung microenvironment is important for predicting disease outcome.

B. multivorans population biology was explored using pan genome analysis, average nucleotide identity and phylogenomic analysis (n = 283). The population split into two genomic lineages, with four *B. multivorans* model strains selected to represent them: soil strain ATCC 17616, BCC1272, BCC0033, and BCC0084. The latter 3 CF strains were completely genome sequenced. Unique *B. multivorans* lineage-specific genes were identified through gene presence-absence for diagnostic PCR design. Genes *ghrB_1* and *glnM_2* selected as the lineage 1 and lineage 2 targets, respectively. PCR showed 100% lineage-specificity against 48 *B. multivorans* strains.

Phenotypic analysis was performed on a *B. multivorans* subset (n = 49) evaluating morphology, growth, motility, biofilm formation, and exopolysaccharide production. The *B. multivorans* phenotype was variable, with no link to genomic lineage. Phenotypic comparison was also performed when *B. multivorans* were mixed with a secondary CF pathogen. Suppression of *P. aeruginosa* LESB58 protease production was identified as an interesting interaction via an unknown mechanism. Three *B. multivorans* strains (BCC0033, BCC0084, and ATCC 17616) were also evaluated in a murine respiratory infection model and all showed good persistence over 5-days.

This work has built a foundation of knowledge on the *B. multivorans* phenotype and genotype, enabling associations between lineage, therapeutics testing, and clinical outcome to be studied.

P337

Characterisation and optimisation of the rumen microbiome

Alice Buckner[1], Laura Glendinning[2], Juan Palma Hidalgo[3], Jolanda van Munster[3], Mark Stevens[2], Mick Watson[2], Jamie Newbold[3]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom. [3]SRUC, Edinburgh, United Kingdom

Abstract

One way of tackling the increased demand for animal protein, while mitigating environmental impacts of production, is to improve the efficiency of the animal production system by focusing on ruminant digestion. Microbes in the rumen assist the host in breaking down ingesta to easily digestible products, but also produce methane that contributes to climate change. To better characterise the composition and functioning of this microbial ecosystem, this PhD project aims to use culture and metagenomic analysis to compile an efficient community of microbes that provide the host with the maximum amount of dietary-derived energy. Rumen fluid was cultured in three different, commonly used media at various dilutions before undergoing 16S rRNA sequencing, along with samples of the original rumen fluid. Initial results suggest that the three different media support distinct communities that cluster by principal component analysis, and the effect of media on community composition is greater than that of the donor cow. Technical replicates of cultures from the same cow and medium show similar community compositions indicating reproducibility of consistent communities. Next steps include applying statistical methods to further explore these initial observations, and to allow informed conclusions to be made. Following this, the genomic data obtained from the microbes that were unable to be cultured will be used to develop novel or adapted media specific for these microorganisms. This will aid in determining which rumen microbial communities digest feed more efficiently while producing less methane, and help improve the output of ruminant production systems.

P338

Next-generation sequencing of Crimean-Congo Haemorrhagic Fever Virus directly from clinical samples: a comparative study between targeted and non-targeted approaches.

Jake D'Addiego, Nadina Wand, Roger Hewson

UK Health Security Agency, Salisbury, United Kingdom

Abstract

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is an orthonairovirus associated with outbreaks across Europe, Middle East, Asia and Africa. Whilst most CCHF cases are subclinical, 12% are severe and sometimes fatal. Next-generation sequencing is poised to play a crucial role in characterising CCHFV genetic diversity in endemic regions. Low titre clinical samples present a challenge for the recovery of complete genome sequences. The aim of this study was to develop methodologies for direct sequencing of CCHFV genomes from clinical samples.

Sets of primers targeting 500bp fragments of CCHFV genomes were designed against Europe 1 sequences and used to enrich the CCHFV genome by PCR, following random hexamer cDNA synthesis. Sequencing libraries for nanopore and Illumina platforms were prepared according to the manufacturers' instructions. Sequencing metrics were determined utilizing SAMTOOLS.

Near complete genome sequences (>99% coverage) were recovered from tested samples up to Ct 31 utilizing the targeted enrichment protocol. In contrast, genome coverage following non-targeted enrichment dropped significantly for samples with low viral titres, after Ct 27. The utility of the amplicon enrichment protocol was demonstrated on Illumina sequencing platform, generating greater read depth (78.9% at 100x read depth) and the point-of-care MinION platform (74.9% at 100x read depth).

We conclude that our enrichment method outperforms metagenomic enrichment protocols for whole genome sequences recovery for samples with low virus titre, offering a tool to further study genetic determinants of virulence and disease severity of CCHFV. The method can be adapted to other clades of interest in the future.

P339

Development of a CRISPR/Cas9 GeCKO library screen to identify pro-viral cellular genes in chicken cells that can be exploited to control avian endemic viruses

Yana Chen[1,2], Abraham Lee[2], Lonneke Vervelde[2], Erica Bickerton[1], Finn Grey[2], Andrew Broadbent[3], Helena Maier[1]

[1]The Pirbright Institute, Surrey, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom. [3]The University of Maryland, MD, USA

Abstract

Infectious bronchitis virus (IBV) and infectious bursal disease virus (IBDV) are endemic in the UK and pose a threat to the poultry industry and food security. Despite their extensive usage, vaccination-based control approaches face a number of challenges, including rapid mutation and immune escape of the target virus. Since IBV and IBDV share similar activities in their replication cycles (e.g. contain RNA genomes, enter through endocytosis, and replicate in the cytoplasm), the aim of this project is to identify common host factors that are involved in chicken-virus interactions through a genome-scale CRISPR/Cas9 knockout (GeCKO) screen. The chicken fibroblast cell-line DF-1, which supports IBV and IBDV replication, was modified to stably express Cas9, and Cas9 activity was measured by flow cytometry and western blot. The editing ability of Cas9 expressing DF-1 clones was validated by creating gRNA-specific knock-out cells. A chicken lentivirus GeCKO library was generated and titrated. The screening strategy for cell-selection and sequencing, including FACS-based and cell survival-based screening, were optimized. We are now ready to conduct the GeCKO CRISPR screens for both IBDV and IBV infection. The findings will improve our understanding on IBV and IBDV replication, and the candidate genes may be exploited to generate virus-resistant chickens in the future.

P340

p-OXA48: a very conserved plasmid helps to shed light on within patient conjugation dynamics.

Alice Ledda, Katie Hopkins, Julie Robotham

UKHSA, Colindale, United Kingdom

Abstract

Background: Plasmids are well known vehicles of HGT, spreading antimicrobial resistance genes to neighbori It is theng bacteria, independently of their species. Finding a plasmid-borne resistance in different hosts in a patient is a sign of within-patient plasmid conjugation. p-OXA48 plasmid is a very conserved plasmid carrying a cabapenem resistance gene. It is the perfect candidate to test this hypothesis using methods from comparative genomics.

Methods: Whole-genome sequencing was performed on OXA48 carbapenemase-producing Enterobacterales (CPE) isolates referred to the UKHSA's AMR & HCAI Reference Unit between 2014 and 2016. More than one isolate per patient was included if they belonged to a different bacterial species than the first isolate, or had a different resistance. Bacterial host strains were confirmed in-silico and time between samples were derived from epidemiological data. Genome sequences were used to reconstruct the full p-OXA48 plasmid using plasmidSPAdes and MUMMER to identify possible plasmid alleles.

Results: 311 OXA48-positive isolates originating from 111 patients were included. Most patients (81) had one resistance (OXA48) in 2 different bacterial species. 53 of them had at least one E. coli. 40 patients had samples taken on the same day, more than 70% (85/110) within one month. Average time between samples was 41 days and median was 9 days. Genomic analysis showed little diversity between the reconstructed plasmid samples, mostly due to putative recombination events.

Conclusions: p-OXA48, being a very conserved plasmid, can be fully reconstructed using short read sequencing data and used to shed light on the dynamics of within-patient conjugation.

P341

High MAG recovery and precision species profiling of a pooled human gut microbiome reference using PacBio HiFi sequencing

Kirren Kaur[1], Jeremy E. Wilkinson[2], Meredith Ashby[2], Siyuan Zhang[2], Khi Pin Chua[2], Kris Locken[3], Shuiquan Tang[3], Brett Farthing[3], Michael Weinstein[3], Martha Carlin[4], Raul Cano[4], Kyle Langford[5], Benjamin Auch[5], Ivan Liachko[5], Daniel M. Portik[2]

[1]PacBio, London, United Kingdom. [2]PacBio, California, USA. [3]Zymo Research Corporation, California, USA. [4]The BioCollective, LLC, Colorado, USA. [5]Phase Genomics, Inc, Washington, USA

Abstract

Mock communities of known composition are often run in parallel with microbial community samples to allow for accurate data evaluation and to facilitate cross-study and inter-lab comparisons, yet they lack the microbial diversity of real-world samples. The ZymoBIOMICS Fecal Reference with TruMatrix Technology (D6323) is a pooled human fecal reference that provides a complex alternative to mock communities. However, the microbial content of this standard is only partially characterized. Here, we explore the content of this sample using highly accurate long-read sequencing. We generated 11.9 million HiFi reads (88.3 Gbases) using the PacBio Sequel IIe system. We performed taxonomic and functional profiling, and metagenome assembly, using analyses tailored to HiFi reads. With taxonomic profiling settings optimized for high precision and recall, we detected 155 species. 92% of HiFi reads were assigned a functional annotation, and resulted in over 66.9 million total annotations from 17,000 unique classes. We used hifiasm-meta to perform metagenome assembly and a PacBio binning pipeline to characterize high-quality (HQ) metagenome assembled genomes (MAGs). This workflow identified 199 HQ MAGs, of which 102 MAGs were >95% complete and included 54 MAGs composed of a single, circular contig. Finally, we downsampled the data to simulate several multiplexing schemes and investigated the effects Taxonomic and functional profiling results were largely robust across data levels. The species-level abundance profiles and highly complete MAGs generated in our study helps shed light on the diverse content of this novel metagenomic reference and the use of PacBio HiFi sequencing for generating high-quality metagenomic data.

P342

Interplay between MarA and FlhDC transcriptional activators in flagellum biosynthesis in Enterotoxigenic *Escherichia coli*

Ksenia Klimova[1], David Grainger[2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Universiry of Birmingham, Birmingham, United Kingdom

Abstract

In many pathogenic bacteria flagellum plays an important role throughout the infection cycle. It helps bacteria to reach the target site, invade, colonise, grow and disperse to the new host. FlhDC is a master flagellar regulator in enteric bacteria related to *E. coli*. Transcriptional activation of all structural elements of the flagellar apparatus rely on FlhDC regulator directly and indirectly.

Multiple antibiotic resistance activator MarA is most known as a global regulator of *acrAB–tolC* expression but it also have an important role in DNA repair, membrane integrity, biofilm formation and virulence. MarA binds over 60 downstream targets known as "marboxes" located in promoter regions of genes that assist in surviving antibiotic exposure.

In this work we show that MarA can increase transcription of some operons involved in flagellum biosynthesis in the presence of FlhDC flagellum master regulator in a competitive way.

P343

In silico investigation of the plasmid composition of over 400 Enterobacteriaceae strains held by the UK's National Collection of Type Cultures.

Jake Turnbull ORCID iD, Sarah Alexander, Jo Dick

The National Collection of Type Cultures, UKHSA Culture Collections, London, United Kingdom

Abstract

The National Collection of Type Cultures (NCTC) is composed of 6,000 medically relevant bacterial strains maintained by the UK Health Security Agency (UKHSA). The genomes of approximately 3,000 NCTC strains have been long-read sequenced, with the genomic reads, available assemblies, and annotations made publicly accessible under NCBI BioProject PRJEB6403. This wealth of genomic data provides an opportunity to refine what is known to date about the plasmid content of the sequenced NCTC bacterial isolates.

Here, we apply a series of bioinformatic tools and approaches to approximately 400 assemblies derived from strains of Escherichia coli and Klebsiella pneumoniae. We identify contigs of plasmid provenance, assess contig circularity, type contigs deemed to be of plasmid provenance based on *rep* and *mob* loci, cross reference these contigs with a database of known plasmid sequences, and determine their AMR gene content. Genome assembly optimization (e.g. re-assembly using different assemblers and the addition of short read data) in a select strain set was also performed to investigate whether the current genomic assemblies adequately reflect the true plasmid content of the strains in question.

The resultant analysis details the most comprehensive description of the plasmid population of NCTC Escherichia coli and Klebsiella pneumoniae strains carried out to date, revealing the NCTC strain set to be a rich source of diverse plasmids. This dataset, containing both previously described and putatively novel structures, has been contextualised using the available meta-data associated with the strains, and allows commentary on plasmid evolution and human history.

P344

Unravelling the role of orphan ParA genes in Rhodococcus erythropolis PR4

SHABNAM PARWIN, Dr Preeti Srivastava

INDIAN INSTITUTE OF TECHNOLOGY DELHI, DELHI, India

Abstract

Orphan parA like genes have been identified in a few bacteria where they have been reported to be involved in diverse functions. Rhodococcus erythropolis PR4 chromosome contains four copies of parA genes in their genome: 1 copy of operonic parA (6509168-6510172) and 3 copies of orphan parA genes, parA1 (2388455-2389252), parA2 (3533210-3534223) and parA3 (4434533-4435387). There are only limited studies on orphan parA genes and none from Rhodococcus spp. The orphan parA genes show 40% – 50% identity with the operonic parA gene. The deletion of parA2 and parA3 gene resulted in an altered elongated morphology as compared to the wild type rod shaped cells. The cell length in ΔparA2 and ΔparA3 deletion mutant was found to be up to 7.5 µm as compared to that of wild type strain whose cell length goes up to 3.5 – 4.0 µm only. Chromosome segregation as observed by DAPI staining, was not affected in ΔparA2 and ΔparA3 deletion mutants. The sub-cellular localisation was found to be at the cell centre or at quarter positions in case of ParA3-GFP. On the other hand, a maximum of three foci were observed in case of ParA2-GFP, where one was found to be at the cell centre and the other two were observed at the cell poles. The elongated cell shape in orphan parA deletion mutants together with differences in localization of the two orphan ParA proteins suggest their distinct roles in cell division and morphogenesis.

P345

A multiomic approach to defining the essential genome of the globally important pathogen Corynebacterium diphtheriae

Emily Goodall ORCID iD[1], Camila Azevedo Antunes[2], Jens Möller[3], Vartul Sangal[4], Paul Hoskisson[5], Andreas Burkovski[3], Ian Henderson[6]

[1]Univsersity of Queensland, Brisbane, Australia. [2]German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany. [4]Northumbria University, Newcastle, United Kingdom. [5]University of Strathclyde, Glasgow, United Kingdom. [6]University of Queensland, Brisbane, Australia

Abstract

Diphtheria is a respiratory disease caused by Corynebacterium diphtheriae. While the toxin-based vaccine has helped control outbreaks of the disease since the mid-20th century there has been an increase in cases in recent years, including systemic infections caused by non-toxigenic C. diphtheriae strains.

Transposon insertion sequencing (TIS) is a high-throughput method used to link phenotype with genotype. It is commonly used to identify genes required for survival or pathogenicity of clinically relevant strains; or to understand poorly characterized organisms.

Here we describe the first study of gene essentiality in C. diphtheriae, providing a dense library and model dataset in the Actinobacteriota phylum. This high-density library has allowed the identification of conserved essential genes across the genus and phylum and enabled the elucidation of essential domains within the resulting proteins including those involved in cell envelope biogenesis.

Integration of the TIS data with proteomics data enabled validation of essential proteins previously described as hypothetical and uncharacterized proteins, which are also represented in the vaccine. These data are an important benchmark and useful resource for the Corynebacterium, Mycobacterium, Nocardia and Rhodococcus research community. It enables the identification of novel antimicrobial and vaccine targets and provides a basis for future studies of Actinobacterial biology.

P346

Population diversity of *Penicillium roqueforti* isolates of Turkish mold-ripened cheeses

Hatice Ebrar Kirtil ORCID iD, Banu Metin ORCID iD

Istanbul Sabahattin Zaim University, Istanbul, Turkey

Abstract

Mold-ripened cheeses are produced locally by spontaneous fermentation in various regions of Turkey, such as Erzurum, Konya, Karaman, and Kars districts. In this study, we aimed to determine the population diversity of *Penicillium roqueforti* (n=120) isolated from 61 traditional mold-ripened cheeses. The isolates were molecularly identified using ITS or *benA* sequencing. PCR screening indicated that all *P. roqueforti* isolates harbored the cheese-related horizontal gene transfer regions, *Wallaby* and *CheesyTer*. The mating genotype distribution of *P. roqueforti* isolates determined by MAT locus PCR was skewed in favor of MAT1-2 (95%). The isolates with opposite mating types were found together in three cheese samples, indicating the possibility of sexual reproduction in the cheese matrix. A population analysis using three microsatellite loci (Proq01_3, Proq02_2, and Proq16) resulted in 36 sequence types (STs), with the most common one (ST2) comprising 35% of all isolates (n=42). The isolates with different STs were identified in some cheese samples, which indicates that different strains from the environment may grow on cheeses during the ripening process. Twenty isolates were selected according to the microsattelite analysis to represent the population and five polymorphic loci (*cmd, benA, proq235, proq631*, and *proq845*) from these isolates were sequenced. Phylogenetic analyses showed that while some Turkish *P. roqueforti* isolates were closely related to the blue cheese isolates around the world, some isolates formed Turkey-spesific clusters.

P347

Identifying Novel Powdery Mildew Susceptibility and Resistance Genes in Strawberry

Samantha Lynn[1], Jim Dunwell[2], Helen Cockerton[3]

[1]NIAB, East Malling, United Kingdom. [2]University of Reading, Reading, United Kingdom. [3]University of Kent, Canterbury, United Kingdom

Abstract

The biotrophic pathogen powdery mildew (Podosphaera aphanis) causes disease on strawberry. Strawberry powdery mildew is a global problem, infecting leaves, flowers and fruit. Epidemics of powdery mildew can result in high economic losses due to unmarketable fruit. Control of powdery mildew disease is mitigated by the application of fungicide sprays, however, the continued application of fungicide has caused concerns regarding fungicide insensitivity and resistance.

Generating disease resistant cultivars offers a favourable solution to reduce the impact of powdery mildew on strawberries. This project will characterise the tissue specificity of the disease response across different strawberry genotypes. The overall aim will be to identify possible tissue specific resistance/susceptibility genes for use in breeding through conducting a Genome Wide Association Study (GWAS). Disease phenotyping data was collected from a field trial consisting of 350 different cultivars with five replicates. Phenotyping was performed in July, August and September in 2021 and 2022. A heritability score of 0.86 in 2021 and 0.8 in 2022 shows that a significant proportion of the variation observed in the field is controlled by genetic components. The genotyping has been performed using an Affymetrix Istraw 35 array. GWAS statistical analysis shows that multiple genetic alleles contribute towards powdery mildew disease resistance in strawberry. RNA sequencing data for Strawberry fruit and leaves collected will be analysed to extrapolate differences in tissues expression during infection of powdery mildew.

P348

IS*Aba*13 transposition in *Acinetobacter baumannii* AB5075

Charles Cooper ORCID iD, David Forrest, James Haycocks, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

Acinetobacter baumannii is a critical priority pathogen for which new antibiotic treatments are urgently required. The commonly studied isolate AB5075 is well documented to undergo a high frequency phase switch between virulent opaque (VIR-O) and avirulent translucent (AV-T) colonies. Such colonies can only be distinguished using oblique illumination microscopy. We have observed stable colonies (here termed “grey” variants) that appear clearly translucent under room lighting. Such colonies differ in biofilm forming capacity, surface motility and natural transformation.

Comparative analysis of transcripts between VIR-O and grey variants shows upregulation of an IS5-like element IS*Aba*13 family transposase. This is coupled with downregulation of an acetyltransferase and glycosyltransferases which form part of the capsule synthesis operon. Long read genome sequencing revealed the insertion of an IS*Aba*13 insertion sequence into this operon, resulting in premature termination of transcription.

To investigate other locations IS*Aba*13 can transpose to, native insertion sites were mapped across the chromosome, revealing a propensity for insertion into AT-rich regions. In many Gram-negative bacteria, AT-rich regions are bound by the histone-like nucleoid-structuring protein (H-NS). Strikingly, in an *hns* mutant, this preference for AT-rich regions is abolished. This suggests a role for H-NS in directing transposition.

These findings highlight the potential to generate heterogeneity within bacterial populations using insertion sequences, and form the basis for understanding the factors which influence transposition in *A. baumannii.*

P349

Comparative genomics of Salmonella spp. from clinical gastroenteritis stool specimens

Steven Rudder ORCID iD[1], Bilal Djeghout[1], Ngozi Elumogo[1,2], Nicol Janecko[1], Gemma Langridge[1]

[1]Quadram Institute, Norwich, United Kingdom. [2]Eastern Pathology Alliance, Norwich, United Kingdom

Abstract

For pathogen identification during clinical and epidemiology studies it is standard to use a single colony isolated from the target sample for pathogen identification. Whilst this approach is effective at identifying the pathogen, no information about the population dynamic of the pathogen(s) is recorded. The aim of this study is to analyse the diversity between 20 Salmonella colonies isolated from individual Salmonella positive stool samples using long and short read sequencing. Seven Salmonella PCR-positive stool specimens from gastroenteritis patients at Norwich and Norfolk University Hospital were collected between 2019 and 2022. Selective media was used to isolate Salmonella from the stool samples with a target of 20 isolates per stool. Automated RevoluGen Fire Monkey high molecular weight (HMW) DNA extraction was used in combination with Oxford Nanopore long read sequencing and Illumina short read sequencing to obtain high quality consensus genome sequences for bioinformatic analysis. Hybrid genome assemblies were constructed, and complete chromosomes were used for structural and nucleotide variation analysis. This confirmed that each patient was infected with a single Salmonella serovar: Java (2), Infantis, Typhimurium, Salamae, Anatum and Enteritidis. Using RESfinder the same AMR genes (aac(6')-laa and mdf(A)_1) were identified in all isolates of Samonella Java across the two sets. The typical SNP distances within isolates from 1 patient was found to be 12. This study demonstrates the genetic variation present within Salmonella populations recovered from individual gastroenteritis stool specimens.

P350

Exploration of Coupled Promoter-Terminators in Gram Negative Bacteria

Andrew Fletcher, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

Many DNA sequences elements are involved in the control of bacterial transcription. The -10 and -35 promoter regions recruit RNA polymerase (RNAP) to the promoter and facilitate initiation, while intrinsic terminator sequences encode for hairpin structures in the nascent RNA, which leaver RNAP off the transcript. Promoters and terminators have long been thought of as separate entities; however, our preliminary data suggests that these regions commonly overlap.

The transcription start sites (TSS) of *Acinetobacter baumannii* were mapped using Cappable-seq and the transcript termination sites (TTS) mapped using Term-seq. With RNAP occupying the 35 bases upstream from a TSS when at the promoter, any occurrence of a TTS within the same region may facilitate interactions between terminating and initiating RNAPs.

Mapping every TSS to its closest upstream TTS, we observe that spacings of 7-12bp are favoured, which positions the TTS within the -10 promoter element. Thus, we suggest that promoter and terminator sequence elements may serve duel regulatory roles. We also note favoured spacing of exactly 20bp, ensuring the TSS and TTS are separated by exactly two full turns of the DNA.

Two coupled promoter-terminators have been further characterised using both *in vivo lacZ* assays and *in vitro* transcription assays. Our results suggest interactions between initiating and terminating RNAPs may be present at the 20bp-spaced *guaB-glmA* interface, but not at the 10bp-spaced *surE-nlpD* interface.

Given the crowded nature of many bacterial genomes, we suggest that coupled promoter-terminators may be a fundamental level of gene regulation which has previously avoided our attention.

P351

Genetic background influences the evolvability of antimicrobial resistance

Roshan Khatri, Jasmin Lawrence, Gareth McVicker ORCID iD, Alasdair Hubbard ORCID iD, Maria Rosa Domingo-Sananes ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Mutations can allow bacteria to acquire resistance to antibiotics and at the same time, they may affect cell fitness and sensitivity toward other antibiotics (collateral effects). The genetic background of a bacteria may also influence the effects of such mutations. For example, the same resistance-causing mutation in two different genetic backgrounds can provide different levels of resistance and fitness effects, due to epistatic interactions. Understanding such interactions could help us predict how resistance evolves and therefore develop methods to slow down or reverse the rapid evolution of antimicrobial resistance in bacterial populations. We are working to understand how genetic background can influence the evolvability of AMR by comparing the mutation-selection windows of strains that vary in genetic background. The mutation-selection window is the range of antimicrobial concentrations for which one-step mutants with increased resistance can be selected. The wider the window, and the higher its top, the more evolvable a strain is. We find that the width of mutational windows to different antibiotics can vary in different clinical isolates of *Escherichia coli*. We also find that mutations that confer increased resistance to one antibiotic affect the evolvability of resistance to a second antibiotic in *E. coli* K-12. The results from these analyses will provide insight into how genetic interactions may affect resistance, fitness and evolvability. This could be important for designing treatment strategies that minimise the risk of resistance evolution.

P352

Predicting evolution using Transposon Directed insertion-site Sequencing (TraDIS).

Mathew Milner, Peter Lund

University of Birmingham, Birmingham, United Kingdom

Abstract

Laboratory-based evolution has become a widely used method to explore fundamental questions about evolution as a process and is also a powerful tool to study the link between genotype and phenotype. We are interested in understanding short and long term adaptations of bacteria to stressful environments, and to study this we have evolved five populations of E. coli MG1655 in a dynamic pH environment, by iterative growth and daily dilution in unbuffered LB, starting at pH 4.5. This was done over five months, keeping a frozen fossil record of intermediate populations during this process. Whole genome sequencing of the evolved populations and of individual clones from each population revealed many striking similarities in the evolutionary trajectories in the evolved strains; for example, mutations in arcA are common and may cause loss or alteration of function. We are interested in exploring the impact of multiple different parameters on evolutionary trajectories, but as evolution experiments take a long time, we are currently investigating whether experiments, using a high density transposon libraries and Transposon Directed Insertion-site Sequencing (TraDIS), can partially replicate evolution experiments in a relatively short time frame. Since TraDIS provides a measure of relative contributions to fitness of each gene (by comparison of read counts after growth in two conditions), in principle it should be possible to use TraDIS to identify genes whose loss of function provides a fitness benefit. Here we compare the outcome of these two techniques and present our latest results.

P353

Robust and versatile ultra-high-throughput single-microbe genome sequencing

Vaidotas Kiseliovas, Duygucan Gul, Giedrius Zolubas, Rapolas Zilionis

Droplet Genomics, Vilnius, Lithuania

Abstract

Whole-genome and targeted sequencing open a window to understanding the function of unculturable microorganisms. On one hand, metagenomic sequencing is attractive for its straightforward sequencing library preparation from bulk environmental samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of individual cells in parallel. To satisfy the need to study such large numbers of single-microbes per sample, well- and droplet-based approaches keep evolving in parallel to provide single-cell compartmentalization required during sequencing library preparation. However, these approaches suffer from a fundamental trade-off between throughput and versatility. Being individually addressable, microwells enable multi-step processing but are not scalable. Droplets offer a throughput of up to a million cells per experiment but only allow a limited number of processing steps to be performed. The later limitation is severely felt went it comes to microbial research, as harsh conditions typically required for lysis are incompatible with downstream nucleic acid barcoding steps. Our Semi-Permeable Capsule (SPC) technology combines the throughput of droplets with the versatility of wells by enabling a virtually unlimited number of processing steps on genetic material from millions of individual microbes in parallel. We demonstrate the use of SPCs for barcoding >100,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality.

P354

Whole genome sequencing to identify most prevalent resistance conferring mutations in *Escherichia coli* strains and evaluation of impact on the three-dimensional structure of protein and drug binding affinity

Priyanka Sharma[1], Arti kapil[2], Punit kaur[2], Harpreet Singh[1]

[1]Indian Council of Medical Research, New Delhi, India. [2]All India Institute of Medical Sciences, New Delhi, India

Abstract

Background:*Escherichia coli* is one of the leading causes of infectious diseases worldwide and the emergence of multi-drug resistant phenotypes in developing countries has raised a concern. Whole genome sequencing is widely being used for the prediction of antimicrobial resistance, virulence markers, sequence types and outbreak analysis.

Methods: A total of 50 strains of *E.coli* were selected for the purpose of WGS analysis from different clinical samples. Whole genome sequencing was done using MiSeq followed by the read quality analysis, trimming and de novo assembly using FastQC, Trimmomatic and Spades respectively. The assembled contigs were used to BLAST against CARD, PlasmidFinder and VRDB databases to identify various resistance markers, plasmid types and virulence genes. Schrodinger was used to prepare the three dimensional structure and molecular dynamic studies and molecular interaction of the ciprofloxacin with wildtype and mutant structures were compared.

Results:The phenotypic antimicrobial susceptibility to third generation cephalosporins, fluoroquinolones, piperacillin-tazobactum and carbapenems was 18, 26, 38 and 60%. The sensitivity and specificity of WGS for prediction of antimicrobial susceptibility ranged from 0.97 to 1 and 0.70 to 0.96 respectively. One or more plasmids were harboured by 45/50 strains. The most prevalent mutation was ser83asp followed by asp87gly in DNA gyrase enzyme. Molecular dynamic studies revealed that these substitutions affect the interaction of carboxylate moiety of quinolone ring in ciprofloxacin.

Conclusion: The novel drug molecules can by identified against DNA gyrase enzyme by bypassing the mutation hotspots in quinolone binding pocket to treat MDR and XDR strains.

P355

An effective pipeline for ancient DNA analysis of the human oral microbiome

Francesca Standeven[1], Gwyn Dahlquist-Axe[1], Camilla Speller[2], Conor Meehan[3], Andrew Tedder[1]

[1]School of Chemistry and Biosciences, University of Bradford, Bradford, United Kingdom. [2]Department of Anthropology, University of British Columbia, Vancouver, Canada. [3]Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom

Abstract

Ancient DNA (aDNA) has emerged as a recognised source of data for evolutionary and archaeological topics, including ancient diets, disease, migratory patterns, culture, and gender studies. In recent years, aDNA has also begun to be used for microbiome studies, looking at the communities of bacteria that existed in different time periods. While this has been done successfully for amplicon-based studies, recovery of full metagenomic assembled genomes is still problematic. This is due to the complex nature of aDNA, such as extensive fragmentation, degradation, and contamination. Furthermore, since ancient microbiome project initiatives are typically interdisciplinary, many specialists in the field are not well versed in the bioinformatics tools needed for such analyses. This study uses a dataset of 155 metagenomic samples from archaeological dental calculus (mineralised dental plaque) sequenced using short read technology spanning over 5000-years (during the Bronze/Iron Age, Roman Period, Viking Age, Anglo-Saxon Period, Medieval Period, and Industrial Age) to evaluate and construct a user-friendly, efficient workflow for ancient human oral microbiome analysis. The runtime, computational resources, ease of use and accuracy of results were all considered to ensure such a pipeline can be used widely by researchers in the field of aDNA.

P356

Development and Application of Genomic Tools for Tracking Viral Outbreaks in Fish

Shayma Alathari ORCID iD

University of Exeter, Exeter, United Kingdom

Abstract

Fish losses and food shortages due to the emergence of viruses are commonly seen in countries dependent on aquaculture, posing a significant threat to fish farmers' livelihoods and income security. Tilapia farming is one of the most important sectors in aquaculture worldwide, with total global production estimated at more than 6 million tonnes in 2018 (FAO; Martínez-Cordero et al 2021). A novel strain of Infectious spleen and kidney necrosis virus (ISKNV) has been identified as an agent of high morbidity and mortality (60-90%), threatening tilapia aquaculture in Lake Volta, Ghana. Losses of more than 10 tonnes of fish per day following infection (Ramírez-Paredes et al. 2019) resulted in the closure of more than 50 farms, loss of over 400 jobs, and the disruption of the livelihoods of the communities along the lake (Okai 2021).

Long read sequencing of tiled PCR products has enabled field-based, real-time genomic surveillance of recent viral outbreaks, providing detailed epidemiology and phylogeography information for disease management. Leveraging similar approaches for tracking ISKNV outbreaks faces challenges, as a much larger genome and much slower evolution rates associated with dsDNA viruses. Here, we developed a new protocol to recover near-complete (96%) ISKNV genomes from tissue samples collected from the Lake Volta outbreak.

This work represents the first use of long read, tiled PCR approaches for real-time surveillance of viruses associated with aquaculture and shows that similar approaches can be used to monitor outbreaks of other large dsDNA viruses.

P357

Comparing prophage carriage across longitudinal isolates of Pseudomonas aeruginosa sampled from Bronchiectasis patients

Jennifer L Coulson, Darren L Smith, Mohammad A Tariq

Northumbria University, Newcastle, United Kingdom

Abstract

A clinical marker of Bronchiectasis (Br) is the permanent widening of the bronchi. Often caused by reoccurring or chronic infection and an inability of the airways to clear mucus and their associated microorganisms. This results in the presence of a thick mucus which is difficult to clear. This enables opportunistic pathogens such as Pseudomonas aeruginosa (Pa) to colonise. Pa is the most common lung pathogen and can lead to lowered lung function and eventual death. Pa can adapt its physiology via factors such as biofilm formation, lipopolysaccharide production and acquisition of antimicrobial resistance and is also known to alter its metabolome.

Temperate bacteriophages are thought to mediate the adaptions Pa makes by transferring genes through Horizontal genetic transfer which aids its survival in the lungs of Br patients. Currently, much remains unknown about the prophage carriage in Pa of Br patients or what accessory genes are carried between the phages in chronic infection. The aim of this work is therefore to understand the carriage of prophages over the progression of Br in isolates collected from a patient with Br over a six year period. These strains have been sequenced and so we will confirm lysogeny by using prophage prediction informatic tools Phaster/ProphET. We aim to identify the number of inducible prophages and their diversity across the longitudinal isolates. We will focus on changes in the phage genome over time that may be linked to bacterial fitness or temperate phage adaptation to the chronic lung.

P358

Antarctic marine invertebrate microbiomes amid a changing climate

Kudzai Hwengwere[1,2], Mathew Upton[2], Kerry Howell[2], Lloyd Peck[1], Melody Clark[1]

[1]British Antarctic Survey, Cambridge, United Kingdom. [2]University of Plymouth, Plymouth, United Kingdom

Abstract

Background: The West Antarctic Peninsula is experiencing the most rapid change in the Southern hemisphere. Ocean warming will affect glacial retreat and sea ice, which together could have catastrophic effects on marine ecosystems. Marine invertebrate communities living in the near-shore will be at immediate risk with these environmental changes, particularly because they are very sensitive to warming. In recent years, the microbiome is becoming increasingly referenced as an additional organ. Microbiome research on tropical marine invertebrates has shown that host-associated microbiomes restructure and respond to seasonal demands of the hosts, supporting nutrition and feeding. Yet there is very little known about microbial populations which inhabit Antarctic marine invertebrates or the role they play for normal physiology and helping the hosts cope with the stress of ocean warming. Therefore, this project will provide novel data on bacterial communities that live within eight species of Antarctic invertebrates and how these communities vary with season and with warming.

Methods: The major aim of this project is to describe the diversity and functionality of invertebrate microbiome using traditional cultivation techniques and 16S rRNA gene Amplicon and Metagenomic sequencing (the latter using the long-read MinION platform).

Results: Data is being generated for the first time regarding these eight species and their microbiomes.

Conclusion: Marine invertebrates living in the Antarctic may harbour unique microbes that help them survive the intense seasonality. This project is narrowing this knowledge gap.

P359

RNA G-Quadruplexes regulate the transcription of the PE/PPE genes in *Mycobacterium*

Ashish Kumar ORCID iD, Perumal Vivekanandan

Kusuma School of Biological Sciences, Indian Institute of Technology, Delhi, India

Abstract

G-quadruplex structures are crucial in many cellular processes like transcription, translation, and replication. The RNA G-quadruplexes are thermodynamically stable and can readily form due to their single-stranded configuration, and their role in prokaryotes remains poorly understood. In this study, we analysed 1624 bacterial genomes and coding sequences for putative quadruplex sequences (PQS). Mycobacteria have significantly higher PQS densities in their genomes and CDS. Interestingly, CDS enriched in PQS were observed only in slow-growing pathogenic mycobacteria. The majority of the PQS in the CDS of pathogenic mycobacteria mapped to PE/PPE genes, a gene family involved in pathogenesis and host immunity evasion. The PE/PPE genes are unique to pathogenic mycobacteria and represent an expanding family of genes in mycobacterial genomes, which are well-known for their genomic down-sizing during evolution. Using RNA oligos for a selected subset of PQS from PE/PPE genes, we demonstrate their ability to form RNA G-quadruplexes in vitro using standard biophysical methods. The addition of BRACO-19, a G-quadruplex binding ligand, stabilized the quadruplexes. It also significantly inhibits the growth of pathogenic mycobacteria at low micromolar concentrations. This effect was less pronounced on non-pathogenic mycobacteria, suggesting a role for RNA G-quadruplexes in regulating mycobacterial growth. Our results suggest that stabilising the RNA G-quadruplexes in pathogenic mycobacteria's PE/PPE genes inhibits their transcription and translation, and growth dynamics of mycobacteria. In sum, this work highlights how RNA G-quadruplex-containing genes are regulated in mycobacteria and lay the groundwork for understanding the biological role of RNA G-quadruplexes in prokaryotes.

P360

Genomic Analysis of Clinical Multidrug Resistant *E. coli* in Nigerian Hospitals

Manal Mohammed[1], Mkpouto Monday Otong[2], Clement Mboto3 Mboto[3], Ben Solomon Asuquo[4], Bernard Okoro[3], Christopher Ekpo[5], Uwem Edet Okon[6], Elizabeth Nkagafel Mbim[7]

[1]School of Life Sciences, University of Westminster, London, United Kingdom. [2]Department of Microbiology, Faculty of Biological Science, University of Calabar, Calabar, Nigeria. [3]Viro-Bio Research Laboratory, Department of Microbiology, University of Calabar, Calabar, Nigeria. [4]Department of Medical Microbiology/Parasitology, University of Calabar Teaching Hospital, Calabar, Nigeria. [5]Department of Science Laboratory Technology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria. [6]Department of Biological Sciences (Microbiology), Faculty of Natural and Applied Sciences, Arthur Jarvis University, Akpabuyo, Nigeria. [7]Department of Public Health, Faculty of Basic Medical Sciences, Arthur Jarvis University, Akpabuyo, Nigeria

Abstract

E. coli is an emerging nosocomial pathogen causing problems in health care settings. The management of human infections caused by multidrug resistant (MDR) *E. coli* poses substantial clinical challenges in hospitals. The study aimed to characterise the resistome and virulome of *E. coli* in Nigerian hospitals. Bacterial identification and antimicrobial susceptibility testing against 9 antibiotics were performed using standard methods. Whole genome sequencing (WGS) was carried out on ten beta-lactam resistant isolates using an Illumina MiSeq platform. *In-silico* analysis revealed several resistance genes against beta-lactams (*bla*OXA-1, *bla*SHV-152, *bla*SHV-158, *bla*SHV-28, *bla*SHV-106, *bla*CTX-M-15, *bla*TEM-1B, *bla*SST-1, *bla*CTX-M170, *bla*CTX-M-216, *bla*TEM-207, *bla*TEM-104, *bla*CTX-M-156, *bla*CTX-M-88, *bla*TEM-230, *bla*TEM-217, *bla*TEM-30, *bla*TEM-234 and *bla*TEM-198). In addition to resistance genes against other antibiotic classes including aminoglycosides, aminocyclitol, folate pathway antagonists, Fosfomycin, macrolides, tetracyclines, quaternary ammonium compounds and quinolones. Genomic analysis of clinical *E. coli* strains also revealed bacterial isolates harbour several virulence genes (including *aap, aatA, agg4A, agg4C, agg4D, aggR, capU, gad, ORF3, ORF4, iss, iucC, iutA, hlyE, sat, sepA, sitA, terC* and *traT*).

Comprehensive analysis of MDR *E. coli* strains provided by WGS detected variable antimicrobial resistance genes and virulence factors. Programs are therefore needed to control spread of MDR *E. coli* in hospitals in Nigeria.

P361

Investigating the molecular genetic mechanisms governing pathogenic *Yersinia* species soil survival

Hannah Ford ORCID iD

The University of Nottingham, Nottingham, United Kingdom

Abstract

Yersinia pestis is the causative agent of human plague and has evolved from its ancestor *Yersinia pseudotuberculosis*. *Y. pestis* is transmitted between rodents and humans via fleas however, plague characteristically disappears from plague foci for decades between outbreaks and is not recovered from rodent or flea hosts. As *Y. pseudotuberculosis* is a soil-borne pathogen, *Y. pestis* may also survive in soil, providing an explanation to how plague remerges in plague foci.

This project aims to investigate what genetic mechanisms drive *Y. pseudotuberculosis* and *Y. pestis* to survive in soil. *Y. pseudotuberculosis* was inoculated and recovered from soil and survival assays found that *Y. pseudotuberculosis* can survive in non-sterilised soil for at least 1 month *in vitro*. Soil infection assays with *Caenorhabditis elegans*, found that *Y. pseudotuberculosis* can survive within nematodes for at least 5 days in non-sterilised soil suggesting that *C. elegans* may act as an environmental reservoir.

Similar experiments are underway using our *Y. pestis* soil infection model and further work is ongoing to identify the genetic variants that underpin *Y. pseudotuberculosis* and *Y. pestis* soil survival. These include genome sequencing and transposon-directed insertion site sequencing to identify genetic/ genomic variants significantly more abundant or absent in soil.

Findings from this study could provide context to the pattern of current and historical plague outbreaks and increase our understanding of the *Yersinia* spp. lifestyle.

P362

Interspecific competition can drive plasmid loss from a focal species in a microbial community.

David Walker-Sünderhauf ORCID iD[1], Uli Klümper[2], William Gaze[1], Edze Westra[1], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]Technische Universität Dresden, Dresden, Germany

Abstract

Plasmids are key disseminators of antimicrobial resistance genes and virulence factors, and it is therefore critical to predict and reduce their spread within microbial communities. The cost of plasmid carriage is a key metric which can be used to predict their ecological fate.

Here, we investigate cost and maintenance of IncP1 conjugative plasmid pKJK5 to focal strain *Variovorax* sp. The presence of certain growth partners increased plasmid cost to this host, and accordingly embedding *Variovorax* in a community context led to rapid loss of pKJK5 compared with culturing this strain in monoculture.

We generalised these findings by tracking maintenance of pKJK5 in all constituents of a synthetic 5-species community and found community-dependent plasmid loss in a second species, as well as loss of a second plasmid lacking payload genes.

We propose that the destabilising effect of interspecific competition on plasmid maintenance may have an impact on plasmid abundance in clinical and natural environments. Once further investigated, this effect may be leveraged to cure plasmids from focal strains by addition of growth partners.

P363

Regulating the sugar factory: How do hybrid two-component systems (HTCS) in gut bacteria regulate global gene expression?

Diana Githwe

Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

The human gut is home to microorganisms that have formed a symbiotic relationship with their hosts to create the human gut microbiota (HGM). A key metabolic function of the bacterial genus *Bacteroides* in the HGM is the ability to break down host and dietary polysaccharides which cannot be broken down by the host, due to polysaccharide complexity and the lack of adequate enzymes. *Bacteroides* use unusual hybrid two-component systems (HTCS) to sense specific oligosaccharides and regulate gene expression for various proteins needed for the catabolic process. The HTCS is a single polypeptide consisting of an oligosaccharide-binding sensor domain located in the periplasm, linked by transmembrane helices to the cytoplasmic histidine kinase (HK) and response regulator (RR) with an araC-type DNA binding domain. This raises questions about how a membrane bound regulator interacts with DNA.

To determine whether HTCS are bound to DNA in both the inactive and activated forms, Electromobility Shift Assays (EMSAs) have been used to investigate binding by native, inactive and phosphorylated RR to target DNA sequences. We have shown that all protein forms of two HTCSs (specific to yeast a-mannan) bind DNA at all predicted HTCS binding sites, suggesting that target DNA is located at the membrane. We hope to fluorescently label the HTCS binding sites to visualise the location of target DNA during growth on polysaccharides.

Understanding how this crucial system is modulated at the cytoplasmic membrane will reveal new insights into how these symbionts are so successful at rapidly detecting and responding to polysaccharide cues.

P364

Evolutionarily novel and difficult to detect putative genes across Escherichia

Zachary Ardern ORCID iD

Wellcome Sanger Institute, CAMBRIDGE, United Kingdom

Abstract

Bacterial genomes contain many putative protein-coding genes which are either inconsistently or never found by the most popular available gene detection methods. These sequences are typically either shorter than the arbitrary thresholds used in gene finding or are overlapping known genes in alternative reading frames e.g directly in antisense. They are also typically evolutionarily novel, i.e. restricted to a very small proportion of bacterial taxa such as a single species or genus. New methods allow better understanding of the sequence features leading to inconsistent annotations, and the properties of the new genes which were previously difficult to discover. In addition to the sequence data from a single genome which is typically used as input for gene prediction, in *Escherichia* there is direct high resolution evidence of translation from multiple ribosome profiling studies across a few *E. coli* strains and other species including new unpublished data from *E. albertii* and *E. fergusonii*. Evidence of selection on putative genes of interest can also be studied across the pangenomes of the genus and the species within it. Using both of these new lines of evidence, i.e. combining expression data from ribosome profiling with inferences about selection made from large scale genomic data, reveals many putative new genes across the genus. Here I introduce the methods used to find them and some characteristics of these interesting sequences which comprise part of the large region of genomic “dark matter” yet unexplored across even the highly studied bacterial genus *Escherichia*.

P365

What's on my food? Exploration of the *E. coli* population structure on retail foods from Norfolk, United Kingdom

Gabriel Astorga ORCID iD[1], Roxana Zamudio ORCID iD[1], Leonardo de Oliveira Martins ORCID iD[1], Raphaëlle Palau[1], Samuel J. Bloomfield ORCID iD[1], Dipali Singh ORCID iD[1], John Wain ORCID iD[1,2], Alison E. Mather ORCID iD[1,2]

[1]Quadram Institute, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Escherichia coli is used as an indicator organism for faecal contamination and antimicrobial resistance (AMR) and is commonly found in many environments, including on food . Understanding the diversity of commensal and pathogenic *E. coli* lineages present on retail foods, as well as the range of AMR genes, virulence genes and plasmid replicons those lineages harbour, is important to understand potential risk to consumers. This study surveyed, isolated, and sequenced the whole genomes of 516 *E. coli* isolates from retail raw chicken, raw pork, raw salmon, raw and cooked prawns, and fresh leafy green vegetables in Norfolk, United Kingdom. Initial findings indicate that commensal *E. coli* belonging to phylogroups A and B1 were predominant in the survey. Additionally, intra-sample and intra-food source *E. coli* sequence type diversity was observed. Based on the phylogenetic core gene tree, *E. coli* isolated from different food sources were phylogenetically heterogenous. Further exploration revealed that *E. coli* from raw chicken meat harboured more AMR genes, virulence genes and plasmid replicons than the other food sources. Genomic surveillance of multiple isolates within a sample can reveal genetically diverse *E. coli*. As food production becomes increasingly more global, there is an increasing need for the ecological and evolutionary surveillance of infectious diseases for the safety of public health.

P366

Identifying genes under positive selection in the core-genome of *Staphylococcus epidermidis*

Callum Rimmer ORCID iD[1], Ferran Navarro ORCID iD[2,3], Jonathan Thomas ORCID iD[1]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. [3]Universitat Autònoma de Barcelona, Barcelona, Spain

Abstract

Staphylococcus epidermidis has been shown to possess a diverse global population comprised of six genetic clusters (GCs) and three genomic groups. However, signatures of positive selection within core-genes of *S. epidermidis* have not been examined. Here we present a candidate list of genes under positive selection from a representative sample of the global *S. epidermidis* population.

Briefly, 1623 publicly available *S. epidermidis* whole-genome sequences (WGSs) were supplemented with the hybrid nanopore/Illumina assemblies of 12 strains belonging to the underrepresented GC2, based on provisional sequence type (ST) data. WGSs were filtered based on average nucleotide identity and assembly quality, leaving 1003 WGS for analysis. Genetic cluster assignments based on sequence type information was performed with BAPS. Roary identified 529 core-genes, with alignments screened for recombination using RDP5. A phylogenetic tree was built using RAxML-ng, which was sub-sampled to prepare a reduced dataset of 100 strains for analysis. Using both a maximum-likelihood and Bayesian-inference approach, PAML site models were used to screen 959 core-genes from the reduced dataset for signatures of positive selection, and candidate genes were confirmed using omegaMap. Sixteen genes were identified as under positive selection, including six hypothetical genes. COG analysis of genes under selection revealed most were linked to metabolic pathways but also included genes linked to defence mechanisms and signal transduction.

This work provides insight into the possible selective pressures placed upon the core-genome of *S. epidermidis* and areas of functional importance within genes under selection.

P367

CarD: Linking Transcription of Minimal Promoters to Supercoiling in *Rhodobacter sphaeroides*

David Forrest, David Grainger

University of Birmingham, BIRMINGHAM, United Kingdom

Abstract

Where RNA polymerase begins transcription in bacteria is dictated by promoters, bipartite DNA sequences composed of -35 and -10 elements. The consensus sequence of the -10 element (TATAAT) has inherent symmetry, resulting in -10-like sequences on the complementary reverse strand. These overlapping promoters can drive transcription from both strands in a process termed bi-directional transcription.

Many E. coli bidirectional promoters consist of two divergent transcription start sites (TSSs) separated by 18bp. This configuration requires the final base of the consensus -10 element to be a T. Whilst highly conserved in most bacteria, over half of Rhodobacter sphaeroides promoters lack this final T. R. sphaeroides compensates for this by utilising a transcription factor, CarD, to activate transcription from these minimal promoters.

To investigate if CarD dependent promoters exhibit bidirectionality, we determined the global TSS in R. sphaeroides. This identified multiple potential bidirectional promoters, with the most frequent configuration of bidirectional TSSs separated by 23 bp. However, we were unable to demonstrate CarD dependant activation of bidirectional transcription in vitro. Whilst troubleshooting, we observed CarD only activates transcription on negatively supercoiled DNA. Levels of supercoiling are linked to external conditions including temperature, osmolarity and oxidative stress. We speculate that CarD may regulate transcription in response to alterations in DNA supercoiling, in turn brought on by environmental changes.

Ongoing work aims to elucidate the involvement of CarD in linking transcription regulation to supercoiling and bidirectional transcription in vivo

P368

Uncovering pre-resistance and rare mutations associated with antibiotic resistant bloodstream infections

Milly Newton, Conor Meehan, Alasdair Hubbard, Maria Rosa Domingo-Sananes

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Discordance between phenotypic and genotypic testing in *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) antimicrobial susceptibility testing is a significant issue in hospitals. As more and more genotypic testing is undertaken, these discordances can result in incorrect drug susceptibility information with downstream effects on patient treatment. Such discordance is also compelling for research as it could uncover rare mutations and pre-resistance.

We used a combination of population genetics and machine learning approaches to understand the discordances found in a large dataset of clinal *E. coli* and *K. pneumoniae* samples. FST, random forest and rare mutation analysis were used in combination to uncover patterns of resistance and pre-resistance that stratify data based on MIC and EUCAST susceptibility profiles.

Our pipeline provides a next step in the use of large public datasets to try to uncover the unknown aspects of antimicrobial resistance. This could set a basis for further research within this subject and it may show the importance of tracking nosocomial antimicrobial resistance using genotypic methods.

P369

Using ancient DNA to investigate the impact of the Industrial Revolution on human oral microbiome communities

Gwyn Dahlquist-Axe[1], Francesca Standeven[1], Camilla Speller[2], Andrew Tedder[1], Conor Meehan[3]

[1]School of Chemistry and Biosciences, University of Bradford, Bradford, United Kingdom. [2]Department of Anthropology, University of British Columbia, Vancouver, Canada. [3]Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom

Abstract

The human microbiome has been recognized as closely linked to many aspects of human health, from dental disease to cancer. The oral microbiome in particular is heavily influenced by diet, disease, and daily habits. While much is known about the modern oral microbiome structure and function, little is known about the evolution of this community and how it changed in relation to human cultural changes.

In this project we use samples of ancient DNA obtained from the dental plaque of 134 ancient individuals from across England dating from the Iron Age until the post-Medieval Era to look at the impact of changes to dietary resources and hygiene practices on the oral microbiome. In particular, we compare the abundances of different bacteria, particularly pathogens, before and after the Industrial Revolution, which brought an increase in pollution and availability of sugar and processed foods. Existing studies investigating the effects of the Industrial Revolution on human oral microbiomes in Europe generally include limited sample sizes that may mask correlations between specific lifestyle factors and changes in the oral microbiome. However, our findings show differences in alpha and beta diversity of the oral microbiome community about this time period, likely due to these sweeping changes in British culture and environment.

P370

Whole genome chemical genomic screening in *Pseudomonas aeruginosa*: proof-of-concept for use in gene function investigation

Manuel Banzhaf[1], Ava Jordan[1], Hannah Doherty[1], Sotirios Kamraminos[1], Nassos Typas[2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]EMBL, Heidelberg, Germany

Abstract

We profiled two single-gene deletion libraries of clinically relevant P. aeruginosa strains (PA01 and PA14) using over 200 chemical and environmental stressors. In these assays, we obtained quantitative measurements of mutant fitness and their ability to form biofilms.

The quantitative phenotypic signature for each mutant strain allows a) identifying novel gene functions; b) mapping of metabolic pathways; c) mapping of interconnections between functional cellular processes; d) addressing the role of horizontal gene transfer in network rewiring and gene repurposing; e) understanding how chemical perturbations affect the secondary metabolite profile; f) identifying novel secondary metabolites; etc.

To validate our data set, we selected and tested experimentally three of our hits. Our data set revealed a fitness relationship between the knockout of a (p)ppGpp hydrolase causing increased sulfonamide sensitivity; the knockout of a hypothetical gene causing sensitivity to nitrogen limitation; and two hypothetical genes on the same operon causing sensitivity to metal stress. We validated these hits by using various assays.

Overall, the results of these experiments were as predicted by the chemical genomics screen. This supports the hypothesis that the screen is a useful resource for predicting gene-phenotype relationships in PA14 and PA01, and can be used to direct further research.

P371

Interactive visual analysis of *Neisseria meningitidis*

Margaret Varga, Holly Bratcher, Keith Jolley, James Bray, Martin Maiden

University of Oxford, Oxford, United Kingdom

Abstract

Bacterial meningitis is an infectious disease of the membranes surrounding the brain that is a key public health challenge worldwide. A major and frequently fatal cause is *Neisseria meningitidis*, the *meningococcus*, which can also grow in the blood, causing septicaemia (sepsis). These two disease syndromes can occur separately or together and are known collectively as invasive meningococcal disease (IMD). *N. meningitidis* can be encapsulated or not, but meningococci causing disease are almost always encapsulated. The capsules are classified into twelve serogroups, of which, six serogroups, A, B, C, W, X, and Y, are responsible for most IMD.

PubMLST.org is a widely-used open-access genomics reference database, containing an integrated collection of databases comprising phenotype and provenance data (metadata) linked to nucleotide sequence data up to and including genome assemblies, for molecular characterization of many bacterial species. Such epidemiological information is crucial for monitoring, tracking, and responding to cases of IMD. The availability of IMD isolates and non-culture diagnosis information also enables effective surveillance and assessment of the effect of meningitis vaccines, providing objective and effective means of comparing and assessing changes in vaccine antigens. The Bexsero Antigen Sequence Type (BAST) scheme, for instance, was developed and implemented under PubMLST.org work.

Here we report our visual analytics and visualization systems developed to support the analysis and exploration of the *N. meningitidis* isolates hosted in PubMLST. We demonstrate how effective visual analytics techniques enable users to assimilate and understand large and complex genomics datasets.

P372

Vibrio cholerae circulating in the Forcibly Displaced Myanmar National population in Bangladesh, in the context of a mass vaccination campaign

Alyce Taylor-Brown ORCID iD[1], Mokibul Hassan Afrad ORCID iD[2], Ashraful Islam Khan[2], Florent Lassalle ORCID iD[1], Md. Taufiqul Islam[2,3], Nabid Anjum Tanvir[2], Nicholas Thomson ORCID iD[1,4], Firdausi Qadri ORCID iD[2]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]International Centre for Diarrheal Disease Research, Bangladesh, Dhaka, Bangladesh. [3]Griffith University, Gold Coast, Australia. [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

The population of Cox's Bazar has increased drastically in the last decade with the influx of Forcibly Displaced Myanmar Nationals (FDMNs) into Refugee Camps (RCs) in Cox's Bazar, Bangladesh. This influx put further pressure on health and sanitation infrastructure, placing the FDMNs and the host population of Bangladeshi Nationals (BGDN) at increased risk of diarrhoeal disease outbreaks including cholera, which was later confirmed during sentinel surveillance of diarrhoea patients.

We sought to understand the epidemic risk of the *Vibrio cholerae* strains circulating in the RCs around the time of a large pre-emptive mass oral cholera vaccination campaign, by assessing the relationship between these strains and those responsible for the current cholera pandemic ("7PET" lineage). We contextualised these findings against the outbreaks in Haiti and Yemen, where reactive vaccination campaigns were conducted.

Our phylogenetic analysis of 222 *V. cholerae* isolates obtained from stool collected from ten surveillance sites between July 2014 and November 2019 showed that these isolates did indeed cluster within two sublineages (one global and one local) of the high-risk epidemic "7PET", branching alongside *V. cholerae* that were present contemporaneously in Dhaka or Kolkata. Interestingly, the globally radiating sublineage was associated with a shorter duration of diarrhoea and lower relative risk of rice watery stool compared to the endemic sublineage.

Our data show that the risk of local or widespread epidemics in this setting were very high but suggest the mass vaccination campaign was successful in preventing a population expansion of the pandemic clone.

P373

Metagenomics for pathogen diagnostics: Problems solved by long read data

Hannah Trivett ORCID iD, Alistair Darby

University of Liverpool, Liverpool, United Kingdom

Abstract

The gold standard method for identifying enteric bacterial infections is stool culture. Whilst some clinical laboratories have transitioned towards molecular techniques such as multiplex PCR panels which are affordable and fast, they provide limited information and require apriori knowledge of the expected cause of infection. Several laboratories are adopting short-read sequencing (SRS) as a form of pathogen identification; however, the advances in metagenomic long-read sequencing (LSR) technology have leveraged a future where LRS has the potential to be incorporated into the suite of diagnostic tools. This project evaluates metagenomic LRS versus metagenomic SRS. Comparing sequencing results to culture-dependent identification methods, we assess the utility of long-read metagenomic sequencing to identify enteric pathogens as an alternative to culture-based detection and SRS methods. After sequencing a selection of enteric PCR-positive stool samples, taxonomic profiles were produced using Kraken2, BUG-SEQ, SourMash and MEGAN-LR to ascertain the most suitable method for clinical application. Metagenome LRS provides comprehensive profiles of the enteric microbiome, and with deeper resolution, novel and low-abundance taxa can be identified. The information from metagenome sequencing goes beyond the scope of the cumulative molecular methods to give a greater depth of knowledge aiding subtyping, surveillance, and outbreak investigations. Rapid developments in sequencing technology have leveraged a situation where metagenomic sequencing costs are approaching the cumulative costs of all molecular and culture-based testing for clinical samples. As we move further into the next-generation sequencing era, culture-free diagnostics could become a reality, with metagenomic-based diagnostics integrated as a routine clinical pathogen diagnostic.

P374

Four principles to establish a universal virus taxonomy.

Peter Simmonds[1], Evelien Adriaenssens[2], Murilo Zerbini[3], Workshop Consortium (39 members)[4]

[1]University of Oxford, Oxford, United Kingdom. [2]Quadram Institute, Norwich, United Kingdom. [3]Universidade Federal de Viçosa, Viçosa, Brazil. [4]Various, Various, Ukraine

Abstract

The International Committee for the Taxonomy of Viruses (ICTV) is an international body tasked with the classification of viruses and the nomenclature of species and other taxa. However, there are major differences in the conceptualisation and approaches to virus classification and nomenclature among virologists, clinicians, agronomists amongst others. Here, we provide recommendations for the construction of a coherent and comprehensive virus taxonomy, based on expert scientific consensus reached by virologists, evolutionary biologists and ICTV members at a workshop held in Oxford in April, 2022.

Firstly, assignments of viruses should be congruent with the best attainable reconstruction of their evolutionary histories, i.e., taxa should be monophyletic. Secondly, phenotypic and ecological properties of viruses may inform, but not override, evolutionary relatedness in the placement of ranks. Thirdly, alternative classifications that consider phenotypic attributes, such as being vector-borne ("arboviruses"), infecting specific hosts (eg. "mycoviruses", "bacteriophages") or displaying specific pathogenicity (eg. "human immunodeficiency viruses"), may serve important clinical and regulatory purposes but often create polyphyletic categories that do not reflect evolutionary relationships. Nevertheless, such classifications should be maintained if they serve needs of specific communities or play practical clinical or regulatory roles. However, they should not be considered or called taxonomies. Finally, while an evolution-based framework enables viruses discovered by metagenomics to be incorporated into the ICTV taxonomy, there are essential requirements for quality control of the sequence data used for these assignments.

Combined, these four principles enable future development and expansion of virus taxonomy as the true evolutionary diversity of viruses becomes apparent.

P375

Ecological genomics of host adaptation of *Escherichia coli*

William Monteith[1,2], Biel Garcias Puigserver[3], Evangelos Mourkas[2], Samuel Sheppard[2]

[1]University of Bath, Bath, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]Universitat Autònoma de Barcelona, Barcelona, Spain

Abstract

Escherichia coli is both the most common member of the commensal microbiota of the vertebrate gut and an opportunistic pathogen causing both intestinal and extra-intestinal infections. Whole-genome sequencing and population genetics-based epidemiology has revealed a diverse population structure of *E. coli* that segregates into distinct phylogenetic lineages. Host characteristics such as diet, gut morphology and physiology are important predictors of the distribution of *E. coli* lineages. However, the population structure is also shaped by host ecology, specifically, the domestication status of the host.

To investigate the genetic basis of *E. coli* host adaptation a collection of >5,500 *E. coli* genomes isolated from diverse host species and ecologies, representing eight major phylogenetic lineages, was constructed. We performed 80 genome-wide association studies (GWAS) in parallel on this collection investigating the genetic variation associated with host species, ecology and physiology.

The GWAS results reveal a genomic mosaic of adaptive regions, whereby in many cases there are variations (i.e. SNPs) associated with complementary host species, physiology and ecology, for example adaptation to cow, ruminant and domestic niches, respectively. This hierarchical approach to understanding host adaption provides new avenues to investigate the genetic basis of phenotypic variation in bacteria. Identification of specific genes underlying phenotypic variation within a given host species, in addition to adaptations linked to broader ecology, are important for deciphering the ecological and evolutionary forces that shape the population structure of *E. coli*.

P376

Uropathogenic *Escherichia coli* population structure and antimicrobial susceptibility in Norfolk, UK

Cailean Carter ORCID iD[1,2], Alexandra Hutchison[3], Steven Rudder[1], Elizabeth Trotter[3], Ngozi Elumogo[3], Gemma Langridge[1]

[1]Quadram Institute, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Norfolk and Norwich University Hospital, Norwich, United Kingdom

Abstract

Half of all women have experienced a urinary tract infection (UTI) in their lifetime, and this remains a persistent issue in rural counties like Norfolk. In alignment globally, Uropathogenic *E. coli* (UPEC) are the main etiological agent for UTIs in Norfolk and are increasingly difficult to treat due to multi-drug resistance associated with clonal groups. We therefore set out to identify which clonal groups and resistance genes are disseminating in the community and hospitals in Norfolk, the first study of its kind for UPEC in this region. 217 isolates were collected between August 2021 and January 2022 from the reference clinical microbiology lab at Norfolk and Norwich University Hospital (NNUH) for isolates equally from the community and hospitals. These were sequenced using the Illumina and MinION platforms for in silico multi-locus sequence typing and antibiotic resistance determinant detection using AMRFinderPlus. Of the 74 STs detected in our dataset, 8 STs were responsible for 57% of UTI infections sampled for this study, 6 of which had broad susceptibility to antibiotics used in UTI screening in Norfolk. The exceptions were multi-drug resistant clonal groups ST131 and ST1193 which were the fourth and eighth most frequently isolated ST, a smaller proportion of the population than for other regions of the UK. Trimethoprim resistance rates were high in community (15.2%) and hospital (36.2%) settings which limits cost-effective treatment for many cases. Similarly, ST131 and ST1193 were the main causes of ciprofloxacin resistance in both hospital and community settings, further limiting cost-effective therapeutic options.

P377

Core Genome Multilocus Sequence Typing Development and Validation for *Haemophilus influenzae*

Made Krisna ORCID iD[1,2,3], Keith Jolley[2], Alexandra Boubour[4], Raph Hamers[1,3], Angela Brueggemann[4], Odile Harrison[2], Martin Maiden[2]

[1]Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom. [2]Biology Department, University of Oxford, Oxford, United Kingdom. [3]Oxford University Clinical Research Unit Indonesia, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [4]Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

Abstract

Haemophilus influenzae (Hinf) is both part of the human nasopharyngeal commensal microbiota and a human pathogen causing invasive diseases. The high genetic diversity observed in Hinf as a result of frequent horizontal gene transfer events necessitates approaches for fine discrimination among strains whilst minimising bias in extrapolating genetic relatedness. Core genome MLST (cgMLST) typing is a highly adaptable and dynamic approach that allows a granular evaluation of the population structure. A cgMLST scheme for Hinf was developed using several bioinformatics tools (PIRATE, chewBBACA, PEPPAN, and Genome Comparator by BIGSdb). An optimal workflow combining different tools was determined and applied to a dataset of complete Hinf genomes (N = 14) and draft genomes (N = 1,059). Initial analyses revealed 1,017-1,309 core genes, after paralog exclusion. Variations in annotation and paralog definitions employed by each software were likely to cause discrepancies in observed results. After implementing the predefined workflow combining different tools and validation using an independent dataset (N = 1,495), over 1,000 core loci were identified. Based on neighbor-joining tree comparison, cgMLST had a better discriminatory capability than 7-loci MLST and ribosomal MLST. The results indicate that genomic comparison using cgMLST provides a more robust population structure characterisation of Hinf. The scheme will be implemented in the publicly available PubMLST database (https://pubmlst.org/), which hosts over 3,000 Hinf genomes, and will improve surveillance and understanding of this increasingly antimicrobial resistant pathogen.

P378

The Kocurious case of Noodlococcus: characterisation of a laboratory contaminant provides genomic insights into Kocuria rhizophila

Siu Fung Ho, Gregory McCallum, Elizabeth Cummins, Alex Wildsmith, Ross McInnes, Samuel Nicholls, Joshua Quick, Willem van Schaik, Robert Moran

University of Birmingham, Birmingham, United Kingdom

Abstract

A laboratory contaminant, Noodlococcus was named for its coccoid cells and unusual colony morphology, which resembled a pile of noodles. On the day of its discovery, we shared photos of Noodlococcus on social media, which attracted international interest from scientists and the general public. Along with laboratory characterisation and electron microscopy, we generated a complete Noodlococcus genome sequence using Illumina and Oxford Nanopore data. The genome consisted of only a 2,732,108 bp chromosome that shared 97.6% Average Nucleotide Identity (ANI) with the *Kocuria rhizophila* type strain TA68. Despite its environmental ubiquity and relevance to agriculture, bioremediation and human medicine, there have been few genomic studies of the *Kocuria* genus. We conducted a comparative, phylogenetic and pan-genomic examination of all 131 publicly-available *Kocuria* genomes, with a particular focus on the 29 that had been called *K. rhizophila*. This revealed two phylogenetically-distinct lineages of *K. rhizophila*, with within-lineage ANI values of 96.8-99.9% and between-lineage values of 89.6-90.5%. With ANI values of <95% relative to TA68, the second lineage constitutes a separate species that we refer to as *K. pseudorhizophila*. Differences in the *K. rhizophila* and *K. pseudorhizophila* pangenomes likely reflect both phenotypic and evolutionary variation. Notably, the type I-E CRISPR-Cas system we identified in Noodlococcus is conserved in true *K. rhizophila* but absent from *K. pseudorhizophila*.

P379

Pseudomonas aeruginosa genetic diversity within and between the lungs of patients with non-cystic fibrosis bronchiectasis

Niamh Harrington[1], Kendall Cagney[1], Anastasia Kottara[2], Matthew Shepherd[2], Taoran Fu[2], Dylan Childs[3], James Chalmers[4], Joanne Fothergill[1], Steve Paterson[1], Michael Brockhurst[2]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Manchester, Manchester, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom. [4]University of Dundee, Dundee, United Kingdom

Abstract

The evolution of antibiotic resistance during chronic bacterial infections is leading to high rates of treatment failure. Several conditions cause increased risk of recurrent infection, including the chronic lung disease bronchiectasis. *Pseudomonas aeruginosa* is the most prevalent pathogen affecting people with this condition and is associated with worsened disease outcomes. The genetic diversity of *P. aeruginosa* infection populations in this context, and the effect on treatment success and infection outcome, is poorly understood. To investigate this, we performed whole genome sequencing of over 2000 *P. aeruginosa* isolates from the sputum of bronchiectasis patients enrolled in a Phase-III randomized clinical trial for an inhaled ciprofloxacin treatment (ORBIT3); and pooled sequencing of each infection population for 183 patients. We constructed core genome phylogenies to define the population diversity within and between patients. Population sequencing revealed the dynamics between *P. aeruginosa* subpopulations during infection driven, in part, by the selection pressure of antibiotic treatments. Throughout the trial, all *P. aeruginosa* populations became resistant to the inhaled ciprofloxacin. Our sequencing analysis revealed mutations in known genes associated with ciprofloxacin resistance including *gyrA*, *gyrB*, and *parE*, some not previously reported, as well as other pathways. Our work provides insight into *P. aeruginosa* infection populations in people with non-cystic fibrosis bronchiectasis, which has not been extensively studied. It also furthers understanding of antibiotic resistance evolution in a clinical setting, which will facilitate novel treatment development.

P380

Discovery, biosynthetic assessment and evolutionary study of *Streptomyces ortus* sp nov. isolated from a deep-sea sponge

Sam Williams ORCID iD[1], Catherine Back[1], Eleanor Best[1], James Stach[2], Tom Williams[1], Paul Race[1], Paul Curnow[1]

[1]University of Bristol, Bristol, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom

Abstract

The deep sea is known to host novel bacteria with the potential to produce a diverse array of undiscovered natural products. Understanding these bacteria is thus of broad interest in ecology and could also underpin applied antimicrobial drug discovery. The *Streptomyces* genus has contributed more to our antibiotic arsenal than any other group of bacteria. Despite decades of exploration, global analysis has suggested they still possess more undiscovered biosynthetic diversity than any other bacterial group. Here, we isolate a new strain of *Streptomyces* from the tissue of a deep-sea sponge found at a depth of 1869 m in the Atlantic Ocean. Phylogenomics determined this strain represents a novel marine species that we name *Streptomyces ortus* sp. nov. The biosynthetic potential of *S. ortus* sp. nov. was assessed relative to other members of its clade via comparative gene cluster family (GCF) analysis and we identified six biosynthetic clusters unique to our deep-sea strain. Culture-based assays were used to demonstrate the antibacterial activity of *S. ortus* sp. nov. against two drug-resistant human pathogens. Finally, we assessed the contribution of vertical evolution to GCF content in this clade and revealed a clear congruent relationship between phylogeny and GCF content, even between strains isolated from vastly different environments. This work demonstrates the utility of isolating novel Streptomyces strains for antibiotic discovery. For the first time, we used species tree-gene cluster tree reconciliation to assess the contribution of vertical evolution on the biosynthetic gene cluster content of *Streptomyces*.

P381

Comparative genomics reveals functional conservation of LysR-type transcriptional regulation in the nosocomial pathogen *Pseudomonas aeruginosa*

Jamie Deery, F. Jerry Reen

University College Cork, Cork, Ireland

Abstract

Signal responsive proteins present a tunable microbial response system that governs changes in nutrient availability, metabolic reprogramming, signalling and cell-cell communication, bioremediation, efflux, and biotransformation. The LysR-Type Transcriptional Regulation (LTTR) system is one of the most abundant classes of transcriptional regulator in gram negative bacteria and it is particularly abundant in *Pseudomonas aeruginosa*, a harmful nosocomial pathogen associated with burn wounds and respiratory infection in Cystic Fibrosis. While several LTTR proteins in this species have been shown to play a role in antimicrobial resistance and signalling, very little is known about how and why these proteins have evolved to such frequency, and whether or not any relationships exist within the distribution. In this study we sought to identify the patterns underpinning LTTR distribution in *P. aeruginosa* and to uncover cluster based relationships within the pangenome. Comparative genomics revealed ~86k LTTRs present across the sequenced genomes (n=699) of this pathogen in the JGI IMG database alone. They are widely distributed across the species, with core LTTRs present in greater than 90% of the genomes, and accessory LTTRs identified in less than 2%. Further analysis showed that subsets of the core LTTRs can be classified as either variable or conserved, with the variable LTTRs being *P. aeruginosa* specific, in contrast to the widely distributed conserved LTTR proteins. These findings provide new insights into the organisation of LTTR proteins within the *P. aeruginosa* species, delivering a scaffold for future studies to determine the functional significance of the cluster, conservation, and distribution patterns identified.

P382

The tRNA-Leu(CAG) gene of Ser2 budding yeasts is used, non-essential, and likely not involved in translation

Eoin O Cinneide ORCID iD, Kenneth Wolfe

UCD Conway Institute, Dublin, Ireland

Abstract

The yeast genera Saccharomycopsis and Ascoidea use a non-standard genetic code in their nuclear genes, where CUG codons are translated as serine instead of leucine. This is due to the presence of a tRNA-Ser with the unusual anticodon CAG, which reads CUG codons. However, these species also retain an ancestral tRNA-Leu(CAG), which theoretically could translate CUG codons as leucine. In Ascoidea asiatica, both tRNAs are active and dual translation occurs. However, all species in the genus Saccharomycopsis appear to only translate CUG codons as serine, despite having both tRNA genes. This raises the question of why the tRNA-Leu(CAG) gene has been retained in Saccharomycopsis, and we hypothesised that it might be required to translate CUG codons as leucine in a specialised subset of genes. To investigate this, we sequenced the genomes of 25 Saccharomycopsis species and analysed their genes for signatures of CUG-Leu translation, and generated gene deletions to test for essentiality. This tRNA-Leu(CAG) deletion mutant displayed no phenotype, and thus appears non-essential. We made alignments of orthologous genes, and applied a heuristic rule to search for columns with a high likelihood of requiring CUG translation as leucine. We detected a strong bioinformatic signal of CUG-Leu translation in genes involved in meiosis and sporulation, a specialised stage of the yeast life cycle. We hypothesised that tRNA-Leu(CAG) may be employed exclusively during sporulation. We performed LC-MS/MS on sporulating cultures to determine if CUG is translated as leucine exclusively during this process, which we could not detect.

P383

Transcriptional regulation in response to low oxygen conditions by ArcA in *Vibrio cholerae*

James Haycocks[1], Tom Guest[2], David Grainger[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Umeå University, Umeå, Sweden

Abstract

Vibrio cholerae is the causative agent of the disease cholera, which is responsible for 4 million cases of diarrhoea and over 100,000 deaths each year (WHO, 2015). Cholera is currently epidemic in 16 countries (ECDC, 2020) and the ongoing outbreak in Yemen has already caused over 2.5 M cases and 4000 deaths (Federspiel and Ali, 2018). *V. cholerae* persists in two habitats; a vast environmental reservoir as aquatic biofilms on chitinous surfaces, and the crypts of the human small intestine. The organism requires a very different transcription programme for each of these situations, and thus timely transcriptional regulation is vital for the success of the pathogen. The roles of various transcription factors responding to different environmental cues (e.g. carbon availability, cell density) in regulating the life cycle of *V. cholerae* have been well-studied, but the role of oxygen concentration is not well understood. Of note, the lumen of the small intestine is largely anaerobic, but the crypts are slightly oxygenated (1-2%).

To remain viable, bacteria must maintain a crucial balance of oxidative and reductive power in the cell. This is controlled by the two-component ArcA/B regulatory system, which shuts off components of the cell depleting reducing power when terminal electron acceptors (e.g. oxygen) become unavailable. In this study, we investigated the role of ArcA in regulating lifestyle switching using a variety of genomic and biochemical approaches. We show that ArcA targets not only genes involved in fine-tuning metabolism for low-oxygen conditions, but also targets genes required for virulence.

P384

Delving into the diversity of highly conserved 16S ribosomal RNAs and their operon architectures in Bacterial and Archaeal genomes

Riya Srivastava[1], Arpita Bansal[1], Gaurav Sharma ORCID iD[1,2]

[1]Past Affiliation: Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru, Karnataka, India. [2]Indian Institute of Technology Hyderabad, Sangareddy, Telangana, India

Abstract

16S/23S/5S rRNAs are integral parts of 70S ribosomes and associated protein synthesis machinery. Predominantly, these subunits are clustered and expressed as a single operon. 16S rRNA sequence is present in multiple copies per organism; however, it is still extensively used as a phylogenetic marker for bacterial taxonomy and classification. Moreover, the number of 16S operons per organism goes beyond any specific taxa, genome size, ecological niche, etc. Here, we explore the diversity, identity, and evolutionary relatedness of 16S/23S/5S rRNAs, their operon structures, and their nearby syntenic architecture among 3,893 genomes belonging to Bacteria (1,271 genera) and Archaea (96 genera). This study revealed that *Tumebacillus avium* has the maximum rRNAs (108; 32 operons), including 37 16S, 39 23S, and 32 5S rRNAs. 95% of identified 16S rRNAs are present as linked cluster with a nearby 23S and 5S rRNAs. No correlation exists between 16S rRNA numbers and the genome size or GC% of the respective organism. Out of identified clusters having one of the 16S/23S/5S rRNAs within a vicinity of ten genes, two types of differently organized abundant clusters, i.e., 16S- $tRNA^{Ile}$-$tRNA^{Ala}$-23S-5S (33.3%) and 16S-23S-5S (29.8%) were distinctly visible which raises concerns behind ITS diversity in such a conserved genomic structure. A few organisms have a mere 80-95% identity within two 16S rRNAs copies, and we deciphered their evolutionary relatedness at inter and intraspecies levels using homology and phylogenetic analysis. Overall, this study aims to understand how this ITS diversity affects the 16S rRNA-based taxonomy and metagenomic studies.

P385

Using Infectious Bronchitis Virus to Characterise Technical Biases in High Throughput Sequencing

Graham Freimanis[1], Michael Oade[2], Ge Wu[1], Sarah Keep[1], Erica Bickerton[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Princeton University, Princeton, USA

Abstract

During the process of high-throughput sequencing, mutations are introduced into viral genome sequences as a by-product of the sample preparation process. When combined with miscalls incorporated from variant-calling, rare mutations are practically indistinguishable from process-introduced error. This confounds useful intra-host diversity information critical for molecular phylogenetic study.

We designed a model system, to characterise these technical biases for established whole genome sequencing, and analysis protocols. We sequenced eight copies of an Infectious Bronchitis virus genome, over 2 MiSeq sequencing runs with high (1µg) and low (100ng) starting input, to identify the level of variation within the resulting pipeline. Comparing high and low input, we assessed genomic profiles, identifying variation using in-house variant calling analysis pipelines. We then applied this data to interpret the level of bias within each component of the pipeline, characterising that DNA based samples showed significantly less variation, compared to their RNA counterparts, and that significant point of introducing variation was at the stage of reverse transcription. To fully assess the variation introduced by variant calling, and treating our bioinformatics pipeline as a gold standard, we then processed this dataset, comparing the output of 4 virus variant callers and 4 global haplotypers, providing a benchmark of the variation between individual software callers. This dataset demonstrated a significant level of variation between callers and global haplotypers.

These results also provides a baseline on the biases inherent in the sequencing and analysis pipelines and highlights the variability, when variant calling from high-throughput sequencing datasets.

P386

The molecular genetics of the virulence plasmid of *E. coli* O104:H4.

Rachel Whelan, Gareth McVicker

Nottingham Trent University, Nottingham, United Kingdom

Abstract

The exchange of mobile genetic elements has previously resulted in the emergence of atypical strains posing a huge risk to human health. One such example was detected in the 2011 European outbreak of *Escherichia coli* O104:H4 which carried an unusual combination of EAEC- and STEC associated virulence factors on a plasmid and phage respectively. The *E. coli* O104:H4 virulence plasmid has exhibited unusual stability when subjected to a range of environmental stresses, in contrast to more frequent plasmid loss in vivo. Following the curing of the virulence plasmid via heat shock, we have identified a significant phenotypic difference between the wild-type and plasmid free variant. These include a dramatic reduction in motility and adherence to human colonic cells with an increased aggregative phenotype in the plasmid free variant. We have further investigated the function of putative TA system genes present on the virulence plasmid by inducing their expression in lab made vectors in order to further understand the mechanisms involved in plasmid maintenance during infection, from which we have identified a functional CcdAB TA system. Furthering our knowledge of these maintenance systems allows us to better understand the systems involved in maintaining crucial virulence factors during infection. This atypical strain had displayed heightened pathogenicity and provided unforeseen treatment challenges and was therefore investigated as a model for understanding MGE carriage to predict and combat future outbreaks of hybrid pathovars through the exchange of mobile genetic elements.

P387

What the phage? A genomic map of the prophage content of domestic UK Shiga-toxin producing *E. coli* (STEC) O157:H7 genomes.

David Greig ORCID iD[1,2,3], Claire Jenkins ORCID iD[1,2]

[1]National Infection Service, United Kingdom Health Security Agency, London, United Kingdom. [2]NIRH Health Protection Research Unit for Gastrointestinal Pathogens, Liverpool, United Kingdom. [3]Division of Infection and Immunity, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Following the recent improvements in long-read sequencing technologies, it is now possible to sequence and generate single contiguous *de novo* assemblies of complex prophage-rich bacterial genomes. We used Oxford Nanopore Technologies to generate complete assemblies of 176 genomes of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and to characterise the accessory genome, including prophage content. The Shiga toxin gene (*stx*), the key virulence factor and defining characteristic of the STEC group, is bacteriophage encoded.

Bioinformatics analyses identified and excised 2,981 prophages from the 176 STEC O157:H7 genomes. Prophage content varied from 14 to 20 prophages per chromosome, with STEC O157:H7 belonging to lineage II having more prophages on average than those in lineage I. For lineage II, genome size was proportional to prophage number, whereas lineage I genomes varied in size independent of prophage content.

Of the 2,981 prophages, 308 were *stx*-encoding prophages each harbouring a single *stx* subtype of either *stx1a*, *stx2a* or *stx2c*. Prophages harbouring *stx1a* had a preferential *stx*-encoding bacteriophage integration site (SBI) of *yehV* (96.6%, 58/60) whereas *stx2c* had a preferential site of *sbcB* (98.25 112/114). *stx2a*-encoding prophages had the most diverse SBI sites, including *argW* (49.2% 66/134), *sbcB* (31.3% 42/134), *yecE* (8.2% 11/134). There were 15 genomes that had multiple copies of stx2a-encoding prophages, inserted at different SBIs.

Accurately mapping the acquisition and loss of *stx*-encoding bacteriophages enables us to predict the virulence potential of the different STEC O157:H7 lineages, and to monitor for emerging threats to public health.

P388

Monitoring Changes in Viral Population Diversity During Pathogenic Infection of Infectious Bronchitis Virus

Ge Wu, Sarah Keep, Erica Bickerton, Graham Freimanis

the Pirbright Institute, Pirbright, United Kingdom

Abstract

The avian coronavirus, Infectious Bronchitis virus (IBV) represents one of the most significant threats to the global poultry industry. Live-attenuated vaccines (LAVs) currently provide a line of defense in protecting flocks from infection and are produced through serial passages of a virulent field isolate through embryonated hens' eggs. Furthermore, LAVs have been documented to have a potential for reversion to virulence, thereby driving outbreaks. It is unclear how these viruses regain pathogenicity. We sought to profile viruses *in vivo* to understand the genomic changes that accompanied transition to pathogenicity.

A IBV D388 viral genome (D388_cl) was synthesized as a clone and inoculated into SPF Rhode Island red (RIR) chickens, which were assessed against wild type for clinical signs. Samples were taken from culled birds at 4, 6 and 7-days post infection. The synthesized virus exhibited a delayed pathogenic phenotype compared to wild type. We hypothesized that as viruses moved to a pathogenic phenotype there would be an accompanying change in the diversity of their swarm structure. To investigate this hypothesis, we used whole genome sequencing to characterise genomic changes between time points *in vivo*. Changes were compared between wild type and clone viruses and patterns between variants were identified. We also compared these results with those changes observed in other attenuated/pathogenic IBV viruses, in addition to exploring the impact at the level of protein expression.

This investigation characterized the changes in genomic profiles in viruses *in vivo* and how these impacts upon their ability to induce a pathogenic phenotype.

P389

In silico exploration of the rumen microbiome potential as source of CGTase enzymes for biotechnological applications

Izabella de Carvalho Batista Muniz ORCID iD[1,2], Renata Ferreira Santana ORCID iD[1,2], Fernanda Godoy Santos[2], Linda Oyama ORCID iD[2], Renata Cristina Ferreira Bonomo ORCID iD[1], Sharon Huws ORCID iD[2]

[1]Universidade Estadual do Sudoeste da Bahia, Itapetinga, Brazil. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

Cyclodextrins have prominent roles in numerous industrial sectors due to their ability to form inclusion complexes or coacervates with non-polar compounds of different sizes. These structures are uniquely synthesized by the enzymatic action of CGTase (EC 2.4.1.19), which converts starch and its derivatives into cyclodextrins. Cyclodextrin production is limited by the high cost associated with obtaining CGTase, hence studies on this field have a high biotechnological appeal. Rumen microorganisms have been widely reported as producers of carbohydrates and might be a good source of new CGTases isoforms. This work evaluates the occurrence of CGTase sequences in ruminal protein sequence databases. Sixteen CGTase amino acid sequences were selected from literature and used to mine ruminal bacterial protein sequences in the Hungate 1000 collection (418) as well as metagenome assembled genomes (MAGs) from MGnify database (5579) using BLASTp. The retrieved putative CGTase sequences were aligned, and subsequent phylogenetic trees were performed in Clustal Omega and visualized with the Interactive Tree of Life (iTOL). BLASTp results showed 242 and 15 matches from the Hungate and MGnify databases respectively, with percentage identity ranging from 40 to 58.42%. Functional annotations of each putative selected sequence were verified using InterPro platform and alignment tools. The highest occurrence of matches from MGnify belongs to the genus *Catonella sp* (2065), while those from Hungate 1000 belong to the genera *Lachnobacterium* (88), *Streptococcus* (76) and *Lachnospiraceae* (42). These preliminary results highlight the rumen microbiome as a potential source for new CGTase isoforms that could expand their biotechnological applications.

P390

Biotechnological potential of *Blastococcus* (*Actinobacteria*) in arid agro-ecosystems

Maria del Carmen Montero-Calasanz ORCID iD[1], Adnan Yaramis[2], Jan P. Meier-Kolthoff[3]

[1]IFAPA-Junta de Andalucía, Alcalá del Río, Spain. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Leibniz Institute DSMZ, Braunschweig, Germany

Abstract

The genus *Blastococcus* (*Geodermatophilaceae*, *Actinobacteria*) comprises species isolated from sediments, Desert soil, decaying monuments and plant leaves. Research studies based on genomic data are still scarce. In particular, just two papers, focused on single strains, were published so far, which revealed numerous genes involved in stress response and adaptation to harsh habitats apart from an exceptional potential to produce novel natural compounds. In this study, we aim to taxonomically characterise four novel species within the genus *Blastococcus* and explore their biotechnological potential to be applied in arid agro-ecosystems. Our results indicate *Blastococcus* genomes encode a variety of mechanisms involved in adaptation to environmental stress and a high versatility in carbon metabolism and extracellular enzymes. It is suggested that *Blastococcus* representatives could have an unexpected and remarkable role in soil carbon fixation but also in the decay of organic residues, transformation of native soil organic matter and mineralisation of nutrients available for plants. Their metabolism of *Blastococcus* could therefore be key into the natural restoration of soil fertility in arid and degraded soils.

P391

The diversity of Staphylococcus aureus Pathogenicity islands (SaPIs) within and between different sequence types

Cosmika Goswami, Stephen Fox, Thomas Evans, Alistair Leanord

University of Glasgow, Glasgow, United Kingdom

Abstract

Background: SaPIs are mobile genetic elements containing a variety of accessory genes important in virulence. Although some individual SaPIs are well characterised, the diversity of these elements within clinical isolates of S. aureus is not known.

Method: Whole genome sequencing of 1525 blood culture S. aureus isolates was performed using the Illumina platform. Reads were assembled using SPAdes and annotated using PROKKA . A manual method for SaPI identification was used, initially searching for SaPI integrase genes and then for other conserved genes contained within SaPIs.

Results: We identified 1320 SaPIs in 65.3% (n=996) of our isolates, including 109 novel SaPIs at five different att sites. 68.6% (n=683 of 996) of isolates had one SaPI each, 30.3% (n=302) with exactly two SaPIs and 1.1% (n=11) with three. Distribution of SaPIs across different sequence type (STs) showed distinct clustering; ST 30 had the highest average number of SaPIs per isolate (1.75), and ST22 the next highest at 1.18. Despite representing 10.8% of the total collection, ST5 had relatively few SaPIs (0.42 per isolate). Class of SaPI, att site usage, and accessory gene content also varied between ST types. For example, tsst-1 was found predominantly in SaPIs in ST30 strains while sec3 and sell genes encoding enterotoxins were mainly in ST22 and ST45. A number of putative novel accessory genes were identified.

Conclusions: This extraordinary diversity of SaPIs is a major contributor to evolution within S. aureus, leading to acquisition of novel virulence and antimicrobial resistance genes with differential distribution amongst different STs.

P392

Microfluidic single cell extraction protocols.

Natalie Sparks, Josh Quick ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Antibiotic resistance is a prominent threat to global health, perpetuated by over-prescription of broad-spectrum antibiotics. A key contributing factor to this issue is the hinderance of culture-dependent identification and antimicrobial susceptibility testing within a time-sensitive context. Without case-specific identification of pathogen and AMR prediction, ineffective antibiotics are often administered and contributes to the rise of multidrug resistance. As an alternative to this cycle, diagnosis based on single cell data presents a potential avenue that could provide more information on a faster turnaround. Droplet microfluidics offers a high throughput technique that allows capture of single cell resolution via micro-scale partitioning. Here, we present two applications of droplet-based single cell protocols. We have developed a rapid multiple displacement amplification, partitioned across picolitre water-in-oil droplets, building upon the current standards for whole genome amplification. We are also demonstrating ultra-long extractions from agarose scaffolds suspended within droplets, providing an alternative approach for samples that are typically sequenced from bead beating extractions. Developing single cell resolution from long read data provides more information for AMR prediction for a broad variety of field applications.

P393

Whole genome sequencing of bloodstream isolates of Staphylococcus aureus reveals prolonged transmission chains within Neonatal Intensive Care Units

Cosmika Goswami, Stephen Fox, Alistair Leanord

University of Glasgow, Glasgow, United Kingdom

Abstract

Background: Staphylococcus aureus is one of the commonest agents of nosocomial bacteremia. To identify potential transmission of this organism, we have completed whole genome sequencing (WGS) of bloodstream isolates of S. aureus from across Scotland.

Material/methods: 1545 bloodstream isolates of S. aureus from across Scotland from the years 2015 to 2017 were collected. WGS using the Illumina platform was performed for all isolates. Single-nucleotide polymorphisms (SNPs) in the core genome were identified using SMALT and VarScan software. A threshold of 50 SNPs or fewer was used to identify highly related strains.

Results: We found 150 pairs of isolates with 50 or fewer pairwise SNP differences. Associated clinical data identified persistent transmission chains within neonatal intensive care units; we focused on those within areas where all bloodstream isolates had been collected within the time frame studied. In one unit over a 20-month period, we discovered 10 neonates with bloodstream S. aureusST30 infection with fewer than 50 SNP differences. 9 of the isolates were methicillin sensitive; one isolate acquired methicillin resistance For 8 of these infections the patients had overlapping hospital admissions but for 2 patients there was no overlap, suggesting intermediate carriers of this strain or environmental contamination. None of these cases were flagged as possible outbreaks. Similar prolonged transmission chains were found in other neonatal intensive care units.

Conclusions: The high resolution of WGS allowed us to identify previously unsuspected transmission of S. aureus strains producing bloodstream infection within neonatal intensive care units within Scotland.

P394

A stable and taxonomy free approach to consolidate microbiome genomic data

John-Paul Wilkins, Chris Creevey

Queen's University Belfast, Belfast, United Kingdom

Abstract

Microbiomes can be studied at genomic levels ranging from single genes/amplicons to community-wide shotgun metagenomes. The choice for researchers is based on varying advantages and disadvantages of each, including specificity and cost, but lead to difficulties making direct comparisons between them. To address this, we have developed a novel barcoding system based on the Life Identification Number (LIN) framework. It allows the consolidation and integration of multiple levels of microbiome genomic datasets.

We applied the approach to link metataxonomic and metagenome-assembled genomic data from ruminant microbiomes and SARS-COV2 sequencing data from clinical samples and wastewater surveillance programs. Direct comparison between existing studies, that utilise different data generation approaches, was aided. Stable classification is robust to changes in taxonomy. Inaccuracies and inconsistencies in reference databases and published studies were bypassed. It is designed to allow the ongoing incorporation of future data.

The fine-grained resolution of the barcoding approach enhances phylogenetic placement. This provides a rationale for its use in leveraging the advantages of full genome sequences in large studies where it would otherwise not be economically viable or practical.

BLOCK B

Display: Wednesday 19 – Thursday 20 April

Presentation session: Wednesday 19 April (16.30-17.40)

Session Topic: Tuberculosis: The forgotten pandemic

P395

Cholesterol-dependent activity of dapsone against non-replicating persistent mycobacteria

Jonathan Cox ORCID iD

Aston University, Birmingham, United Kingdom

Abstract

One third of the world's population is estimated to be latently infected with Mycobacterium tuberculosis. This reservoir of bacteria is largely resistant to antimicrobial treatment that often only targets actively replicating mycobacteria, with current treatment for latent infection revolving around inhibiting the resuscitation event rather than preventing or treating latent infection. As a result, antimicrobials that target latent infection often have little to no activity in vivo. Here we report a method of in vitro analysis of physiologically relevant non-replicating persistence (NRP) utilising cholesterol as the sole carbon source, alongside hypoxia as a driver of Mycobacterium bovis BCG into the NRP state. Using the minimal cholesterol media NRP assay, we have observed an increased state of in vitro resistance to front-line anti-tubercular compounds. However, following a phenotypic screen of an approved-drug library, we have identified dapsone as a bactericidal active molecule against cholesterol-dependent NRP M. bovis BCG. Through an overexpression trial of likely antimicrobial target enzymes, we have further identified FolP2, a non-functional dihydropteroate synthase homolog, as the likely target of dapsone under cholesterol-NRP due to a significant increase in bacterial resistance when overexpressed. These results highlight the possible reason for little in vivo activity seen for current front-line anti-NRP drugs, and introduces a new methodology for future drug screening as well as a potential role for dapsone inclusion within the current treatment regime.

P396

Mycobacterial fluxomics as a powerful tool for measuring metabolism and identification of drug targets in tuberculosis and leprosy

Khushboo Borah Slater[1], Martin Beyß[2], Karina Girardi[3], Jacque-Lucca Kearney[1], Tom Mendum[1], Ramanuj Lahiri[4], Dany Beste[1], Flavio Lara[3], Katharina Nöh[5], Johnjoe Mcfadden[1]

[1]University of Surrey, Guildford, United Kingdom. [2]Forschungszentrum Jülich,Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany, Jülich, Germany. [3]Instituto Oswaldo Cruz, Rio de Janeiro, Brazil. [4]United States Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Program, Baton Rouge, Louisiana, USA. [5]Forschungszentrum Jülich,Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany

Abstract

Fluxomics is an emerging technology that uses experimental and computational approaches to measure metabolism in any living organism. Our research focuses on developing new fluxomic tools to measure the metabolism of carbon and nitrogen (two primary nutrients and building blocks of a cell) in two of the most devastating human pathogens that causes tuberculosis (TB) and leprosy. TB caused by *Mycobacterium tuberculosis* (Mtb) is one of the world's biggest infectious killers causing >1 million deaths every year. Leprosy caused by *Mycobacterium leprae* has plagued humanity for thousands of years and continues to cause morbidity, disability, and stigmatization with ~200,000 cases detected globally.

We applied 13C-metabolic flux analysis (MFA) and demonstrated the flexible use of the methyl citrate cycle by Mtb during growth on cholesterol and acetate. Our 15N-flux spectral ratio analysis tool (FSRA) identified multiple nitrogen sources for Mtb replicating in human macrophages. We recently developed 13C15N-MFA to provide the first measurements of carbon-nitrogen dual metabolism in *M. bovis* BCG using a single one-shot dual isotopic labelling experiment, dual atom modelling and Bayesian statistics. We developed the first genome-scale metabolic model GSMN-ML and formulated a nutrient media for *in vitro* growth of *M. leprae*.

Our fluxomics research provided the carbon and nitrogen metabolic profile for intracellular Mtb and M. leprae growing in human cells and *in vivo*. We identified the target nodes: phosphoserine transaminase in Mtb and phosphoenolpyruvate carboxylase in *M. leprae* for drug development, which is an urgent need to develop effective treatments to end TB and leprosy.

P397

Antivirulence therapy as a drug development strategy for tuberculosis

Divya Tiwari ORCID iD

Queen Mary University of London, London, United Kingdom

Abstract

Tuberculosis treatment strategies have developed into a two-pronged approach involving the development of novel antimicrobials as well as agents that stimulate host responses to the pathogen (host-directed therapies). The presence of complex lipids in Mycobacterium tuberculosis (Mtb) cell wall results in a thick waxy coat around Mtb that acts as a structural barrier protecting the bacteria from host factors and rendering the commonly used antibacterial compounds ineffective against Mtb. The cell wall also plays an important role in Mtb interaction with the host immune system with complex lipids involved in modulating the immune response to support bacterial survival throughout the course of infection. We have previously reported that silencing biotin protein ligase (BPL) expression in Mtb impacts cell integrity leading to a loss of acid fastness and enhanced accumulation of rifampicin inside Mtb cells (PMID: 29695454)

Here we present novel data that indicate that incomplete genetic inhibition of BPL expression is not bactericidal and does not impact bacterial growth in vitro. However, it alters bacterial virulence factors that alter host-pathogen interaction and lead to an enhanced pro-inflammatory host immune response. This results in early clearance of these strains from the host lungs in a murine model of aerosol infection. This study identifies key virulence factors that are dispensable for bacterial growth but are critical for the survival of Mtb bacteria in the host. This study, therefore, provides a window into novel pathways and anti-virulence mechanisms that can be targeted for novel anti-tubercular drug therapies.

P398

Investigating vanoxerine as a novel Mycobacterial efflux inhibitor

Asti-Rochelle Meosa John ORCID iD, rAlexander Kingdon ORCID iD, Gurdyal Besra ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Mycobacterium tuberculosis, the causative agent of tuberculosis, accounted for an estimated 10.6 million infections in 2021, with 1.6 million fatalities. Further, the rise of multidrug-resistant and extensively drug-resistant tuberculosis has increased the need for novel and effective treatments against this infection. Drug repurposing is considered a promising alternative, which involves reprofiling existing drugs from different therapeutic uses and has already been used to identify anti-tuberculosis drugs, such as linezolid and the fluoroquinolones. Based on previous research, the drug vanoxerine was identified as a possible antimycobacterial drug, however the exact target was yet to be elucidated. In this project, using *M. smegmatis* as a model organism, the aim was to investigate the possible targets of vanoxerine. Several targets were overexpressed in *M. smegmatis*, to look for shifts in the MIC. The investigated targets included several membrane proteins and stress response proteins. To further investigate the mode-of-action, a fluorescence assay was used to measure both the efflux and accumulation of ethidium bromide in the presence of vanoxerine. *M. smegmatis* efflux was inhibited by vanoxerine. Drug efflux is a key resistance mechanism used by mycobacteria, hence, the identification of vanoxerine as an efflux inhibitor highlights a possible avenue to diminish efflux, increasing the efficacy of current antimicrobials. Hence, further investigation into the potential use of the drug as a therapeutic adjuvant must be undertaken, to confirm if vanoxerine has the potential to be used in a clinical setting.

P399

Pangenome analysis of *Mycobacterium tuberculosis (Mtb)* complete genomes revealed genetic differences among lineages

Mahboobeh Behruznia[1], Maximilian Marin[2,3], Maha Farhat[2,4], Conor Meehan[1,5]

[1]Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom. [2]Department of Biomedical Informatics, Harvard Medical School, Boston, USA. [3]Department of Systems Biology, Harvard Medical School, Boston, USA. [4]Pulmonary and Critical Care Medicine, Massachusetts General Hospital, Boston, USA. [5]Unit of Mycobacteriology, Institute of Tropical Medicine, Antwerp, Belgium

Abstract

The pangenome size and function of the *Mycobacterium tuberculosis* complex has been greatly debated in recent years. Here we used a highly curated and complete data to investigate the gene content variation in the human lineages of *Mtb* (L1 to L8) to identify factors that may affect virulence, antimicrobial resistance, and evolutionary characteristics.

A total of 297 complete, closed genomes were used in this study. TB-profiler was used for lineage assignment and BUSCO for genome quality control. PGAP was used for genome annotation, and Panaroo was used for pangenome analysis. The accessory genomes were assigned COG functional categories using eggNOG-mapper.

The *Mtb* pan-genome contained a total of 4,276 genes, including 3,853 core and 423 accessory genes. The small accessory genome size further supports the clonal evolution of Mtb. A principal component analysis (PCA) based on the accessory genomes showed that genomes clustered by their lineage, further suggesting vertical patterns of gene loss and gain but also showing that within lineage differences in gene content are common. Genes with unknown function (S), followed by genes involved in defence mechanisms (V), replication, recombination and repair (L), were the most important COG functional groups that explained most of these variations in the PCA. Analysis of the accessory gene content also showed the presence of lineage-specific genes that can explain the metabolic variations and virulence potential in *Mtb* lineages.

P400

Rv0365c is a glycoside hydrolase family 76 enzyme responsible for cleaving lipoarabinomannan in *Mycobacterium tuberculosis*

Aaron Franklin[1], Abigail Layton[1], Albel Singh[1], Sudagar Gurcha[1], Gurdyal Besra[1], Elizabeth Lowe[2], Patrick Moynihan[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]university of Newcastle, Newcastle, United Kingdom

Abstract

Mycobacterium tuberculosis, the causative agent of Tuberculosis, is responsible for the deaths of around 1.5 million individuals each year. Mycobacteria have a uniquely complex cell envelope which contributes to the pathogens ability to survive and thrive within macrophages. A key component of this cell envelope are the glycolipids as they have important roles in both structural support and modulation of the host immune system. One of the major phospholipids produced by mycobacteria is phosphatidylinositol. This molecule can be glycosylated to produce the phosphatidylinositol mannosides, as well as lipomannan and lipoarabinomannan. These mannosylated glycolipids are typically found in the periplasm anchored to the cell membrane. However, present in the mycobacterial capsule are molecules that are thought to be derived from lipoarabinomannan, but which lack their lipid carrier. There is no explanation as to how arabinomannan is released from its lipid carrier and trafficked from the periplasm to the capsule where it interacts with the host immune system. Using biochemical assays, here we provide evidence that a glycoside hydrolase of family 76, Rv0365c, is the enzyme responsible for hydrolysing lipoarabinomannan and allowing the derived glycolipid to be trafficked to the cell surface. Through thin-layer chromatography we show that Rv0365c is first capable of digesting mannan purified from Saccharomyces cerevisiae, and then confirm that the enzyme is active on lipoarabinomannan purified from Mycobacterium bovis BCG. Furthermore, through mass spectrometry, we provide evidence that one of the products released from this reaction has a mass spectrum consistent with the lipid carrier of the glycolipid.

P401

Structural Basis of Glycerophosphodiester Recognition by the *Mycobacterium tuberculosis* Substrate-Binding Protein UgpB

Jonathan Fenn[1], Ridvan Nepravishta[2], Collette Guy[1], James Harrison[1], Jesus Angulo[2], Alexander Cameron[1], Elizabeth Fullam[1]

[1]University of Warwick, Coventry, United Kingdom. [2]University of East Anglia, Norfolk, United Kingdom

Abstract

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB) and has evolved an incredible ability to survive latently within the human host for decades. The *Mtb* pathogen encodes for a low number of ATP-binding cassette (ABC) importers for the acquisition of carbohydrates that may reflect the nutrient poor environment within the host macrophages. *Mtb* UgpB (Rv2833c) is the substrate binding domain of the UgpABCE transporter that recognizes glycerophosphocholine (GPC), indicating that this transporter has a role in recycling glycerophospholipid metabolites. By using a combination of saturation transfer difference (STD) NMR and X-ray crystallography, we report the structural analysis of *Mtb* UgpB complexed with GPC and have identified that *Mtb* UgpB not only recognizes GPC but is also promiscuous for a broad range of glycerophosphodiesters. Complementary biochemical analyses and site-directed mutagenesis precisely define the molecular basis and specificity of glycerophosphodiester recognition. Our results provide critical insights into the structural and functional role of the *Mtb* UgpB transporter and reveal that the specificity of this ABC-transporter is not limited to GPC, therefore optimizing the ability of *Mtb* to scavenge scarce nutrients and essential glycerophospholipid metabolites via a single transporter during intracellular infection.

P402

tris-(1,10-phenanthroline)iron(II) complex potentially targets cell wall remodelling in *Mycobacterium tuberculosis*

Fernanda Demarqui ORCID iD, Rachel Machado ORCID iD, Débora Campos ORCID iD, Fernando Pavan ORCID iD

São Paulo State University, Araraquara, Brazil

Abstract

The emergence of multi and extensively-resistant tuberculosis makes the discovery of new chemical entities ultimately important to treat the disease. Our group showed the tris-(1,10-phenanthroline)iron(II) complex as a great potential new drug, active at low concentrations against H37Rv and resistant clinical isolates and not cytotoxic to different cell lines, but its mechanism of action was unknown. To identify its target, two spontaneously resistant mutants isolated in 16x and 2x MIC of the complex had their whole genome sequenced. The variant caller breseq was employed to assess the Single Nucleotide Polymorphisms. The possible effects of the tris-(1,10-phenanthroline)iron(II) complex in the cell wall were assessed with scanning electron microscopy. The H37Rv strain was exposed to 1 and 2x the MIC of Isoniazid, Ethambutol, and the tris-(1,10-phenanthroline)iron(II) complex, non-treated bacteria was used as control. In the WGS data, 8 mutations were present exclusively in the mutants. Excluding those in highly polymorphic genes, most of them occurred in genes related to the cell wall or transmembrane proteins. A mutation identified in both mutants pointed to a possible effect of the complex in the cell wall remodeling process. From the SEM images, it was possible to observe that mycobacterial cells treated with the tris-(1,10-phenanthroline)iron(II) complex exhibited a flattened shape, corroborating with the findings from WGS data. In conclusion, the results point to the cell wall as the target for the tris-(1,10-phenanthroline)iron(II) complex, leading to impaired cell wall remodeling.

P403

Novel synergistic combinations of bedaquiline and antimicrobial peptides

Ye Xu[1], Winnie Akwani[1], Suzanne Hingley-Wilson[1], Johnjoe McFadden[1,2]

[1]Department of Microbial Sciences, University of Surrey, Guildford, United Kingdom. [2]Quantum biology doctoral training centre, University of Surrey, Guildford, United Kingdom

Abstract

Multi-drug resistant (MDR) TB remains a global health issue. This is despite novel drugs including bedaquiline (BDQ). BDQ does exhibit severe side-effects in 50% of patients. Lowering the dose whilst maintaining the bactericidal activity may result in few side effects. Antimicrobial peptides (AMPs) are natural products which display a wide-spectrum antimicrobial activity. Lynronne, the AMPs which were selected from the rumen microbiome, was found to bind to cellular membrane lipids and induce cell lysis in methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*.

In this study, a synergistic reduction of the minimum inhibitory concentration (MIC) of BDQ and LYN-1 and LYN-2 was noted in *mycobacterium tuberculosis*. Scanning electron microscopy (SEM) revealed variations in cellular structure in bacteria treated between single drugs and the combination. Membrane pores were found in mycobacterial cells treated with BDQ and LYN-1 which indicates the compromising cell membrane induced by the combination. While with BDQ and LYN-3, a polar aggregation of cellular contents was observed in mycobacterial cells. Results showed BDQ combined with AMPs have a synergistic inhibition and bactericidal effect on mycobacterial cells. The possible synergistic mechanism could be that the cellular membrane lysis induced by AMPs facilitates the uptake of BDQ into cells. However, varied cellular structures indicate the various synergistic mechanisms of BDQ with LYN-1 or LYN-3. This study identifies a promising novel synergistic drug combination to further improve the clinical practice of BDQ.

P404

Heavy metal bystander effect during mycobacterial infection

Claire Davison ORCID iD, Dany Beste ORCID iD, Melanie Bailey ORCID iD, Jordan Pascoe ORCID iD, Mónica Felipe-Sotelo ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Understanding the battle for metals is a key focus of host-pathogen research. Scarce metals are essential for intracellular pathogens to survive within their hosts however host cells use these metals as bacteriostatic/bactericidal weapons and therefore understanding metal homeostasis may lead to novel therapeutics. To achieve this goal sensitive methods to accurately detect and quantify metals are essential. Here we developed single particle - inductively coupled plasma - mass spectrometry (SP-ICP-MS) methods to analyse the intracellular concentration of zinc during a macrophage model of tuberculosis. Our results show a significant increase in zinc concentration in the mycobacteria infected macrophages but highlighted that there is significant heterogeneity in the concentrations between individual cells. This is significant as zinc availability impacts on the ability of Mycobacteria to withstand oxidative stresses and therefore may be a driving factor in the development of phenotypic heterogeneity and affect antibiotic susceptibility and survival. Using flow cytometry to measure the numbers of infected cells we also demonstrate an elemental bystander effect with uninfected cells also having elevated levels of zinc. Together these findings establish SP-ICP-MS as a powerful approach to study metal homeostasis during the interaction of Mycobacterium tuberculosis with its human host cell.

P405

MTS1338 in *Mycobacterium tuberculosis* promotes the detoxification of Reactive oxygen species under oxidative stress

Reena Nirban

Indian Institute of Technology, Delhi, India

Abstract

Various mechanisms present in *Mycobacterium tuberculosis* for its persistence under stress conditions in the hostile environment of macrophages. Small RNA-mediated regulation is one of the mechanisms. Small RNA (sRNA) plays a vital role in regulating bacterial survival under an altered environment by changing the gene expression primarily at post-transcriptional levels. Our research is focused on the regulatory role of oxidative stress-induced small RNA in *Mycobacterium tuberculosis*. We focused on MTS1338 (MTB00077), an already known sRNA present only in the pathogenic strain of mycobacterium, induced during infection. We observed that MTS1338 is induced under oxidative stress and detoxifies Reactive Oxygen Species (ROS) formed during the stress. Our main approach is to investigate the regulatory mechanism behind the induced expression of MTS1338 under oxidative stress. Although the ROS detoxification triggered by MTS1338 did not promote the cell growth rate, could maintain the sustainability of the cell's growth by adjusting and adapting to the stress, which perhaps leads to the dormancy of M. tuberculosis cells. Investigation into the mechanism of how MTS1338 detoxifies ROS revealed that increasing the intracellular abundance of MTS1338 increases Catalase peroxidase (KatG) and alkyl hydroperoxide reductase activity (AhpC). Ectopic expression of MTS1338 has also been found to enhance KatG and AhpC activity in M. tuberculosis cells. Thus, it is conceivable that these two enzymes attribute MTS1338-mediated ROS detoxification.

P406

Quantification of differentially culturable Mycobacterium tuberculosis cells in serial sputum samples to predict treatment failure or success in TB.

Jonathan Decker[1], Galina Mukamolova[1], Michael R Barer[1,2]

[1]University of Leicester, Leicester, United Kingdom. [2]University Hospitals of Leicester, Leicester, United Kingdom

Abstract

Background: Treatment failure in TB has been associated with the persister phenomenon. Differentially culturable (DC) M. tuberculosis (Mtb) bacilli grow in liquid medium supplemented with Mtb culture supernatant (CSNs) containing resuscitation promoting factors but do not form colonies when directly plated onto solid media. DC cells are tolerant to antimicrobials and cause disease relapse in the Cornell mouse model. We hypothesised that a high proportion of these DC bacilli detectable during TB treatment might predict treatment failure.

Methods: Snap-frozen sputum samples from 34 patients participating in TB treatment trials were kindly provided by the TB Alliance; all had drug-sensitive Mtb and 7/32 had unfavourable outcomes. Operator-blinded most probable number (MPN±CSN) and CFU counts were determined on decontaminated samples taken 0, 2, 4 and 8 weeks into treatment. DC counts were assessed as the log10 excess of MPN +CSN counts over CFUs.

Results: Of the 136 cultures successfully completed, 62 showed >1 log10 excess of DC bacilli. This proportion was greatest at (n=21/32) at baseline and declined as treatment progressed. DC excess was strongly associated with treatment failure at 4 and 8 weeks (p=0.0017 & 0.0179 respectively). DC values in excess of 1.55 log10 over CFU were associated with a 28-fold excess risk of treatment failure.

Conclusion: High levels of DC Mtb bacilli in sputum from patients on TB treatment associate with treatment failure in this small scale retrospective study. If validated, this approach could facilitate rapid assessment of new treatment regimens in clinical trials.

P407

Developing an enzymatic mycobacterial lysis reagent

Joseph Manion[1], Josh Quick[2], Patrick J Moynihan[2], Elisabeth Lowe[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Mycobacterial disease kills over a million people world-wide each year. A key part of mycobacterium resistance to antimicrobials and sanitation products is the 'mycomembrane' made up of three major components: peptidoglycan, arabinogalactan and mycolic acids. This impermeability due to the multi-layer membrane has caused issues in the extraction of high quality gDNA; often involving toxic chemical or mechanical methods that can aerosolise the pathogens.

We have developed a cocktail of novel enzymes which hydrolyse the arabinogalactan component of the mycobacterial cell wall. We show the cocktail can completely hydrolyse arabinogalactan to constituent monosaccharides. An enzymatic lysis step of 15-120 minutes prior to commercial genomic extraction kits results in yields higher than those achieved by bead-beating for both M. smegmatis and M. abscessus complex strains, and less fragmented DNA. Purified genomic DNA is suitable for downstream applications such as multiplex PCR or long-read sequencing.

Cheaper, more effective lysis technology for mycobacteria, which does not shear DNA to the same extent as bead-beating, will allow improved tracking and a greater insight into the spread and potential mutations that can affect the treatment course.

P408

Transition metal homeostasis is key to metabolism and drug tolerance of *Mycobacterium abscessus*

Yi Liu ORCID iD, Ivana Pennisi, Ashleigh Cheyne, Nadia Fernandes, Richard Williams, Rebekah Moore, Valeria Garbin, Mark Rehkämper, Gerald Larrouy-Maumus

Imperial College London, London, United Kingdom

Abstract

Antimicrobial resistance (AMR) is one of the major challenges that humans are facing this century. Understanding the mechanisms behind the rise of AMR is crucial to tackle this global threat. Among the triggers of phenotypic antimicrobial resistance, the contribution of transition metals has been understudied in *Mycobacterium abscessus* (Mabs), a fast-growing non-tuberculous mycobacterium known for its extreme AMR levels. Deeper understanding of the effects of transition metal ions will be beneficial for our knowledge in AMR and the discovery of potential therapeutic targets. Here, we investigated the impact of transition metal ions, nickel (Ni), cobalt and copper on the physiology and drug susceptibility of Mabs. The minimum inhibitory concentrations (MICs) of different ion-antibiotic combinations to Mabs were quantified, and surprisingly we found that Ni was able to reduce MIC to clarithromycin, a cornerstone antibiotic used for Mabs treatment, while increase MIC to other antibiotics such as amikacin. To determine the underlying mechanisms, we used a multi-pronged approach including RNA sequencing, bioenergetics and metabolomics. Upon Ni exposure, we observed that Mabs altered its transcriptome with upregulation of the transcriptional regulator WhiB7, leading to a remodelling of its central carbon metabolism via upregulation of the glyoxylate shunt. Meanwhile, we found that Ni treatment enhanced the uptake of clarithromycin in Mabs cells and caused a reduced MIC. Taken together, this study not only provides new insights into the tolerance mechanisms of Mabs against antibiotics and transition metals, but also suggests potential of Ni in adjuvating the efficacy of clarithromycin during Mabs infection treatment.

P409

The Phenotypic profiling of mycobacterial mutant libraries through chemical-genetic screening.

Rudi Sullivan ORCID iD[1], Hannah Doherty[1], Micheal Alao[1], Luke Kidger[1], Danesh Moradigaravand ORCID iD[2], Manuel Banzhaf ORCID iD[1], Patrick Moynihan ORCID iD[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

Abstract

The *Mycobacterium tuberculosis* complex is comprised of closely-related species of mycobacteria that are able to cause tuberculosis in both humans and animals. These bacteria are exhibiting an increased resistance to conventional antibiotics. Some mechanisms of drug resistance can involve the activation of new biochemical pathways and compensatory mutations in target genes. However, the genetic basis for these mechanisms remains uncertain as much of the genome has yet to be fully annotated. In addition, recent genomic profiling using CRISPRi has shown that a large number of genes of unknown function are potentially druggable. It is therefore clear that new methods for high-throughput phenotyping and functional assignment are required. Here we present the optimisation of a high-throughput chemical-genomic method for characterising mycobacterial gene-networks. By subjecting mycobacterial single gene deletion libraries to an array of chemical stresses, this method generates a genome-wide stress-response map to identify comprehensive fitness signatures for each mutant and stress tested. These phenotypic profiles allow for (i) the functional classification of orphan genes (ii) the identification of pathways and/or processes involved in antimicrobial resistance and (iii) the discovery of primary and secondary drug targets. Furthermore, by implementing techniques developed for genome-wide approaches and applying them to individual mutants we demonstrate the utility of our methods. We hope that this technology will help advance the mycobacterial field and provide an opportunity to identify new anti-microbial targets in mycobacteria.

P410

Determining the mode of action of vanoxerine and repurposing against Mycobacteria

Alexander Kingdon ORCID iD, Asti-Rochelle Meosa John ORCID iD, Gurdyal Besra ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Mycobacterium tuberculosis is the causative agent of tuberculosis and has led to 1.6 million deaths in 2021. Both a complex treatment regimen and the increasing issue of drug resistance, necessitates the need for novel drug development. Drug repurposing is one approach used to identify novel antimycobacterial compounds, and screening of the Prestwick Library was undertaken with this aim. Vanoxerine, a dopamine re-uptake inhibitor, was identified as a promising compound from this screen but had an unknown mechanism of action. A lack of spontaneous resistance mutants led to a wide range of approaches being undertaken to determine the mechanism. These included cell envelope lipid analysis, RNA-sequencing and phenotypic assays to study the membrane. Herein, we show vanoxerine dissipates the membrane electric potential leading to inhibition of Mycobacterial growth. Downstream effects included inhibition of cellular energetics, efflux, and membrane transport. Overall, this study has identified the mechanism of action of a novel antimycobacterial compound, allowing it to be used as a tool compound for membrane electric potential disruption. In addition, repurposing of vanoxerine or its analogues may allow a faster route to clinical use compared to traditional drug discovery.

P411

Assessing and optimising tuberculosis drugs for non-adherence to treatment: An interdisciplinary project using pharmacodynamic modelling and wet laboratory approaches

Roo Nicola Rose Cave[1], Madeleine G. Moule[2], Pia Abel-zur-Wiesch[3], Helen R. Stagg[4]

[1]Edinburgh, Edinburgh, United Kingdom. [2]The University of Edinburgh, Edinburgh, United Kingdom. [3]Norwegian Institute of Public Health, Oslo, Norway. [4]Usher Institute, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Mycobacterium tuberculosis is one of the leading causes of death from an infectious agent globally and causes tuberculosis (TB) disease. In 2020, around 10 million people developed TB with over 1.5 million deaths. The main transmission route of TB is through aerosolised droplets, leading to infection in the lungs. While 80% of TB cases are susceptible to antibiotics, treatment is a gruelling and complex for patients to follow causing social, financial and health impacts. The initial two-month phase comprises rifampicin, pyrazinamide, isoniazid, and ethambutol hydrochloride, followed by a four-month continuation phase of isoniazid, and rifampicin. A contributing factor to negative treatment outcome and greater TB recurrence is when an agreed plan of prescribed drugs is not followed and doses of drugs prescribed are missed (non-adherence). Current research into non-adherence groups patients by percentage of doses missed, but does not capture the dose taking patterns. Therefore, the impact of these patterns of dose-taking on patient outcomes is not known.

Digital adherence technologies allow granular dose-by-dose monitoring of non-adherence of patient adherence patterns. Harnessing this new data source we explore the implications of adherence patterns on rifampicin efficacy. In this study we combine pharmacodynamic mathematical models and laboratory experiments to show the impact of real-world adherence patterns on bacterial growth to expand our knowledge of regimen performance. This interdisciplinary approach to furthering our understanding of variable drug exposure on bacterial levels will allow us to identify patterns of adherence that are impacting patient bacterial load to inform future drug improvement and patient support.

P412

Multiform AMR in mycobacteria due to altered epitranscriptional modification of tRNAs

Andrea Majstorovic ORCID iD, Julien Vaubourgeix ORCID iD

Imperial College London, London, United Kingdom

Abstract

Antimicrobial resistance (AMR) is the ability of bacteria to avoid or delay being killed by an antibiotic. AMR can manifest in three ways: resistance, tolerance, or high persistence. Tolerance and persistence complicate treatment of many bacterial infections, including tuberculosis, by contributing to treatment length, treatment failure, disease recurrence, and the emergence of resistance. Therapeutically targeting tolerant and persistent cells could improve outcomes, but the molecular mechanisms underlying tolerance and persistence in mycobacteria are not well understood. To fill this gap, we developed a forward genetic method for the isolation of high survival (HS) mutants—which display tolerance and/or high persistence—based on their spatial separation from resisters upon exposure to a drug in vitro. Using this method in the model mycobacterial species *Mycobacterium smegmatis*, we isolated two HS mutants with mutations in tRNA (adenine-N(1)-)-methyltransferase, a gene that modifies tRNAs; both mutants display multiform AMR manifesting as tolerance to aminoglycosides and resistance to macrolides. Our work has linked these AMR phenotypes to upregulation of the transcriptional regulator WhiB7, and we are currently investigating the hypothesis that loss of tRNA methyltransferase activity activates WhiB7 through formation of a ribonucleic complex composed of a polynucleotide phosphorylase, a novel chaperone, and a Y RNA. Collectively, our work suggests that epitranscriptional tRNA modifications may mediate multiple forms of AMR in mycobacteria and that WhiB7 may serve as a point of convergence in the regulation of multiple mechanisms of survival in mycobacterial species.

P413

Determining the architecture of the Mycobacterium tuberculosis MCE4 cholesterol transporter complex

Joshua Ari Kaplan[1], Katherine Fenn[2], Ruth Rose ORCID iD[1], Isha Mohan[3], Maha Alharpi[1], Camila Aguilera Suarez[1], Miklos Kralik[1], Vidya Chandran Darbari ORCID iD[1]

[1]Queen Mary University of London, London, United Kingdom. [2]University of Leeds, Leeds, United Kingdom. [3]University of Leeds, London, United Kingdom

Abstract

Mycobacterium tuberculosis (Mtb) is one of few bacterial species known to completely degrade cholesterol, which it uses as a carbon source for metabolism and biosynthesis to cause and maintain disease. It induces infected macrophages to differentiate into foam cells and translocates from the phagosome into the resulting lipid bodies, where the pathogen has plentiful access to host-derived lipids. Movement of lipids across the diderm mycobacterial cell envelope requires transporters to bypass the hydrophilic periplasm. Proteins from the mammalian cell entry (MCE) 4 operon in Mtb are thought to form a membrane-bound ATP-binding cassette (ABC) -like complex that is involved in the import of cholesterol. Using AlphaFold2, we have modelled the MCE4A-F heterohexameric complex, providing insights into a potential MCE4 complex assembly driven by co-evolutionary data. Docking studies using this model suggest possible preference for cholesterol as a substrate versus typical phospholipids. To validate this model experimentally, we have recently cloned, expressed and purified a minimal core complex derived from the six proteins MCE4A-F, for structural studies. Low-resolution negative stain transmission electron microscopy (TEM) at 50000 times magnification shows promising images of particles resembling a ring of MCE proteins, with dimensions that correspond to the E. coli MlaD hexamer. The sample preparation has been optimised for CryoTEM and we are in the process of acquiring high-resolution CryoTEM data with the LonCEM Titan Krios microscope. Structural characterisation of the minimal core complex will reveal and confirm the order of assembly in the MCE4 transporter, and provide insights into the substrate binding pocket.

P414

Discovery and characterisation of exo-D-arabinofuranosidases that degrade mycobacterial arabinogalactan

Jennifer Ross[1], Omar Al-Jourani[1], Samuel Benedict[2], Patrick Moynihan[2], Elisabeth Lowe[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Complex polysaccharides from a range of sources can be broken down by multiple members of the human gut microbiota. We exploited the diversity of microbiota to find enzymes capable of degrading the elaborate cell walls of acid-fast organisms, such as Mycobacterium tuberculosis. Mycobacterial cell walls contain a complex glycan called arabinogalactan (AG), with no known enzymes known to cleave its D-arabinan component.

We have discovered members of a glycoside hydrolase family (GH172) from gut bacteria which have exo-D-arabinofuranosidase activity on AG. We have characterized three GH172 enzymes from the gut Bacteroidetes species Dysgonomonas gadei, and used a combination of X-ray crystallography and cryo-EM to gain insight into their structure. This reveals an unexpected diversity of oligomerization states within the family, with trimeric, hexameric and dodecameric structures being observed.

Additionally, we have shown enzymes from this family are also found in Nocardia brasiliensis, M. avium paratuberculosis, and parasitic bacteria, suggesting that the ability to degrade mycobacterial glycans plays an important role in the biology of diverse organisms.

P415

The role of the protein formerly known as pyruvate phosphate dikinase in the life and death of *Mycobacterium tuberculosis*

Jane Newcombe[1], Jordan Pascoe[1], Amit Singh[2], Amitava Sinha[2], Somenath Shee[2], Miraz Rahman[3], Mark Laws[3], Celia Goulding[4], Apoorva Bhatt[5], Albel Singh[5], Dany Beste[1]

[1]University of Surrey, Guildford, United Kingdom. [2]Indian Institute of Science, Bangaluru, India. [3]Kings College London, London, United Kingdom. [4]University of California Irvine, Irvine, USA. [5]University of Birmingham, Birmingham, United Kingdom

Abstract

The development of new tuberculosis treatments is a formidable challenge due to the high prevalence of antibiotic resistance strains and the remarkable antibiotic tolerance of the causative agent, *Mycobacterium tuberculosis*. Microbial metabolism has an active role in every stage of tuberculosis pathogenesis and therefore presents an attractive reservoir for therapeutic targets. However, many metabolic reactions are orphaned or their enzymes misannotated and this presents a bottleneck in studying suitable targets. We identified that Rv1127c, annotated as a pyruvate phosphate dikinase (PPDK) is essential for *M. tuberculosis* to survive in the presence of the physiological relevant carbon source cholesterol and intracellularly within human macrophages. Using medicinal chemistry, we designed and synthesised several lead compounds which target this protein and kill *M. tuberculosis* validating Rv1127c as a bone fide drug target. PPDK enzymes catalyse the inter-conversion of ATP, Pi and pyruvate with AMP, PPi and phosphoenolpyruvate. Whilst Rv1127c has the entire N-terminal PPDK domain that binds ATP it completely lacks the C-terminal PEP/pyruvate binding domain, which questions the annotated function of this enzyme. *M. tuberculosis* Rv1127c is in genomic proximity to essential enzymes and regulators of the methylcitrate cycle, which like Rv1127c are essential for cholesterol and propionate detoxification. Here, we show that Rv1127c functions as a novel phosphotransfer system which is important for redox homeostasis and control of metabolic flux through central carbon metabolism when growing on substrates which yield propionate and during intracellular growth. This work provides drugs and biological functions for Rv1127c and stimulates future research.

P416

Killing TB from the outside by glycan targeting

Collette Guy[1], Ruben Thomas[2], Matthew Gibson[2,3], Elizabeth Fullam[1]

[1]School of Life Sciences, University of Warwick, Coventry, United Kingdom. [2]Department of Chemistry, University of Warwick, Coventry, United Kingdom. [3]Warwick Medical School, University of Warwick, Coventry, United Kingdom

Abstract

Tuberculosis (TB) is a major global health threat and is the world's leading cause of death from a single infectious agent, with 1.6 million deaths in 2021. Recently there has been a rise in drug-resistant TB that cannot be successfully treated by any therapeutic regimens. Therefore, there is an urgent requirement for next generation antibiotic agents to treat TB with new modes of action. The causative agent of TB, *Mycobacterium tuberculosis* (*Mtb*), possesses a unique cell envelope consisting of a diverse range of complex carbohydrates and lipids, many of which have been shown to have fundamental roles in pathogenesis and virulence. As such, pathways involved in the biosynthesis of the mycobacterial cell wall are the target of multiple current anti-tubercular agents.

Here, we introduce a new concept for the development of anti-TB agents: direct targeting of the unique glycans located on the mycobacterial cell envelope. We designed and synthesised a panel of multimeric boron containing compounds which target glycans and found that these killed mycobacteria, including the *Mtb* pathogen. In contrast, these compounds were non-toxic to Gram-negative bacteria and mammalian cells. Shotgun proteomics, genomics, biophysical measurements and imaging were undertaken to elucidate the mode of action. Our results indicate that these compounds bind and recognise specific glycans within the mycobacterial cell envelope, opening up direct targeting of the *Mtb* cell surface glycans as a new avenue to explore in the on-going pursuit for new TB therapeutic agents.

P417

Metabolic incorporation of fluorinated trehalose probes for the detection and visualisation of *Mycobacterium tuberculosis*

Collette Guy[1], Jonathan Ramirez Carenas[2], James Gott[3], Chris Furze[1], Chris de Wolf[1], Jesus Angulo[4,5,2], Elizabeth Fullam[1]

[1]School of Life Sciences, University of Warwick, Coventry, United Kingdom. [2]Instituto de Investigaciones Químicas, Sevilla, Spain. [3]Warwick Manufacturing Group, University fo Warwick, Coventry, United Kingdom. [4]Instituto de Síntesis Química y Catálisis, Zaragoza, Spain. [5]Departamento de Química Orgánica, Universidad de Sevilla, Sevilla, Spain

Abstract

Sensitive, rapid and accurate diagnosis of Mycobacterium tuberculosis (*Mtb*) infection is a central challenge in controlling the global tuberculosis (TB) pandemic. The detection of mycobacteria is made difficult by the low sensitivity of current diagnostic tools, with over 3.6 million TB cases estimated to be missed each year. To overcome these limitations there is an urgent need for next-generation TB diagnostic technologies. Fluorinated probes have recently shown promise for use in diagnostic imaging in neurodegenerative diseases and cancer. Here we exploited the mycobacterial specific trehalose LpqY-SugABC transporter, which does not have a human homologue, to import fluorinated-trehalose analogues for the direct visualisation of *Mtb*.

A mixture of chemoenzymatic and synthetic methods were used to synthesise a panel of trehalose analogues, fluorinated at either the 2-/3-/4- or 6-position. Binding studies revealed that LpqY tolerates trehalose modified with fluorine at either the 2-, 3- or 6-position, but not the 4-position, and STD-NMR studies established the critical features of fluorotrehalose analogue recognition. Uptake studies found that *Mtb* can import 2-fluorotrehalose, 3-fluorotrehalose and 6-fluorotrehalose, but not 4-fluorotrehalose, indicating that recognition of the fluorinated trehalose analogues by LpqY influences import. Lipid analysis showed that following uptake the modified fluorinated sugars are incorporated into mycobacterial cell-surface glycolipids, allowing the use of fluorine imaging for pathogen specific detection by using Secondary Ion Mass Spectrometry (SIMs). These results suggest there is potential for fluorinated-trehalose analogues to be used in diagnostic imaging of *Mtb*.

P418

Amoxicillin/Relebactam combination supresses intracellular *Mycobacterium abscessus* persistence in lung alveolar macrophage-like cells *in vitro* in a morphotype dependant manner

Sean Kelly[1], Justyna Lopatecka[1], Sarah Bastyans[1], Simon Jackson[1], Mathew Upton[1], Jonathan Cox[2], Gyorgy Fejer[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]Aston University, Birmingham, United Kingdom

Abstract

Mycobacterium abscessus (*Mab*) is one of the most important pathogens in cystic fibrosis and replicates in lung alveolar macrophages (AMs). *Mab* has smooth (S) and rough (R) morphotypes which are associated with different pathogenesis. Recently the combination of Amoxicillin and Relebactam was found very effective against *Mab*. Nevertheless, intracellular *Mab* persistence may influence antibacterial therapy. AM specific studies would be important to study *Mab* infection and treatment, but the limited availability of AMs restricts such approaches. Previously we have established a novel, primary, continuous AM model (MPI cells). Here we used this system to study *Mab* infection and antibiotic sensitivity. MPI cells have been infected with *Mab* and intracellular bacterial loads were assessed at different time-points after infection. The amounts of released bacteria have been also measured in the cell free supernatants. The Mab infected macrophage cultures have also been treated with Amoxicillin/Relebactam and the extra and intracellular bacterial loads have been quantified from these cultures as well.

Mab persisted in MPI cells and the bacteria was predominantly intracellular at least for 6 days. The R *Mab* morphotype replicated more strongly than the S form of the bacteria.
Amoxicillin/Relebactam treatment reduced the intra and extracellular bacterial loads but intracellular bacteria still persisted at lower levels. Surprisingly, the antibiotic treatment was much more effective against the R than the S morphotype.

Our findings show MPI cells are valuable tools to study *Mab* infection and test potential drugs against the intracellularly persisting pathogen.

P419

Capture and isolation of mycobacteria from blood using bacteriophage-bound magnetic beads

Christopher Shield ORCID iD, Benjamin Swift ORCID iD, Alexandra Bartlett

Royal Veterinary College, London, United Kingdom

Abstract

Despite advancements in the mycobacterial diagnostic field, there are still unmet needs in the diagnostic armoury. True point-of-care detection remains elusive and current reference tests struggle to detect non-pulmonary tuberculosis. We aimed to develop and investigate the ability of bacteriophage bound to magnetic beads to capture, concentrate and lyse mycobacteria, to make an assay capable of detecting viable mycobacteria from blood samples.This Phagomagnetic separation (PhMS) method could capture and subsequently lyse *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium bovis* BCG cells from media and artificially inoculated blood samples with an average capture efficiency of 56.8 ± 7.12%. Incorporation of loop-mediated isothermal amplification (LAMP) created a sensitive (LOD ≤ 10 cell/ml) and specific (0/18 non-target species detected) diagnostic that could detect viable cells from blood samples within 6 hours. Johne's disease, bovine TB and human TB cases were correctly identified by testing clinical blood samples with PhMS-LAMP. This novel technology harnesses the power of bacteriophages to selectively bind, infect and lyse mycobacteria with the sensitivity and robustness of LAMP to bring mycobacterial diagnostics closer to a point-of-care, reducing costs, equipment and decreasing the time to detection. Detection of non-pulmonary tuberculosis is still a diagnostic niche yet to be met. Combining the ability to detect traditionally hard to diagnose patients and the move closer to the point-of-care means the PhMS method could lead to a better understanding and control of mycobacterial infection. No diagnostic is "one size fits all", but these advancements add an important piece to the diagnostic mosaic.

P420

A Point-of-Care Diagnostic for Tuberculosis

Suzan Alzeer, Les Baillie

Cardiff University, Cardiff, United Kingdom

Abstract

Tuberculosis (TB) is thought to infect one third of the world population. It is challenging to diagnose and treat and the emergence of antibiotic resistant variants of Mycobacterium tuberculosis (Mtb) has provided added pressure to ensure that the correct treatment is started as soon as possible. Current methods can take weeks to generate results or are to expensive for low resourced countries where the disease is endemic and any delay in appropriate treatment can negatively impact on patients' outcomes. The aim of this project is to advance the development of a portable, low cost, easy to operate, assay capable of identifying Mtb and of determining its antibiotic sensitivity in minutes to facilitate same day treatment.

Over the course of a number of years researchers in Cardiff have been developing a process in which focused microwaves can be used to release target specific DNA sequences from bacteria in clinical samples which are subsequently captured by magnetic beads. In this project we will use the BCG strain of M.bovis, a harmless close relative of M.tuberculosis which shares considerable genetic homology, to develop a system capable of detecting M.tuberculosis in real time. We will design and validate DNA probes with specificity for M. bovis and M.tuberculosis and will optimise their binding to magnetic beads so as to maximise the capture of target DNA liberated by the action of microwaves.

P421

Evaluation of riboflavin biosynthesis pathway as a new TB drug target

Enkai Jin, Dimitrios Evangelopoulos

Department of Microbial Diseases, UCL Eastman Dental Institute, University College London, London, United Kingdom

Abstract

New and more effective drugs are desperately needed to combat tuberculosis (TB) and inhibiting new targets that are different from those of current TB drugs is favoured to tackle drug resistance. The riboflavin biosynthesis pathway in *Mycobacterium tuberculosis* is of particular interest as the enzymes involved in the pathway are expected to be essential for survival and they are not present in humans. To investigate the potential of targeting the initial steps of the riboflavin biosynthesis pathway, we constructed two mutants lacking either the 3,4-dihydroxyl-2-butanone-4-phosphate synthase/GTP cyclohydrolase II (*ribA2*) or deaminase/reductase enzyme (*ribG*) in the model organism *Mycobacterium smegmatis* mc^2155. Δ*ribA2* and Δ*ribG* can only grow when complemented with external riboflavin, whereas they failed to multiply in riboflavin-deficient media. Riboflavin starvation experiments *in vitro* lead to killing with no appearance of suppressor mutants. We also developed a coupled assay to measure the enzymatic activity of RibA2 and RibG from *M. tuberculosis* that can be used to identify inhibitors in future studies. Our findings demonstrate the essentiality of *de novo* riboflavin biosynthesis in the survival of *M. smegmatis* and provide an approach to identify inhibitors targeting the riboflavin biosynthesis pathway of *M. tuberculosis*.

P422

Development of novel anti-tuberculosis inhibitors

Laura Boardman-Slack[1], Saki Raheem[2], Christopher Lawson[3], Mark Odell[1]

[1]University of Lincoln, Lincoln, United Kingdom. [2]University of Westminster, London, United Kingdom. [3]University of Strathclyde, Glasgow, United Kingdom

Abstract

Mycobacterium tuberculosis, one of the several aetiological agents of tuberculosis (TB) led in the number of deaths caused by a single infectious organism in 2019 with an estimated 10 million people contracting TB, 1.7 million of these cases ending in fatality. TB also has significant zoonotic potential and is highly prominent in both agricultural and wild animal systems. Current treatments are lengthy, and the burden of drug-resistant TB is extensive, new treatments are desperately needed.

Novel compounds CL-54-02 and CL-54-04 (based on a new lead chemical scaffold and previously shown to be non-toxic to mammalian cells) have been developed to target the metabolic enzyme isocitrate lyase 1 (ICL1), an enzyme shown to be crucial for the persistence of TB infection. The efficacy of these novel compounds was assessed through NMR and UV-spectroscopy assays. Cl-45-04 is the most potent with an IC50 in the nM range. Kinetic studies suggest this to be non-competitive/mixed inhibition. Initial inhibition of mycobacterial growth in complete medium has also been observed with CL-54-04 inhibiting with an MIC again in the nM.

Binding Affinity has been assessed through thermal Shift and Isothermal calorimetry with results confirming a binding relationship between the compounds and enzyme. Co-crystallisation studies were carried out to determine the nature of this binding relationship. Crystal growth was successful, and the three-dimensional structure solved. This revealed presence of our compound bound and ICL1s distinctive active site loop in the open conformation.

Session Topic: Teaching Symposium

P423

Art and Science Case Study: Increasing public engagement in a non-science space and the impact on the audience demographic with students as presenters.

Melissa Lacey ORCID iD[1], Kelly Capper-Parkin ORCID iD[1], Rachel Schwartz-Narbonne ORCID iD[1], Katherine Hargreaves ORCID iD[2], Catherine Higham[3], Catherine Duckett ORCID iD[1], Sarah Forbes ORCID iD[1], Katherine Rawlinson ORCID iD[1]

[1]Sheffield Hallam University, Sheffield, United Kingdom. [2]Sheffield Teaching Hospitals, Sheffield, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom

Abstract

Science capital, the science-related resources an individual has access to, are key to influencing participation and engagement.[1] Disparities in science capital exist across society, particularly in ethnic minority and socioeconomically disadvantaged communities, and are suggested to contribute towards explaining inequalities in science participation.[2]

Science public engagement events often report the same core demographics of visitors, despite innovations to attract traditionally hard to reach audiences.[3] Previously we have reported that community [4] and university-hosted [5] events increase knowledge and components of science capital amongst participants, however we are still failing to attract audiences representative of society.

This study assessed the impact of using a diverse group of scientists to attract an audience representative of local ethnic diversity and measures the impact of participation on science capital amongst different groups of visitors.

Data was collected via exit questionnaires from our integrated arts and science public engagement event in March 2022, hosted in collaboration with Museums Sheffield. Students representative of the wider society were recruited to participate in delivery of the event. From qualitative and quantitative data, statistical and/or thematic analysis was completed to determine the impact of the intervention on visitor demographics and science capital.

Attendance at this event was more representative of Sheffield's ethnic diversity than at previous events.[4,5] Increases in perceived knowledge and factors of science capital were observed. Utilising a diverse student body in public engagement events has benefits to both student participation and audience engagement.

1. https://doi.org/10.1007/s11422-015-9667-7
2. https://doi.org/10.1080/21548455.2017.1360531
3. https://doi.org/10.1177%2F1075547019832312
4. https://dx.doi.org/10.1099%2Facmi.0.000231
5. https://doi.org/10.1080/21548455.2021.1971319

P424

Student-generated board game to promote understanding of the antibiotic development pipeline

David Negus ORCID iD, Jody Winter

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Game-based learning can enhance student engagement with complex topics and foster creativity, communication and team working skills. Here, we present a game-based learning activity that we developed to support undergraduate Microbiology students in understanding the complexities and challenges of the antibiotic development pipeline.

Students work in groups, each researching a different phase of antibiotic development between preclinical testing and market. Students write possible positive and negative outcomes from their pipeline phase on post-it notes and assign each outcome a number 1-6. The groups then share their research findings and possible outcomes with the whole class and assemble the board game, using a provided template and their post-it notes.

To play the game, students are informed that each roll of a 6-sided die will cost them £100 million. Their total budget to develop their candidate drug to market is £2bn (20 die rolls). Students must navigate the phases of antibiotic development sequentially by rolling the die to achieve successful outcomes at each phase, without exceeding their budget.

We run this activity annually with undergraduate Microbiology students in their Professional Skills in Microbiology module, linked to their lab-based antibiotic discovery projects and study of antimicrobial resistance. We have also used the activity successfully with visiting international students from a range of disciplines (including pharmacy, nursing, environmental and public health) to promote antimicrobial stewardship. Participants reported that the activity is enjoyable and the key learning outcomes – that antibiotic development is time-consuming, expensive, and challenging, with numerous hurdles – are surprising and memorable.

P425

The Impact of using Virtual Reality Lessons to Teach Microbiology Online

Claire Donald ORCID iD[1], Nicola Veitch[1], Andrew Judge[1], Christopher Carman[1], Pamela Scott[1], Sonya Taylor[1], Leah Marks[1], Avril Edmond[2], Imants Latkovskis[1], Nathan Kirkwood[3], Neil McDonnell[1], Fiona Macpherson[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Dizzyfish, Glasgow, United Kingdom. [3]Sublime, Glasgow, United Kingdom

Abstract

Virtual Reality (VR) is gaining increasing interest as an exciting technology for integration into Higher Education. The University of Glasgow has been working with immersive technology company, Edify, to embed bespoke VR applications within various courses. This collaboration was fortuitous during the COVID-19 pandemic as it provided teachers with a training platform from which to remotely lead classes within realistic virtual environments.

We developed the Disease Diagnostic VR app which was implemented within a Junior Honours life sciences course. To accommodate the pandemic restrictions, the course pivoted online and included an interactive virtual learning environment comprised of videos, quizzes and interactive animations. The VR experience was delivered by-proxy on the final day to further support consolidation of the intended learning outcomes.

To investigate if the integration of VR technology influenced the student learning experience, we compared the inclusion of VR by-proxy (VR-test group) to less immersive teaching material (control group). Our findings indicate that although there is no observable difference in learning outcomes between the 2 groups, students who received the VR rated their experience more favourably than the control group. In addition, student confidence in practical skill elements was greater in the test group than in the control.

While studies into the effectiveness of VR for teaching have expanded recently, this area of research is still in its infancy. Therefore, examining the efficiency of VR within Higher Education is an important topic that will not only inform future practice but further the frontiers of an evolving field of research.

P426

Engaging trainee pharmacists as science communicators through the Antibiotics Guardian schools' ambassadors

Jordan Charlesworth[1], Tanya Miah[1], Tracey Thornley[2], Kirti Solanki[3], Richard Dunne[3], Adeola Henry Ayeni[4], Ellie Gilham[1], Diane Ashiru-Oredope[5,2]

[1]UK Health Security Agency, London, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom. [3]Boots UK, London, United Kingdom. [4]Teesside University, Middlesbrough, United Kingdom. [5]UK Health Seccurity Agency, London, United Kingdom

Abstract

Background

A multisectoral (one health) approach may be needed to curb the emergence of new strains of resistant bacteria and create awareness about the misuse and overuse of antimicrobials, which is one of the driving forces of increasing AMR. One of the approaches used to reduce AMR and preserve the effectiveness of antibiotics is the Antibiotic Guardian campaign.

Method

A UK wide survey was developed and shared among trainee pharmacists who are serving as Antibiotic Guardian schools ambassadors during 2022/23. The survey aimed to assess their knowledge of and to evaluate their confidence in disseminating information regarding AMR and antibiotic usage.

Results

One hundred and thirty-four trainee pharmacists registered to participate in the programme. Most participants planned to deliver sessions in one school (64.2% (86/134)), with 21.3% (26/122) and 18.0% (22/122) planning to approach primary and secondary schools respectively; 59.8% (73/122) participants were undecided on the type of schools they planned to approach. Most participants decided to engage with small groups (86.4% (108/125)) whereas only 2.4% (3/125) decided to engage students individually. Furthermore, 62.4% (78/125) of participants intended to teach in person. Less than half of the trainee pharmacists were confident speaking to colleagues, the public and young people (46.7% (56/120), 47.9% (57/119) and 41.5% (46/118) respectively) about antibiotic use and AMR.

Conclusion

Trainee pharmacists are interested in engaging in scientific communication with young people. However, the confidence to deliver the sessions was relatively low. Post participation feedback will be used to assess whether their confidence increased following the programme.

P427

Peer group interactions within a blended learning space, how students use social media to support their learning and student experience.

Mel Lacey ORCID iD

Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Covid-19 has moved us to a new way of working with delivery and assessments blended between in person and online. Students bridge physical and digital spaces using social media, with these groups predominantly being closed groups that academics cannot access. Absence from these spaces, and the subsequent inability to help direct students learning, has resulted in misconceptions and responses being propagated within peer groups. The use of the blended digital learning space for incoming and current students is a grey area for many with academics forced to make assumptions on how the students are interacting.

Within this study a mix methods approach has been employed whereby students across the Department of Biosciences and Chemistry were questioned on their use of social networks regarding assessment and personal learning. Preliminary finds show that students are seen to establish a variety of personal learning networks depending on need. Large groups form early during a course around finding friends but then dissipate and are replaced with short lived groups linked to assessment tasks or modules. These short-lived groupings are seen to be replicated those we observed in the physical environment as students seek out those with similar levels of attainment or background to for support. Students highlighted that time taken to allow group formation to occur would be a clear benefit. Social networks are being used during on-line assessments and ideas around assessment design are considered. The long-term impacts from this study will be changes to interventions and working practices.

P428

Problem Based Learning in undergraduate immunology teaching

Bridget Kelly ORCID iD

Dundalk Institute of Technology, Dundalk, Ireland

Abstract

Problem based learning (PBL) is a pedagogical approach that has been widely used in many diverse fields to engage students in their learning. In PBL, students solve problems via peer learning in a group situation through real life situations. Immunology, taught at Dundalk Institute of Technology as part of third year science courses, is found to be a challenging subject for students. Initially, PBL was introduced as a pilot for one particular topic in immunology that students were struggling with in order to improve student learning and engagement. Following the success of this pilot, PBL has been adopted as part of the teaching strategy for the past four academic years and accounts for 20% of the module marks. There are now two separate tasks to complete per group: one where student groups research and prepare presentations based on the same topic for all groups; and the other where each student group produce creative learning resources based on a different topic randomly assigned. Each year, students were asked to complete a short online survey consisting of a mixture of 10-12 closed and open ended questions, to gather feedback and get some suggestions for future iterations of this PBL section in immunology. In general, responses to the survey were mainly positive, students could see the benefits of peer learning in PBL and felt they gained worthwhile experience of working collaboratively on a task. Challenges encountered related to general team work and personal time management issues.

P429

Building verbal participation confidence and student community with an in-person, blended teaching approach.

Victoria Easton ORCID iD

University of Leeds, Leeds, United Kingdom

Abstract

There is some controversy within pedagogic literature regarding online learning during the SARS-CoV-2 pandemic. Some publications show that online learning resulted in lower attainment of both course work and exams, with links drawn to a lack of connection and community to both their peers and lecturers as a contributing factor. However, there have been some positive impacts to digital learning, with students reporting that they feel more independent and in control of their learning; and lecturers feeling they that they learnt new pedagogic techniques and have become more innovative. By taking digital teaching developments, can we build a better in-person teaching environment?

Colleagues in the University of Leeds Language Centre found that often a lack of verbal participation is due to a feeling of fear and embarrassment rather than a language issue. Inspired by this, I looked at how an in-person, blended teaching approach can be used to develop a positive learning environment. Specifically, if a mix of digital and verbal response gathering methods can encourage verbal communication confidence and build a sense of cohort community.

Changes to content delivery were implemented in a range of classes from 1st year microbiology to MSc level immunology, and in a range of taught formats from lectures to tutorials. While this is still ongoing, students' impressions are positive with between 85% - 95% of students agreeing or strongly agreeing with the statement that the blended approach to teaching made them feel more confident verbally participating.

P430

Escape Lab: Bringing the investigative approach of antiviral drug discovery to the public in an escape room activity

Maitreyi Shivkumar ORCID iD

Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom

Abstract

The COVID-19 pandemic has shone a light on virology and highlighted the need for public engagement to convey both results and processes behind the research. We designed a 20-minute escape room (Escape Lab) to ask 'How can antiviral drugs be identified and developed?'

Escape Lab is aimed for children (secondary school-aged and above) and adults, with no expectation of prior scientific expertise. The goal is to find the structure of a potent antiviral compound that a scientist in the lab has discovered. The activities mimicked some of the key stages of our drug-discovery research: (1) screening compounds for antiviral activity; (2) checking cytotoxicity; (3) chemically modifying hit compounds to improve potency. Teams interpreted protocols and results from the scientist's lab notebook and undertook 'experiments' to generate clues for each stage. The team 'escaped' the lab when they built a molecular model of the antiviral compound. Successful teams were given certificates and finish times recorded on a leaderboard to generate a sense of competition. Participants could also talk with current researchers to relate the activities to real data generated in our lab.

Escape Lab was run at two events (British Science Festival, Leicester and Leicester Business Festival) in 2022. We had overwhelmingly positive feedback (95% 5-stars) and ~50% of participants seeking to learn more about our research.

Overall, this offers a blueprint for a fun and engaging way to bring research to the public, with the flexibility to update the scenario to incorporate novel research and target different age groups.

P431

Student Perspectives on the Co-creation of Virtual Reality Simulations for Teaching and Learning in the Molecular Sciences

F. Jerry Reen (on behalf of ELEVATE UCC)

University College Cork, Cork, Ireland

Abstract

Virtual pedagogical immersion describes an approach whereby students actively engage with elements of course curriculum through bespoke 3D environmental representations. Being both experiential and constructivist, this innovative approach to teaching molecular biology can open new opportunities for students to engage with key structures and processes that are difficult to comprehend via traditional 2D representation and narrative description. However, in evaluating the potential impact of such virtual simulations it is important to establish how current students, as supposed “digital natives”, may engage with same. The question arises whether a student may benefit from a virtual approach simply because they are more adept today at accessing information through such platforms? In this scenario, increased engagement may derive from the virtual pedagogical immersion conforming to more common experiences/modalities of knowledge interaction for students, outside of educational settings (e.g. online social networks, gaming, Google earth/street view, virtual tours etc.). This is distinct from a student deriving the benefits of novel conceptual insights and deeper learning though new vistas of understanding/modes of engagement exclusively provided by a virtual pedagogical immersion approach. To address these and other questions, a student partnered approach was undertaken to evaluate how virtual simulations can be designed and implemented in the teaching of molecular and cellular biology. A mixed methods approach was pursued, involving surveys, assessments, and online simulation data capture. Students expressed a consensus view towards the application of this innovative teaching modality and appear well equipped to adopt well to this new teaching and learning approach.

P432

Endemicity of B.Sc. and M.Sc. Microbiology Degrees?!

Adenike Ogunshe ORCID iD

Pegasus-Zion Community & Environmental Health, Nigeria, Ijebu Ode, Nigeria

Abstract

Background: According to National Universities Commission (NUC), B.Sc. Microbiology Degree Programme was designed with main objectives of broadly educating students for positions in industry, health sectors, research institutes, and to prepare them for graduate and professional studies in applied areas of Microbiology. Philosophy of postgraduate Microbiology programme is reportedly anchored on unbiased and systematic observations, accurate documentation and interpretation of facts and phenomena, with view of generating body of knowledge.

Methods: Holistic review of B.Sc. and M.Sc. degrees programmes (2007), with respect to their aims and philosophy was undertaken.

Results & Conclusion: Endemicity concerning Microbiology degrees, students, and graduates include, NUC curriculum and Benchmark Minimum Academic Standards (BMAS) for undergraduate programmes in Nigerian universities; legislative discrimination against Microbiology graduates to practise in medical laboratories, lack of National Academy of Microbiology or professional recognitions, non-functional professional societies, minimal funding; teaching and learning deficiencies. More females enroll for Microbiology, mostly to avoid compulsory or required mathematical, physical, statistical (>16.0%), zoological (>13.0%), and chemical (>13.0%) courses, while only 40.0-50.0% are Microbiology courses, taken for graduation requirement. Failure of certain non-Microbiology courses significantly affect students' grading, and class of degree at graduation, in some cases, leading to loss of honours or withdrawal from universities. Lack of regular courses' and Academic staff performances' evaluations by students, External Examiner System, periodic visitations by relevant professional bodies, and Microbiology-related entrepreneurial skills/studies, employability clauses endemically affect B.Sc. degree. Restricted areas of specialisation, lopsided classifications of core and elective Microbiology courses, lack of sophisticated equipment affect M.Sc. Microbiology degree.

P433

Pedagogy Collection: An Innovative Teaching and Outreach Toolkit for Microbiology Educators

Georgios Efthimiou

Hull York Medical School, HULL, United Kingdom

Abstract

Innovative teaching is now considered a crucial factor when designing and delivering curricula of high quality and includes activities such as games, teamwork workshops and problem-solving sessions. In addition, higher education institutions are now expected to build strong bonds with the society via various outreach activities with schools or other community groups. In order to help modern microbiology educators stand up to these challenges, *Access Microbiology* will launch a Pedagogy Collection acting as an innovate teaching and outreach tookit, which will be a handy database of all pedagogy papers published in *Access Microbiology* since 2019. This collection aims to help microbiology educators to improve their teaching by introducing new innovative teaching and outreach ideas, often generated by members and friends of our Society, to their curricula. It can also act as a useful paper repository for such ideas, which will be easy-to-find and used by colleagues in the UK and abroad. This could also stimulate more pedagogical submissions to *Access Microbiology*, helping us to grow our collection. Finally, there is the possibility that the Pedagogy Collection may in the future go under the National Teaching Repository. The National Teaching Repository was recently developed by AdvanceHE and is an open access, online, searchable database for tried and tested strategies and ideas.

Session Topic: Microbial physiology, metabolism and molecular biology forum

P434

Analysis of virulence genes of Staphylococcus aureus, Streptococcus, E. coli isolated from bovine subclinical mastitis

SUDEEP SOLANKI ORCID iD, Durga Devi

RAJUVAS, Bikaner, India

Abstract

Background: The major mastitis-producing organisms are Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, and Escherichia coli. Molecular characterization of the major bacterial pathogens for some genes responsible for their virulence should be considered the reduction of risk factors responsible for the presence and spread of contagious pathogens through milk.

Methods: Out of 200 milk samples, 74 milk samples that were found positive for SCM were cultured for primary isolation of predominant bacterial pathogens. Out of these 74 positive samples, total of 97 isolates were recovered from these milk samples either as a single or mixed infection. To genotypically characterize S. aureus isolates, genes encoding virulence determinants (spa-IgG-binding and Coa), Staphylococcal enterotoxins (bac and bca), and E. coli Shiga toxins (stx1 and stx2) were investigated.

Results: The etiological prevalence of SCM caused by different bacteria was, S. aureus, (27 %), Streptococcus spp. (15%), and E. coli (6.5%) respectively either as single and or as mixed infections. All S. aureus isolates were tested by PCR for the presence of the spa gene and coa gene results revealed that 40 isolates (74.0 %) carried both spa (IgG-binding) and coa gene. 11 isolates of S. agalactiae (36.6 %) carried the bca gene. The bca gene codes for Alpha-C protein, a surface protein that helps the bacteria to enter the host cells. E. coli isolates were also screened for the presence of virulence gene stx1and stx2 gene. Out of 13 isolates tested, 6 isolates harboured both stx1 and stx2 genes.

P435

Mycoflora and Aflatoxins associated with stale retail Pepper consumed in Ogun State, Nigeria

Eniola Oni, Adebukunola Omemu

Federal University of Agriculture, Abeokuta, Nigeria

Abstract

Fungi constitute a major problem in the production, storage and processing of agricultural products, recent concern about the consumption of stale retail pepper in Nigeria necessitated the need to determine the fungal population and also to quantify Aflatoxin produced by the fungi. Fungi species were isolated from 20 pepper samples (Capsicum annum) bought from different markets in Abeokuta, Ogun State using standard microbiological procedures. High Performance Liquid Chromatography (HPLC) was used to quantify the aflatoxin present. Isolated fungi were Aspergillus flavus (55%), Mucor sp (10%), Sacharomyces cerevisiae (20%), Aspergillus fumigatus, Rhizopus sp and Penicillium sp (5%) respectively. Results shows that fourteen of the pepper samples had no detectable aflatoxin and the aflatoxin content in the remaining six samples was 23ng/kg, 18 ng/kg, 15 ng/kg, 9 ng/kg, 6 ng/kg and 2 ng/kg,. five of these samples had aflatoxin content above the European Union maximum tolerance level of 5ng/kg. The presence of toxin producing Aspergillus flavus capable of causing food poisoning raises concern over public health risks that may be associated with the consumption of stale pepper.

P436

Predacious Properties of Myxobacteria Against Yeasts

Michael McCarthy, Benita Arakal, Paul Livingstone

School of Sport & Health Science, Cardiff Metropolitan University, Cardiff, United Kingdom

Abstract

Myxobacteria are unusual prokaryotic organisms that demonstrate an interesting co-operative behaviour, working together to devour bacteria of other species to absorb their biomass to use as energy source. However, the predatory properties of myxobacteria have not been extensively researched against fungi or more specifically yeasts. Yeasts cause a variety of infections in humans most often isolated from recurrent, difficult-to-treat infections owing to their antimicrobial resistance. This study used predation assays to test the effectiveness of 39 myxobacteria isolated from soil against 15 different yeast species, with zones of predation measured at four and seven days after incubation.

The results of this study indicate that myxobacteria have the capability to effectively prey upon yeast species. The dendrogram of Hierarchical clustering illustrated that most myxobacteria performed adequately as moderate predators while some were inefficacious. However, there were some myxobacteria that were particularly good predators that performed well against many of the tested yeasts. RDP092/CA was the best of all Myxobacteria which had the highest predatory activity against all yeast species. This study enabled us to decipher the predatory role of myxobacteria against yeasts, killing the much hardier fungal cells. Further studies to investigate the potential of myxobacteria as biological antifungal agents in yeast related infections are warranted.

P437

Exploiting the modularity of bacterial two-component systems to create synthetic biosensors for drug screening targets

Rahmi Yusuf ORCID iD[1], Roger Draheim[2]

[1]De Montfort University, Leicester, United Kingdom. [2]University of Portsmouth, Portsmouth, United Kingdom

Abstract

The continuous emergence of antibiotic resistant bacteria has been a major issue facing health care systems worldwide. Various strategies in tackling this problem have been put forward, and here we purpose a novel antibacterial target called the Two-Component System (TCS), which consists of a histidine kinase (HK) and a response regulator (RR). Thought to be the progenitor of more complex signalling systems, the TCS is ubiquitous in bacteria but rarely found in plants and mammals, therefore making them an ideal drug target.

Considering the well-conserved nature of TCSs, we attempt to exploit the possible modularity of its sensory domain by fusing the virulence-involved sensor of the S. enterica ser. Typhimurium HK, PhoQ, with the canonical HK, EnvZ of E. coli, which pairs with the RR, OmpR. Previous studies have alluded to the importance of the transmembrane domains during HK signal transfer; therefore, the linker domain was chosen as the fusion point between the two HKs. Immunoblotting was conducted to monitor the expression of PhoQEnvZ, while Fluorescence Spectrophotometry was done to detect signal output generated by OmpR. Aromatic tuning was conducted to restore the magnesium-sensing function of PhoQ, which resulted in a dose-responsive baseline signal output with a dynamic range of approximately two-fold.

Having successfully tested the modularity of our model EnvZ/OmpR with the PhoQ fusion, we have been working on creating other chimeras using the same template. The collection of chimeras will then be used as targets in a high-throughput screening platform to identify potential new antibiotics.

P439

Transcriptional profiling of THP1 cell line stably expressing Esx-4 secretory-pair of *Mycobacterium tuberculosis*

Muhammad Ahsan Naeem ORCID iD[1], Prof. Gang Cao[2], Waqas Ahmad[3], Qaiser Akram[4]

[1]University of Veterinary and Animal Sciences (Section of Pharmacology- Narowal Campus), Lahore, Pakistan. [2]State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, wuhan, China. [3]University of Veterinary and Animal Sciences (Section of Epidemiology- Narowal Campus), Lahore, Pakistan. [4]University of Veterinary and Animal Sciences (Section of Microbiology- Narowal Campus), Lahore, Pakistan

Abstract

The Esx-4 cluster is the most primitive one in the Esx secretory system of Mycobacteria. Its secretory-pair (Rv3444c/Rv3445c) is functionally-unknown, so their regulatory role was explored by stably expressing them in the host cells to get the up- or down-regulated host-responsive proteins by applying the RNA sequencing technique. This work provided transcriptional profiling data of the host cells in response to the Esx-4 secretory pair. Lentiviruses of both genes were harvested on HEK293T cells and were used for human macrophage (THP1) stable cell line generation keeping GFP expression in THP1 as a control. RT-qPCR validated the stable expression of secretory-pair in the THP1 cells. RNA-sequencing was performed and the data analysis on R package provided differentially expressed genes (DEGs), KEGG pathway analysis, and highly up-and down-regulated genes. PANTHER protein classification database exposed differentially expressed genes, KEGG pathways, and the host's up-and-down-regulated genes. Overall, 244 genes were up-regulated and 54 were down-regulated after RNA-seq analysis in Esx-4 secretory-pair stably expressing THP1-cells. The data of the same cell line presented host nucleic acid binding proteins (SRSF12, CPSF3, MCM9, CLUH, and H2B) as the highly up-regulated and (ATXN2 and ZNF3) as the highly down-regulated genes. Pathway enrichment analysis classified the whole RNA-seq data of Esx-4 secretory-pair stably expressing THP1 cell line into 19 pathways, of which CXCL2, IL-1β, and CXCL3 were significantly down-regulated in the immune-related pathway. In short, the Esx-4 secretory-pair induces high up-and down-regulation of nucleic acid binding protein in the host cell and down-regulation of the host immune pathway.

P440

The biofilm matrix of *Pseudomonas aeruginosa*

Rahan Nazeer, Martin Welch

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa (PA) is a WHO “critical priority pathogen”. PA is the ultimate opportunist and exhibits a worrying level of multi-drug resistance. The organism is also exceptionally versatile, and readily “rewires” its basic metabolism allowing it to adapt to challenging environments, such as those in the human airways. Not only does PA adapt to its environment; it also adapts the environment to it, using an arsenal of secreted factors to break down organic matter, communicate with other cells, sequester scarce nutrients and kill hostile interlopers.

Perhaps the most basic way in which PA modifies its immediate environment is through close colonisation, mediated by the formation of biofilms. This entails the secretion of an array of biomolecules which form an ordered, protective compartment. This shields the encapsulated cells from immunological factors and antibiotics, making biofilm formation an integral component of PA’s colonisation strategy.

The extracellular matrix which characterises the biofilm is a complex network of proteins, secondary metabolites, nucleic acids and other exopolymers. Using a selection of defined deletions in key biofilm-associated genes, we have been interrogating the matrix structure and proteome, allowing us to understanding better which components interact and how the matrix might function as a discrete extracellular “compartment”. In particular, we are using quantitative proteomics to understand how the "matrixome" differs in composition in the presence and absence of key linchpin protein(s). Moreover, we are using a novel fluorescent dye to monitor how the dynamics of the biofilm structure change over time.

P441

Mechanism of ammonium transport is critical in yeast filamentation

Gordon Williamson[1], Ana Sofia Brito[2], Adriana Bizior[3], Gaëtan Dias Mirandela[4], Thomas Harris[3], Paul A. Hoskisson[3], Ulrich Zachariae[5], Anna Maria Marini[2], Mélanie Boeckstaens[2], Arnaud Javelle[3]

[1]UEA, Norwich, United Kingdom. [2]ULB, Brussels, Belgium. [3]University of Strathclyde, Glasgow, United Kingdom. [4]ULB, Brussels, United Kingdom. [5]Dundee University, Dundee, United Kingdom

Abstract

Nitrogen is often a limiting growth factor for microorganisms, thus growing in nitrogen limiting conditions is a challenge. To scavenge for nitrogen fungi will begin to grow hyphae or psuedohyphae which are also part of the induction of pathogenicity. The signal for this change in growth has been observed to come from the Mep2 ammonium transport membrane protein, a member of the Ammonium transport proteins (Amt), Methylamine permeases (Mep) and Rhesus proteins (Rh) superfamily found in all kingdoms of life. The overall structure of these proteins is highly conserved including specific amino acids lining their hydrophobic pore. Using in vivo yeast complementation, in vitro electrophysiology and in silico molecular dynamics simulations it has been found that the twin his motif, found in the centre of the pore, plays an important role in the mechanism and selectivity of Mep2. Mutations to the twin his motif switches Mep2 from a highly selective ammonium transporter to an unselective ion channel. This change has also been shown to abolish the induction of filamentous growth. This leads to the conclusion filamentous growth is not triggered by a signal cascade following ammonium translocation but rather by the specific mechanism of transport.

P442

The involvement of CiaR and the CiaR-regulated serine protease HtrA in thermal adaptation of *Streptococcus pneumoniae*

Ozcan Gazioglu ORCID iD, Hasan Yesilkaya, Peter W. Andrew

University of Leicester, Leicester, United Kingdom

Abstract

The in vivo temperature can vary according to the host tissue and during infection. Streptococcus pneumoniae has evolved mechanisms to survive these temperature differences, but neither the consequences of different temperature on pneumococcal phenotype nor the genetic basis of thermal adaptation are known in detail. Previously, we found that CiaR, which is a part of CiaRH TCS, and 17 genes known to be controlled by CiaRH, were identified to be differentially expressed with temperature. One of the CiaRH regulated genes shown to be differentially regulated by temperature is the high temperature requirement protein (HtrA). In this study, we hypothesised that CiaRH system plays an important role in pneumococcal thermal adaptation through its control over *htrA*. This hypothesis was evaluated by testing strains either mutated or overexpressing *ciaR* and/or *htrA* in *in vitro* and *in vivo* assays. In the absence of *ciaR*, the growth, haemolysis, amount of capsule, and biofilm formation were diminished at 40°C only, while the cell size and virulence was affected at both 34°C and 40°C. The overexpression of htrA in the Δ*ciaR* background reconstituted the growth at all temperatures, and the haemolytic activity, biofilm formation, and virulence of Δ*ciaR* partially at 40°C. Also, the overexpression of *htrA* in the wild type promoted pneumococcal virulence at 40°C, while the increase of capsule was observed at 34°C, suggesting that the role of *htrA* changes at different temperatures. Our data suggest that CiaR and HtrA play an important role in pneumococcal thermal adaptation.

P443

Evaluation of the Codon usage, rare codons and molecular phylogeny of Betelactamase gene as a virulence factor of Acinetobacter baumannii

Farzin Minafard[1,2], Forough Samanifar[1], Nima Bahador[1], Abdolrasool Malekpour[2], Mojtaba Mortazavi[3], Saeid Gholamzadeh[2]

[1]Islamic Azad University, Shiraz Branch, Shiraz, Iran, Islamic Republic of. [2]Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran, Islamic Republic of. [3]Department of Biotechnology, Institute of Science and High Technology and Environmental Science, Graduate University of Advanced Technology, Kerman, Iran, Islamic Republic of

Abstract

Background and Objective: Acinetobacter is one of the most important opportunistic nosocomial pathogens. The aim of this study was to investigate the codon preference, rare codons, and molecular phylogeny of the beta-lactamase gene in as a causative agent of nosocomial infection.

Materials and Methods: In a field study, after isolating Acinetobacter baumannii and evaluating the presence of the beta-lactamase gene, the characteristics of rare, preferential codons, total purines and pyrimidines were calculated, and drawing its graphic diagram by Swiss-Model and Molecular Dynamics Simulation method were performed.

Results: In this study, out of 100 samples taken, 27 samples were positive for Acinetobacter baumannii culture. In these positive samples, 11 cases were positive for PCR in terms of the gene encoding beta-lactamase. The gene is about 1,044 nucleotides long and encodes 387 amino acids. Frequency of adenine nucleotides 26.92%, cytosine 22.22%, thymine 26.44% and guanine 24.43%, percentage of guanine nucleotides with cytosine (46.65%), guanine with adenine (34.51%), guanine with thymine (50.86%), adenine with thymine (53.35%), adenine with cytosine (49.14%) and cytosine with thymine (48.66%) were identified. The abundance of amino acids was calculated and the amino acids alanine (10/10%) and arginine (6.60%) had the highest frequencies in this enzyme.

Conclusion: The results of the present study indicated genetic diversity and codon differences in the structure of beta-lactamase isolated from Acinetobacter baumanniiconsidering the specific frequency of codons used in the structure of the beta-lactamase gene, its results can be used in the design of new drugs related to Acinetobacter resistant to antibiotic treatments.

P444

Characterising putative effectors associated with the *Campylobacter jejuni* Type Six Secretion system

Zahra Omole[1], Janie Liaw[1], Geunhye Hong[1], Cadi Davies[1], Abdi Elmi[1], Dong Xia[2], Nicolae Corcionivoshi[3], Brendan Wren[1], Nick Dorrell[1], Abderrahman Hachani[4], Ozan Gundogdu[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]The Royal Veterinary College, London, United Kingdom. [3]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [4]University of Melbourne, Melbourne, Australia

Abstract

Background: Bioinformatic analysis revealed 19.5% of *C. jejuni* genomes, particularly phylogenetic groups associated with human infection and chicken isolates, harbour Type Six Secretion system (T6SS) operons. Despite this prevalence and previous investigations, no *C. jejuni* T6SS effectors have been formally identified. Our recent bioinformatic analysis, identified *Cj0980* (Tox-REase-7 domain containing protein) and *Cj1003* (metalloprotease) as putative *C. jejuni* T6SS effectors which we aim to characterise.

Methods: Two *Cj0980* and *Cj1003* mutant strains were generated in the T6SS-positive *C. jejuni* strain 488. Co-culture experiments were conducted using T6SS-positive and -negative strains to examine bacterial interactions and possible anti-bacterial properties. Additionally, human intestinal epithelial cells (IECs), T84 and Caco-2, were infected with 488 wild-type and mutant strains to observe influence on host-cell infection.

Results: During co-culture, the *Cj0980* mutant strain no longer displayed a selective advantage against prey T6SS-negative strains. Infection of IEC lines with *Cj0980* mutant resulted in reduced interaction and invasion with respect to 488 wild-type strain, conversely intracellular survival and invasive properties were improved during *Cj1003* mutant infection. Both effector mutants elicited weaker induction of the chemokine IL-8 after infection; a phenotype which has been observed in *C. jejuni* strains unable to adhere and invade IECs.

Conclusion: Mutation of *Cj0980* abolished co-culture competitive advantages observed with 488 wild-type and *Cj1003* mutant strains suggesting a role for *Cj0980* in intrastrain antagonism. *Cj1003* mutant strain assays identified interesting phenotypes proposing a function in IEC infection. These preliminary findings warrant further investigation to help fully elucidate the function(s) of these putative effectors.

P445

Vibration hinders surface attachment of *E. coli* via a decrease in membrane hyperpolarization

Dario Bazzoli, Nasim Mahmoodi, Paula Mendes ORCID iD, Tim Overton ORCID iD

School of Chemical Engineering, The University of Birmingham, Birmingham, United Kingdom

Abstract

Biofilm formation presents a massive public health and economic issue, with significant impact on multiple aspects of human life. As removal of biofilms is very difficult, prevention of biofilm formation remains the best option for biofilm control in many cases. However, chemical control (using antimicrobials) can lead to development of resistance, and nanopatterned anti-biofilm surfaces are prone to being coated with conditioning layers. Instead, we used vibration as a method of preventing surface attachment of bacteria.

A device was constructed to evenly deliver piconewton vibrations to bacteria. We discovered that attachment of *E. coli* to the surface was reduced by vibrational stimulation at certain combinations of frequency and intensity. This reduction was not observed in dead cells or beads, suggesting a biological response to the mechanical stimulus. Response to vibration required a proton motive force but not de novo protein synthesis. Measurement of membrane potential revealed that vibration reduced membrane hyperpolarization, which we believe is part of a signalling cascade leading to reduced attachment.

Our work complements recent studies of mechanosensing in *E. coli* and specifically events that occur as bacteria approach solid surfaces. This strategy could enable development of novel biofilm-prevention devices for multiple applications.

P446

Resistance plasmids can facilitate the transfer of virulence in *Shigella sonnei*

Qian Lin Lee, Giulia Pilla, Rachel Exley, Christoph Tang

Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a major public health problem which can be spread by conjugative and mobilisable plasmids.

Shigella sonnei requires a 220 kb virulence plasmid (pINV) to cause dysentery. pINV is non-conjugative but has been mobilised in the laboratory by a helper plasmid previously. As plasmid-borne AMR is an emerging problem in *S. sonnei*, we investigated whether pINV could be transferred by naturally-occurring AMR plasmids, and determined the mechanism of any transfer.

An *S. sonnei* donor with pINV and a conjugative 100 kb *S. sonnei* AMR plasmid (pRES) can transfer both plasmids to an *S. sonnei* strain lacking plasmids; pINV is transferred in the presence of pRES at low frequency. Different plasmids were identified in transconjugants harbouring pINV sequences, including: i) pINV and pRES as separate plasmids; ii) only pINV; and iii) hybrid pINV/pRES plasmids. This suggests that multiple genetic events can mediate pINV transfer.

The origin of transfer (oriT) on pRES was deleted to investigate the role of pRES in driving pINV transfer. Deletion of oriT abolished pRES and pINV transfer, while *recA* deletion in the donor markedly reduced pINV transfer, indicating that the formation of large pINV/pRES fusion plasmids is necessary for pINV transfer. We also detected pINV/pRES fusion plasmids in recipients lacking *recA*. Interestingly, *recA*-independent recombination events can resolve fusion plasmids into hybrid plasmids which were detected post-conjugation in the absence of *recA*.

In conclusion, conjugative AMR plasmids facilitate the transfer of the *S. sonnei* virulence plasmid which could impact spread of virulence and resistance.

P447

A rapid and safe antibiotic discovery platform for Gram-negative pathogens

KAMESHWARI CHENGAN[1], Christopher Mark Smales ORCID iD[1], Simon Moore ORCID iD[1,2]

[1]University of Kent, Canterbury, United Kingdom. [2]Queen Mary University of London, London, United Kingdom

Abstract

Antimicrobial resistance is a global health crisis. We urgently need new antimicrobials to treat resistant bacterial infections before they become incurable. Gram-negative bacteria are problematic due to a "phalanx" of membrane-associated defences that restrict intracellular access of the pathogen. Critically, there has been no new clinical antibiotics developed for Gram-negative bacteria since the quinolones in the 1970s.

The outer and inner membranes present a physical barrier resulting in low permeability or increased efflux of antimicrobials, preventing detection in growth inhibition assays. This can mask antimicrobial hit rates in high-throughput screening (HTS) campaigns of purified compounds or natural products. To address this limitation, we present a synthetic biology approach for identifying antimicrobials against *Klebsiella pneumoniae*. Here, we present a cell-free gene expression tool for rapid detection of transcription and translation inhibitors. This platform is amenable to HTS with a strong signal-to-noise ratio and Z'-factor (greater than 0.7) in 394-well format. Our cell-free system harnesses the native core metabolic enzymes to drive protein synthesis, providing a plethora of intracellular targets in a one-pot reaction. Hence, our assay eliminates the membrane barrier, provides a low-cost (less than £0.006 per drug) and sensitive primary screen to detect antimicrobials in *K. pneumoniae* while tolerating up to 5% DSMO. We are currently leveraging the system to explore novel antibiotics produced by microbes from under-explored habitats. In summary, the cell-free system allows rapid detection of antibiotics and provides a safe (non-living) screening tool for *K. pneumoniae* and its resistant strains, a major problem in nosocomial settings.

P448

Enhanced assimilation of glucose by *Pseudomonas bharatica* CSV86^T with low catalytic efficiency Zwf isozymes through heterologous expression of gluconate oxidative route

Bhavik Shah ORCID iD, Sravanti Kasarlawar, Prashant Phale

Indian Institute of Technology Bombay, Mumbai, India

Abstract

Pseudomonas bharatica CSV86^T preferentially utilises aromatics over glucose. The strain metabolises glucose *via* the intracellular phosphorylative route of Entner-Doudoroff pathway, while oxidative routes (gluconate and 2-ketogluconate) are absent. Consequently, the strain displays a lower growth rate on glucose. Glucose-6-phosphate dehydrogenase (Zwf) plays an important role in the Entner-Doudoroff pathway and is involved in NAD(P)H generation. The present study attempts to understand the biochemical aspects of glucose utilisation and unique substrate hierarchy by characterisation of three Zwf isoforms (ZwfA, ZwfB, and ZwfC) and heterologous expression of oxidative route genes in strain CSV86^T. Purified Zwfs showed varied cofactor preferences where ZwfA was found to be dual-cofactor specific, while ZwfB and ZwfC were $NADP^+$-preferring/specific. ZwfA displayed highest catalytic efficiency and cooperativity for glucose-6-phosphate binding. However, among functionally characterised Zwfs, the isoforms from CSV86^T displayed poor catalytic efficiency, which along with the absence of oxidative routes, might lead to limiting concentrations of downstream pathway intermediates and slower glucose utilisation. Further, cloning of gluconate metabolism genes (*gnu*L-*gnu*K-*gnu*T-*hyp* from *P. putida* KT2440) in pSEVA624 and heterologous expression in CSV86^T led to increased growth rate and biomass on glucose ($0.32h^{-1}$, OD_{540}=~2.6) compared to wild-type ($0.25h^{-1}$, ~1.6). The transformed strain CSV86^T also utilised gluconate as a sole carbon source. In wild-type CSV86^T, possessing catalytically less efficient Zwfs and lacking oxidative routes, incorporation of the gluconate route aids in utilisation of glucose *via* both phosphorylative and oxidative routes, which might increase the concentrations of lower pathway metabolites, thus enhancing efficiency and growth rate on glucose.

P449

Developing ppGpp Target Capture Compounds to Map Stress response Signalling Networks in Staphylococcus aureus

Mark Gainford ORCID iD, Rebecca Corrigan ORCID iD, David Williams

Sheffield, Sheffield, United Kingdom

Abstract

The alarmones guanosine tetra-phosphate (ppGpp) and guanosine penta-phosphate (pppGpp), collectively termed (p)ppGpp, are universally conserved second messenger nucleotides in prokaryotes. (p)ppGpp control the “stringent response” a complicated survival response that occurs in bacteria exposed to external stresses. This response ultimately promotes cell survival through a form of quiescence and has been implicated in increased antimicrobial tolerance and persistent/chronic infections. Despite the discovery of these alarmones in Escherichia coli in 1969, as well as several of their protein targets in later years, numerous binding targets are unknown, and how they facilitate this dormant state is not completely understood. Here, we describe the synthesis of a (p)ppGpp target-protein capture compound using the chemical synthesis of a biotinylated linker fused to enzymatically produced p(s)pGpp. This compound is currently being used to map the alarmone-protein signalling network and reveal (p)ppGpp binding partners in Staphylococcus aureus under various stress conditions such as mupirocin induced amino acid starvation. A similar approach has previously been implemented in E. coli to yield a plethora of (p)ppGpp targets. However, as the protein pathways utilised by E. coli are fundamentally different to that of S. aureus, applying this method to different species will allow for the identification of new protein targets that could aid in the understanding of this stress response.

P450

Does Response Regulator MtrA work alone or as part of a team to control antibiotic production?

Kelly-Rose O'Reilly, Nicolle Som, Rebecca Devine, Barrie Wilkinson, Matt Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

Antimicrobial resistance is an ever-growing threat that continues to burden society. One approach to this issue is novel antimicrobial agent discovery. Promising contenders for antimicrobial exploration and discovery are the Gram-positive, filamentous bacteria in the genus *Streptomyces*, which produce an array of specialised metabolites (SM), including 55% of the antibiotics used today.

Generally, SMs are encoded by locally clustered genes participating in a specific metabolic pathway, known as biosynthetic gene clusters (BGCs). Around 90% of BGCs in *Streptomyces spp*. are not expressed under laboratory conditions, meaning many SMs, including antibiotics, are awaiting discovery. Further understanding of the regulatory pathways controlling antibiotic expression is required to 'unlock' these cryptic BGCs and potentially unveil novel antimicrobials.

To approach this, we investigate MtrA; a master regulator that binds to 85% of BGCs in the model organism *Streptomyces venezuelae*, suggesting it is likely to be an important component in unlocking cryptic BGC expression.

MtrA is part of a highly conserved two-component signalling system, named MtrAB, which coordinates antibiotic production with sporulation. MtrA heavily contributes toward specialised metabolism in *Streptomyces spp*. e.g., through repression of chloramphenicol in *S. venezuelae* and modulation of the antibiotics, actinorhodin, and undecylprodigiosin, in *Streptomyces ceolciolor*. Beyond this, MtrA controls the expression of more than 500 genes in *S. venezuelae*, yet no conserved MtrA consensus binding sequence has been found.

In this work, we investigate whether MtrA interacts with other regulatory proteins to control different regulons of genes. This will help us further understand MtrAB and its influence on secondary metabolism.

P451

Understanding the secretion mechanism and activity of a type VIIb secretion substrate and its inhibition by a cognate immunity protein.

Eleanor Boardman ORCID iD[1], Yaping Yang[2], Tracy Palmer[2]

[1]Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Staphylococcus aureus is a commensal bacterium that can turn against and attack its mammalian host. Infections by *S. aureus* are especially important due to their resistance against many antibiotics. The type VIIb protein secretion system exports substrates that have been implicated in not only *S. aureus* virulence but interbacterial competition. We set out to determine the structure of a DNase toxin used to attack other S. aureus strains. This toxin (EsaD) is known to be inhibited by an immunity protein and export is aided by a specific chaperone. By studying the EsaD toxin in complex with its partner proteins by X-ray crystallography, we have found an unusual inhibition mechanism involving splitting of the nuclease domain down its centre by its cognate immunity protein. We also find a role for small helical proteins (WXG-like) in secretion and/or stability of EsaD and propose a “pre-secretion” toxin complex that is the first of its type for the type VIIb secretion systems found in Firmicutes. We also compare this to the more well studied type VIIa secretion systems of Mycobacteria with unexpected similarities.

P452

Understanding the antimicrobial activity of ovotransferrin in egg white toward *Salmonella* Enteritidis membranes

Julie Legros[1,2], Sylvie Bonnassie[3], Marie-Françoise Cochet[2], Sophie Jan[2], Simon Andrews[1], Florence Baron[2]

[1]School of Biological Sciences, Health and Life Sciences Building, University of Reading, Reading, United Kingdom. [2]INRAE, Institut Agro, Rennes, UMR1253 STLO, Rennes, France. [3]UFR Sciences de la vie et de l'environnement, Université de Rennes 1, Rennes, France

Abstract

Eggs are frequently mediators of foodborne disease resulting in salmonellosis outbreaks which are mainly caused by *Salmonella* enterica serovar Enteritidis. A prominent antimicrobial egg-white factor is the glycoprotein, ovotransferrin. Ovotransferrin belongs to the transferrin family, members of which are known for their powerful iron-chelating activity. However, several studies have also described the ability of these proteins to disrupt bacterial membranes.

In order to better understand the mechanisms of action of ovotransferrin toward *S*. Enteritidis in egg-white conditions, several experiments were performed. The capacity of ovotransferrin to permeabilize membranes was studied by measuring the β-lactamase and β-galactosidase activity of *S*. Enteritidis Sep129-1, a strain expressing these enzymes in the periplasm and the cytoplasm, respectively. Membrane potential change on *S*. Enteritidis PT4-PT124109 was achieved using a spectrofluorescence along with the 3,3-dipropylthiacarbocianine (DiSC3(5)) probe. To mimic egg white conditions, ovotransferrin was tested at pH 9.3 using a synthetic permeate that has the same ionic composition as egg white but has no other egg-white proteins. In parallel, the impact of ovotransferrin iron-saturation was also tested on membrane damage.

The results show that ovotransferrin induces outer-membrane permeabilization and inner-membrane depolarization, although the permeability of the inner membrane towards macromolecules was not apparently impacted. In conclusion, the results indicate that ovotransferrin exerts two antibacterial activities against *S*. Enteritidis, membrane damage and iron restriction. Current work focuses on elucidating the mechanism of ovotransferrin-induced membrane damage in *S*. Enteritidis

P453

EnvR is a potent repressor of *acrAB* transcription in *Salmonella*

Pauline Siasat ORCID iD, Jessica M A Blair ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

RND efflux pumps confer MDR in Gram-negative bacteria by pumping out clinically-relevant antibiotics. From the TetR family of transcriptional regulators (TFTRs), EnvR is an effective repressor of *acrB* transcription, the primary RND pump in Enterobacteriaceae. However, expression of envR is constitutively suppressed due to H-NS silencing. To determine its effects upon the efflux activity of AcrAB-TolC, Salmonella enterica serovar Typhimurium SL1344 was used to construct a strain which overexpressed EnvR from the chromosome via homologous recombination. Under the control of a constitutively active promoter, EnvR is produced at high levels. Due to its regulatory nature and high affinity for the acrAB promoter, *acrB* expression is effectively reduced. The phenotypic effect of this transcriptional regulation was observed by measuring the rate of compound removal from within bacterial cells, where EnvR overexpression led to significantly lower AcrB efflux activity compared to wild-type SL1344. The minimum inhibitory concentration (MIC) values of this strain also showed increased antimicrobial susceptibility against an array of substrates. Taken together, this work indicates that EnvR regulation of AcrB expression can significantly render bacteria avirulent and susceptible to antibiotics. Exploiting the functions of regulators like EnvR seems to be a promising avenue to combat antibiotic resistance.

P454

How does the *Streptomyces* genus translate megasynthases for natural product biosynthesis?

Lakshmeesha Kempaiah Nagappa ORCID iD[1,2], Tobias von der Haar ORCID iD[1], Simon Moore ORCID iD[1,2]

[1]University of Kent, Canterbury, United Kingdom. [2]Queen Mary University of London, London, United Kingdom

Abstract

Megasynthases are incredibly large proteins, reaching up to 2.6 MDa in size – that's one to two orders of magnitude larger than most bacterial proteins and even exceeding the size of a ribosome itself. These colossal proteins are involved in the biosynthesis of a range of secondary metabolites, including antibiotics. Despite their impressive size, we know very little about how bacteria make these proteins. Our research aims to uncover the mysteries of these incredible proteins and learn more about how they're made. To understand this question, we study model megasynthases from *Streptomyces venezuelae* and *Streptomyces* S4, using a combination of *in vivo* and *in vitro* experiments. We have optimised *E. coli* and *S. venezuelae* cell extract to measure megasynthase elongation rate and ribosome processivity, and our initial results show that elongation rates for megasynthetase genes in *S. venezuelae* (2 s/codon) are comparatively slower than that in *E. coli* (0.5 s/codon). To study the production of megasynthase proteins, we used a transcriptional gene expression reporter, generated by CRISPR/Cas9-based genome editing. This allowed us to determine when and under what conditions these genes are expressed. We also validated our findings by monitoring for the production of secondary metabolites using high-performance liquid chromatography. In the future, we plan to conduct proteomics studies to identify the key players involved in megasynthase translation and potential post-translational activation. By combining *in vitro* and *in vivo* approaches, we hope to gain a comprehensive understanding of how these proteins are made and discover new natural products from megasynthase biosynthetic pathways.

P455

Investigating the role of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in lactoferrin iron uptake in *Campylobacter jejuni*

Molly Webster[1], Julian Ketley[1], Tim Knowles[2]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

Campylobacter jejuni is a gram-negative, microaerobe, considered to be the leading bacterial cause of human gastroenteritis. Iron is a crucial metabolite for many processes including host gut colonisation, however it is restricted in hosts as a non-specific defence mechanism. Lactoferrin is a globular glycoprotein that can sequester iron. The CtuA outer membrane receptor of *C. jejuni* enables the *C. jejuni* to utilise this iron source. When ctuA is mutated, growth with lactoferrin as a sole iron source is not completely abolished, indicating there is an alternative lactoferrin utilisation mechanism. GAPDH is a strong candidate as it has been found to interact with lactoferrin. Encoded by the essential *gapA* gene, GAPDH plays a crucial role in *C. jejuni* gluconeogenesis so cannot be mutated. To bypass the need for GAPDH, we have attempted to insert a gene locus encoding the enzymes and GlcP glucose transporter of the Entner–Doudoroff (ED) pathway from a clincal *C. jejuni* isolate into the well-studied NCTC11168 *C. jejuni* strain. Although the transformation was successful each recombinant had at least one inserted gene with a SNP resulting in an amino acid substitution or frameshift. This indicates there may be a metabolic conflict in NCTC11168 with a functioning ED system. Attempts are underway to insert the ED system locus into alternative well characterised *C. jejuni* strains. The GAPDH and CtuA proteins have been purified and their interaction with each other and lactoferrin will be investigated using QCMD. This will allow incorporation of CtuA into a phospholipid bilayer to better mirror its natural environment and conformation.

P456

Developing and optimising a culturomics strategy to uncover the hidden microbiome in human milk

Ranran Li, Julianne Megaw, Simon Cameron

School of Biological Sciences- Queen's University Belfast, Belfast, United Kingdom

Abstract

Human milk (HM) provides the optimal microbiome for infants. To date, only a small proportion of HM microbes have been isolated using conventional culture methods. Culturomics has successfully isolated previously uncultured microbes from the human gut. However, less research has focused on the low-biomass HM microbiome. This study sought to develop and optimise a methodology for HM microbial cultivation to allow further functional analysis. Culturomics conditions were designed to maximally mimic the natural environment in HM. This included (1) sample dilution from 10^{-1} to 10^{-88}; (2) liquid enrichment culture for fastidious microbes; (3) diverse culture broths and media, including non-selective, selective, and enriched; (4) broad culture atmosphere and temperature, e.g., aerobic, 5% CO_2 aerobic, 8-10% CO_2 microaerophilic, and anaerobic from 30-37℃; and (5) incubation duration from 3 to 14 days. The combination of different conditions was implemented into 96-well microplates for liquid enrichment and solid agar culture. The methodology was developed on glycerol stocks of frozen HM samples before being deployed for freshly collected samples. This optimised culturomics strategy has been validated by analysing glycerol frozen HM samples, generating more than 100 isolates for further identification, including from selective media (e.g., Bifidobacterium, Lactobacillus, and fungus selective media), enriched pasteurised donor HM medium, and general media. The application of this optimised method created the high-resolution culture conditions for low-biomass microbial milk, maximally isolated HM microbes for further functional and metabolic research, and minimised laboratory resources.

P457

In vitro degradation of mucin by SslE in commensal and pathogenic E. coli strains identifies important enzyme recognition sites

Leanne Cleaver[1], Saima Rehman[1], Katherine Fenn[2], Vidya Darbari[2], James Garnett[1]

[1]King's College London, London, United Kingdom. [2]Queen Mary University of London, London, United Kingdom

Abstract

SslE is a mucinolytic enzyme secreted via the type II secretion system of virulent, and some commensal, Escherichia coli strains. SslE is an important virulence factor in gastrointestinal infections, allowing bacteria to degrade the mucin layer of the gut to promote adhesion to host cells. We recently determined the structure of SslE and its subdomains by cryo-EM and crystallography, which suggests a unique mechanism of mucin recognition and processive degradation through its N-terminal domains. Here, we investigated this mechanism using wild type SslE and SslE mutants of the Waksman and Enterotoxigenic E. coli strains. Competent SslE knockout (KO) cells were transformed with either an intact SslE complement or SslE complements containing point mutations, designed to target sites that we anticipated are important for mucin binding or catalysis. All strains were grown in LB broth aerobically at 37ºC while shaking, then added to a transwell permeable insert loaded with 10% (w/v) type I-S mucin from bovine submaxillary glands, with LB broth in the well below. After 4 hours growth at 37ºC, CFU/ml counts were performed from the media at the bottom of the well to assess translocation. All strains, except KO, expressed SslE when assessed by Western blotting and levels of penetration through the mucin layer supported the importance of these sites for the binding and/or breakdown of mucins. This study validates our proposed mechanism for SslE recognition and breakdown of mucins and provides a useful assay to test further mutations that affect SslE-dependent mucin degradation.

P458

Chewing the fat: characterization of fatty acyl-CoA dehydrogenases from *P. aeruginosa*

Meng Wang, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

Lung surfactant-derived long chain fatty acid (LCFA) is the primary carbon source for *Pseudomonas aeruginosa* (PA) when the organism infects the airways of people with cystic fibrosis (CF). However, our understanding of how fatty acids are catabolized in PA remains limited, although we do know that this is a lot more complicated than the biochemistry in *Escherichia coli*. To investigate this further, we have used quantitative TMT-proteomics to analyze the proteome of a Manchester epidemic strain (MES) Pa10348 grown on three different fatty acids (octanoate, palmitate and oleate). This revealed that two fatty acyl-CoA dehydrogenases (encoded by PA0506 and PA0508) are likely to be the dominant enzymes in the first step of LCFA-related β-oxidation in PA. However, growth of the corresponding mutants on a range of fatty acids with different chain lengths suggests that PA0506 is mainly responsible for LCFA degradation whereas PA0508 plays a more important role in medium chain fatty acid (MCFA) catabolism. We have purified each enzyme and determined its kinetic parameters, which confirm the distinct substrate specificity profiles. To further investigate the molecular basis of the substrate specificity in each enzyme, we have solved their X-ray crystal structure, and we are using these data to identify potential inhibitors. Current efforts are aimed at investigating the function of the transcriptional regulators controlling PA0506 and PA0508 expression.

P459

A novel fold for acyltransferase-3 (AT3) proteins provides a framework for transmembrane acyl-group transfer

Kahlan Newman ORCID iD[1], Sarah Tindall ORCID iD[2], Sophie Mader ORCID iD[3], Syma Khalid ORCID iD[3,1], Gavin Thomas ORCID iD[2], Marjan van der Woude ORCID iD[2]

[1]University of Southampton, Southampton, United Kingdom. [2]University of York, York, United Kingdom. [3]University of Oxford, Oxford, United Kingdom

Abstract

Acylation of diverse carbohydrates occurs across all domains of life and can be catalysed by proteins with a membrane bound acyltransferase-3 (AT3) domain. In bacteria, these proteins are essential in processes including symbiosis, resistance to viruses and antimicrobials, and biosynthesis of antibiotics, yet their structure and mechanism is largely unknown. In this study, evolutionary co-variance analysis was used to build a computational model of the structure of a bacterial O-antigen modifying acetyltransferase, OafB. The resulting structure exhibited a novel fold for the AT3 domain, which Molecular Dynamics simulations demonstrated is stable in the membrane. The AT3 domain contains 10 transmembrane helices arranged to form a large cytoplasmic cavity lined by residues known to be essential for function. Further Molecular Dynamics simulations support a model where the acyl-coenzyme A donor spans the membrane through accessing a pore created by movement of an important loop capping the inner cavity, enabling OafB to present the acetyl group close to the likely catalytic resides on the extracytoplasmic surface. Modelling suggests the SGNH domain is mobile and can both accept acyl-groups from the AT3 domain and then reach beyond the membrane to reach acceptor substrates. Together this new general model of AT3 function provides a framework for the development of inhibitors that could abrogate critical functions of bacterial pathogens.

P460

Elucidating the substrate profiles of the *S. aureus* peptide transporters using phenotype microarrays

Imran Khan ORCID iD[1], Gavin Thomas ORCID iD[1], Marjan Van Der Woude ORCID iD[2]

[1]University of York, York, United Kingdom. [2]Hull York Medical School (HYMS), York, United Kingdom

Abstract

During an infection, growth of the Gram-positive pathogen *Staphylococcus aureus* is heavily reliant on the acquisition of host-derived peptides and amino acids as essential nutrient sources. Peptide utilisation in this organism is known to be facilitated by at least two transport systems: the ATP-binding cassette system Opp3 and the proton-dependant oligopeptide transporter DtpT. Although both systems contribute to bacterial fitness in a mouse infection model, the peptide transporters of *S. aureus* remain poorly characterised regarding their substrate specificities and physiological roles.

To understand their relative roles in more detail, Biolog Phenotype Microarray technology was applied to compare peptide utilisation by *S. aureus* strain JE2 compared to strains with disruptions in the known peptide transporters. This approach allowed for high-throughput screening of 282 di- and tripeptides, revealing a strong dependence for utilisation of some peptides on DtpT, as well as a small number that required Opp3 and some for which either or both transport systems were utilised. To support our findings, growth of peptide transporter mutants was compared in media depleted of the necessary amino acid arginine and supplemented with arginine-containing dipeptides. These assays provide complimentary evidence for transporter-specific utilisation of the tested peptides and provide a basis for future experiments utilising site-specific transporter mutants to probe the structural basis of substrate specificity in these systems. Overall, our findings represent the first detailed analysis of substrate preferences for the peptide transport systems of *S. aureus*.

P461

Transcriptional response of *Burkholderia cenocepacia* H111 to significant zinc depletion

James Barnett ORCID iD

Birmingham City University, Birmingham, United Kingdom

Abstract

The *Burkholderia cepacia complex* (*Bcc*) is a group of 22 species of bacteria widely distributed in the environment. They first emerged as opportunistic pathogens in the 1980s, causing severe respiratory infections in people with cystic fibrosis (CF). Whilst all species within the *Bcc* have been identified in sputum from CF patients, *B. cenocepacia* and *B. multivorans* account for 85-97 % of cases. In CF patients, infections have a variable clinical course, although the prognosis is generally poor. Infections often result in an accelerated decline in pulmonary function and increased morbidity and mortality. *Bcc* bacteria can also lead to the development of 'cepacia syndrome', characterised by a fatal necrotising pneumonia and bacteraemia. *Bcc* bacteria are naturally resistant to major classes of antibiotics in clinical use, making infections particularly difficult to eradicate. The epidemiology of *Bcc* bacteria is not limited to CF, with infections also occurring in people with chronic granulomatous disease, and reports of nosocomial infections, often from contaminated medical supplies.

To survive, pathogens must be able to acquire the essential nutrients they need for growth in competition with the host. Zinc is an essential nutrient for bacteria, functioning as a catalytic cofactor or structural component of numerous proteins. However, during infection, the host attempts to deprive bacteria of the essential zinc they need to grow in a process termed nutritional immunity. Using RNA-seq, the transcriptional response of *B. cenocepacia* to significant zinc-depletion has been determined, identifying putative zinc importers and zinc sparing as a mechanism to overcome zinc deprivation.

P462

New tools for visualising transcription and translation in tandem in *Streptomyces*.

Jack W Stone, Glenn A Burley, Iain S Hunter, Paul A Hoskisson

University of Strathclyde, Glasgow, United Kingdom

Abstract

The bacterial genus Streptomyces are responsible for the production of clinically relevant antimicrobial agents and understanding control of their biosynthesis will help addressing the challenge of antimicrobial resistance. The bald (bld) mutants in Streptomyces are blocked at an earlier stage of development and are unable to erect aerial hyphae, with many mutations in bld loci pleiotropically blocking antimicrobial production. Perhaps the most severe bld phenotype is found in the bldA locus, encoding a rare leucyl-tRNA, where mutations result in complete loss of morphological development and specialised metabolite production.

In our attempts to create new molecular tools for studying Streptomyces, we have been utilizing the 'vegetable aptamer' systems to develop synthetic genetic probes that allow for quantification and imaging of transcription and translation simultaneously in live Streptomyces. These fluorogenic aptamers are RNA equivalents of green fluorescent protein (GFP) where a fluorescent complex is formed in the presence of 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI). Unlike traditional transcriptional reporter systems (Beta-galactosidase, GFP etc), there is no need for translation of the mRNA to measure reporter activity, providing a direct readout of the RNA of interest. As proof-of-concept for these tools, we have exploited the Broccoli aptamer and coupled this to a TTA-leucine codon containing the mCherry fluorescent protein reporter to create a system for simultaneously visualizing bldA transcription and translation to revisit the role of bldA in antimicrobial biosynthesis control. Here, we discuss a novel genetic tool for Streptomyces, which will assist in the development of current and future antimicrobials.

P463

Evidence of plasmid-mediated transfer of genes associated with DNA methylation in a *Listeria monocytogenes* strain of serovar 1/2a

Ogueri Nwaiwu, Helen Onyeaka

University of Birmingham, Birmingham, United Kingdom

Abstract

The epigenetic phenomenon, DNA methylation, involves the addition of a methyl group to specific regions of the DNA. Methylation of DNA can inhibit the binding of transcription factors and affect gene expression. Other scientists found a nucleotide unit (AJ302030.1) which harboured novel methylase genes in serotype 4b strains of *Listeria monocytogenes* in the past. In this report, we show for the first time that a plasmid from a strain of serotype 1/2a possesses the same methylase gene sequences earlier believed to be present in only serotype 4b strains. The plasmid contains sequences that are 100% homologous to the reference nucleotide unit. Analysis carried out showed that it contained fragments of genes that encode for methyltransferase, a heat shock protein, phosphomethyl pyrimidine kinase and thiamine phosphate pyrophosphorylase found in *L. monocytogenes* methylase strains. We posit that this acquisition was most likely through horizontal gene transfer in the environment. This finding is of epidemiological importance because the presence of the aforementioned genes could confer a different undesirable pathogenic potential to *L. monocytogenes* serotypes mainly associated with the environment. Surveillance for the occurrence and spread of this plasmid is required to establish if there is any clinical or food safety significance.

P464

Regulation of RyjB expression and mechanism of its action

Namra Siddiqui

Indian Institute of Technology Delhi, New Delhi, India

Abstract

Small RNAs modulate a wide range of physiological pathways in bacterial cells in response to changing environmental conditions for their survival. Bacterial small RNAs (sRNAs) attune gene expression at post-transcriptional levels to help adapt altered conditions. These small RNAs are encoded as -*cis* and -*trans* relative to their mRNA targets. Current study is focused on to understand the regulation of expression of a novel 80 nt long sRNA, RyjB, in *Escherichia coli*. We took a systematic approach to investigate *ryjB* expression at different growth phases and at low pH as *E. coli* frequently experiences low pH during its transient passage through stomach. Interestingly, RyjB was identified low pH inducible sRNA in E. coli. A novel DNA binding protein PhoP, a component of PhoP/PhoQ regulatory system was recognized to induce *ryjB* expression under acid stress, as the phoP mutant cells showed the basal level *ryjB* expression under acid stress. Ectopic expression of PhoP in *phoP* mutant cells restored the nimiety of RyjB in the cell. A putative phosphotransferase IIA component, SgcA, was identified to share 4-nt overlap with *ryjB*. Overexpression of RyjB was found to increase *sgcA* expression under normal conditions as well as under acid stress conditions. Furthermore, *ryjB* overexpression with the elimination of overlapped 4-nt sequence shows a basal level of *sgcA* expression. RyjB binds to 5' end of *sgcA* and blocks the RNase E cleavage sites. This suggests that RyjB might function as an antisense RNA.

P465

Characterisation of high-affinity human milk oligosaccharide transporters from *Bifidobacterium longum subsp. infantis*

Rory Elston[1], Manos Papadakis[2], Dani Ungar[1], Gavin Thomas[1]

[1]University of York, York, United Kingdom. [2]Glycom A/S, Copenhagen, Denmark

Abstract

Human milk oligosaccharides (HMOs) are a group of prebiotic sugars that form an important component of human breast milk. They play important roles in shaping the microbial composition of the infant gut and have been shown to impart health benefits to the developing infant. Certain species of bacteria, such as Bifidobacterium longum subsp. infantis can catabolise HMOs to promote their growth in the infant gut, which relies on uptake of whole HMOs into the cell before their metabolism. High affinity transport of sugars is often mediated by ATP-binding cassette (ABC) transporters and here we use an experimental approach to characterise predicted HMOs transporters present in B. longum subsp. infantis. Through purification and analysis of ligand binding to the substate binding protein (SBP) component, an early picture of the substrate specificity of these transporters is presented, providing new insight into the function of bacterial transport during early human life.

P466

Role of the *Campylobacter jejuni* Type VI secretion system and *in silico* identification of putative T6SS effectors

Janie Liaw[1], Zahra Omole[1], Geunhye Hong[1], Cadi Davies[1], Luca Robinson[2], Brendan Wren[1], Nicolae Corcionivoschi[3], Abderrahman Hachani[4], Nick Dorrell[1], Ozan Gundogdu[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]Imperial College London, London, United Kingdom. [3]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [4]University of Melbourne, Melbourne, Australia

Abstract

Background:

The Type VI Secretion System is a contractile nanomachine that injects effector proteins into bacterial or eukaryotic cells. However, knowledge of the roles of T6SS in *Campylobacter jejuni* and identity of its secreted effectors remain limited despite a high prevalence of T6SS in human and chicken isolates.

Methods:

The whole genome sequence of novel human isolate 488 was compared with previously sequenced strains. Expression of T6SS genes and secretion of TssD were analysed and T6SS mutants were constructed. Oxidative stress assays were performed to determine whether T6SS is associated with the oxidative stress response. Roles of T6SS in interactions with chicken cells were examined using in vitro and in vivo models. A comprehensive bioinformatic analysis was performed using publicly available genomes to further gain an understanding into genetic architecture of *C. jejuni* T6SS.

Results:

A T6SS cluster was identified to be highly conserved between human and chicken isolates and presence of a functional T6SS was demonstrated. Presence of T6SS increased oxidative stress resistance in *C. jejuni*, indicating T6SS is associated with the oxidative stress response. T6SS enhanced *C. jejuni* interaction and invasion of chicken primary intestinal cells and enhanced ability of *C. jejuni* to colonise chickens. The bioinformatic analysis, in combination with analysis of T6SS secretome, led to identification of a second canonical VgrG and a number of putative effectors.

Conclusion:

These studies highlight the importance of T6SS during in vivo survival of *C. jejuni* and also provide a framework for our current characterisation of *C. jejuni* T6SS effectors.

P467

Characterisation of plasmid stability elements in a pathogenic *Klebsiella michiganensis* isolate

Jordan Shutt-McCabe, Lesley Hoyles ORCID iD, Gareth McVicker ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Klebsiella michiganensis PS_Koxy4 (ST138) is a GES-5-positive, ertapenem-resistant clinical strain isolated from a rectal swab taken from a patient in a haematology unit. After Illumina and Nanopore hybrid sequencing, the genome of PS_Koxy4 was found to comprise a circular chromosome (6,075,855 bp) and five circular plasmids (pPSKoxy4_1 - 130,876 bp, IncFII; pPSKoxy4_2 - 109,858 bp, IncA; pPSKoxy4_3 - 76,870 bp, IncR; pPSKoxy4_4 - 8,300 bp, IncQ; pPSKoxy4_5 - 4,448 bp). Four putative toxin-antitoxin (TA) systems were discovered to be encoded by pPSKoxy4_1 (CcdAB), pPSKoxy4_2 (HipBA) and pPSKoxy4_3 (VapBC, ParDE). These TA systems have been functionally characterised via toxicity and anti-toxicity experiments. Preliminary assays in *Escherichia coli* have shown ParE to be non-toxic (likely due to premature translation termination), whereas VapC showed mild toxicity and both CcdB and HipA were highly toxic. HipA is of particular interest due to its supposed relationship to persister cell formation and relatively high toxicity in our experiments. Furthermore, genes encoding variants of CcdAB and VapBC have also been found in the chromosome of the strain, suggesting possible complex interactions. Ongoing investigation into the toxicity and plasmid stabilisation functions of these TA systems in their native host will clarify their respective roles. Since prior genetic characterisation has found that PS_Koxy4 encodes the kleboxymycin biosynthetic gene cluster, and therefore has the potential to cause antibiotic-associated haemorrhagic colitis, these results advance our understanding of plasmid carriage in a highly virulent and antimicrobial-resistant pathogen.

P468

Stress responses and tolerance mechanisms of *Pseudomonas putida* to the plastic industry monomers ethylbenzene, styrene and methacrylate ester.

Charles Begley[1], Eilidh Terras[1], Thomas Hender[1], Nicholas Tucker ORCID iD[1,2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Suffolk, Ipswich, United Kingdom

Abstract

The plastics industry is almost exclusively reliant on petrochemical feedstocks for the synthesis of large-volume commodity chemicals. Collectively, the amount used for plastic production is equivalent to approximately 1.3 billion barrels of crude oil per annum. To improve the sustainability of materials such as acrylic, the chemical industry is investing heavily in biological production methods.

Pseudomonas spp. are tolerant to a wide range of chemical stresses and we have demonstrated that this genus is significantly enriched in metagenomic experiments where complex populations of bacteria are challenged with toxic plastic monomers. In particular, *Pseudomonas putida* has gained traction as a synthetic biology chassis organism and is ideally suited to the sustainable production of plastic monomers because of its inherent solvent tolerance. To further understand and optimise *P. putida* for biosynthesis of plastic monomers we have taken a transcriptomic approach to understand its response to chemical stress associated with ethylbenzene, styrene and methacrylate esters. In addition to several efflux pumps, we identified TetR and MarR class transcriptional regulators that are strongly derepressed in the presence of all three solvents. These regulators are capable of binding diverse substrates and in silico docking experiments indicate the plausibility of solvent ligand binding. Deletion of each regulator facilitated assessment of their regulons by RNA-Seq revealing that the MarR type regulator may contribute to the generalised envelope stress response of *P. putida*. Applications of these regulators as biosensors for combinatorial synthetic biology will be discussed as well as recent insights gained from integrating RNA-Seq and Tn-Seq datasets.

P469

Post-translational regulation of glucose metabolism in *Streptomyces*

Kaisha Murray, Daniel E Larcombe, Iain S Hunter, Paul A Hoskisson

University of Strathclyde, Glasgow, United Kingdom

Abstract

Streptomyces are prolific producers of bioactive specialised metabolites which are biosynthesised from primary metabolic building blocks. Understanding the regulation of primary metabolism will enable engineering of metabolism to increase production of antibiotics and other clinically important metabolites from *Streptomyces*. One recently emerged regulatory mechanism in *Streptomyces* is post-translational modification (PTM) by crotonylation. The role of crotonylation as a post-translational modifier of glucose-kinase (Glk) and carbon metabolite repression (CCR) in *Streptomyces* metabolism is poorly understood. A previous study suggested the importance of crotonylation in the regulation of CCR in *Streptomyces roseosporus*, however, no evidence currently exists to suggest this mechanism occurs widely in *Streptomyces* species. To study this the crotonylation machinery in *S. coelicolor* and *S. clavuligerus* strains, the putative crotonylation and decrotonylation machinery from *S. coelicolor* was introduced to the industrial species *S. clavuligerus*. Additionally, over-expression of each component of the crotonylation/decrotonylation machinery was also performed and the antibiotic yield determined. The role crotonylation plays in PTM of *Streptomyces* metabolism was also determined in relation to the regulation of CCR in *S. coelicolor* and *S. clavuligerus*. This will allow for an enhanced understanding of the complex control mechanisms involved in *Streptomyces* primary and specialised metabolite production. Through this understanding there is potential to increase the yield of these vital natural products in industrial strains and make steps towards the identification and development of new clinically useful bioactive metabolites.

P470

Life cycle-specific secondary metabolites in the model eukaryotic microbe, *Dictyostelium*

Arwa Selim ORCID iD, Bill T. Ferrara ORCID iD, Elinor P. Thompson ORCID iD

University of Greenwich, Chatham Maritime, United Kingdom

Abstract

The social amoeba *Dictyostelium discoideum* is an environmental eukaryote that detects and engulfs bacteria and yeast as a food source. Its life cycle progresses from unicellular to multicellular stages, with cAMP as the chemoattractant and the signal controlling the developmental programme. Chemosensing and signalling, cell-type differentiation, the similar gene content to higher animals, and its phagocytosis of other microbes have all led to *Dictyostelium* becoming a useful biomedical and cell-biology model organism. Its secondary metabolism is less explored, although interest is increasing. The amoeba encodes genes involved in quite diverse biosynthetic pathways, through to those encoding the proteins carrying out final transport mechanisms. Two multidrug and efflux (MATE) transporters are encoded, orthologues of which are notable for sequestering flavonoids in plant vacuoles, among other functions. Having demonstrated the ability of *Dictyostelium* MATE proteins to efflux specific polyphenolic substrates, genes were identified in the amoeba that are similar to those for synthesis of plant polyphenolics. These enabled targeted analysis of 'plant' compounds in the amoeba. Life cycle analysis reveals peaks of genetic expression and chemical content at specific stages in development, augmenting the currently limited knowledge of the physical roles of *Dictyostelium* secondary metabolites.

P471

The effects of co-fired blended ash on the metabolism of *Shewanella oneidensis*

Ifeoluwapo Olumekun, Ismini Nakouti, Iacopo Carnacina, Andy Shaw, Ana Bras

Liverpool John Moores University, Liverpool, United Kingdom

Abstract

Sustainability in construction involves using co-fired blended ash, a waste problem in India arising from co-combusting coal and rice husk, in the production of concrete and mortars. This ash was investigated for its effects on *Shewanella oneidensis* with a view to optimising the microbiologically induced calcium carbonate precipitation process. Different percentages (5%, 10%, and 15%) of sterile 500ml tryptone soy broth (TSB) were replaced with autoclaved ash, and a bottle of sterile 500ml TSB containing no ash were inoculated with *S. oneidensis*. These were incubated for 48 hours at 30^0C using static incubation. Cells counts were performed with a haemocytometer and a biological microscope every 4 hours for 24 hours, then every 2 hours till 48 hours. Experiments were duplicated for validity. The highest growth achieved for 5% (2.24 x 10^7 cells/ml broth), 10% (2.8 x 10^7 cells/ml broth), and 15% Ash (2.54 x 10^7 cells/ml broth) was at 24 hours. In contrast, *S. oneidensis* only (1.8 x 10^7 cells/ml broth) was at 16 hours. The lag phase was 4 hours for all the cultures, whilst the log phase lasted for 20 hours for the ash cultures and 12 hours for *S. oneidensis*. The death phase was 32 hours for *S. oneidensis* but shortened to 24 hours for the ash cultures. This work highlights the potential of using co-fired blended ash as a metabolic catalyst for *S. oneidensis*. Future research is needed to further explore its potential in changing the process of culturing bacteria.

P472

Copper dependent activation of nitrite reductases; an insight into bacterial denitrification

Samantha Firth[1], Denis Thaqi[2], Lotte O'Hern[1], Daniel Owen[1], YoungJin Hong[1], Louisa Stewart[3], Alastair McEwan[2], Bostjan Kobe[2], Kevin Waldron[3], Karrera Djoko[1]

[1]Durham University, Durham, United Kingdom. [2]The University of Queensland, St Lucia, Australia. [3]Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Microorganisms derive energy required to live, grow, and reproduce through respiration. Devoid of O_2, many bacteria respire nitrogen oxides through denitrification. Denitrification is a globally important microbial process. Soil bacteria denitrify fertiliser-bound nitrates, contributing to climate change by generating nitrous oxide. Pathogenic bacteria use denitrification to survive in oxygen-limited host tissues and from harmful nitric oxide.

Nitrite reductases (Nir) are central enzymes to denitrification. These enzymes reduce nitrite to nitric oxide. A subclass of Nir requires copper (Cu) for enzyme activity. This work examines how Cu-containing Nir acquire Cu co-factors and become activated inside cells. Our aim is to design antibacterial strategies that disrupt this process.

Using Neisseria gonorrhoeae as our model system, we showed that a periplasmic Cu protein, AccA, delivers Cu to the gonococcal Nir (AniA). This study finds that knockout mutant strains of accA fail to grow in microaerobic conditions and to reduce nitrite, consistent with an inactive AniA. Cu-supplementation of growth media reverses this phenotype, suggesting that AniA is expressed without Cu co-factors and can directly acquire periplasmic Cu. Strains in which AccA's conserved Met and His residues are mutated to Ala phenocopy the accA knockout strain confirming the role of these residues in Cu binding and transfer to AniA. Biochemical measurement of Cu affinity and transfer from AccA to AniA further confirm the role of AccA in activating AniA.

AccA homologues load Cu to bacterial cytochrome c oxidases which catalyse aerobic respiration. Therefore, this work highlights the importance of cellular Cu trafficking during respiration.

P473

Decipher, Disarm and Disengage: Understanding The Biosynthetic Gene Cluster Of Aurodox, A Type III Secretion System Inhibitor From *Streptomyces.*

Robyn E Braes ORCID iD[1], Rebecca E McHugh ORCID iD[2], Andrew J Roe ORCID iD[3], Paul R Hoskisson ORCID iD[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom. [3]Univeristy of Glasgow, Glasgow, United Kingdom

Abstract

Aurodox, an Elfamycin antibiotic produced by *Streptomyces goldiniensis*, exhibits antibiotic activity against Gram-positive bacteria, by inhibiting translation via elongation factor EF-Tu. A novel mode of action was discovered for aurodox, where inhibition of Type III Secretion Systems of Enteropathogenic (EPEC) and Enterohemorrhagic (EHEC) *Escherichia coli* was shown to be an effective antivirulience treatment. The *S. goldiniensis* genome was sequenced and putative aurodox biosynthetic gene cluster (BGC) identified. Cloning and heterologous expression of the putative aurodox BGC (AurI) in *Streptomyces coelicolor* M1152 confirmed its responsibility for aurodox biosynthesis. Computational analysis of the BGC revealed a multimodular polyketide synthase pathway similar to the BGC of kirromycin. We hypothesised that the final step of aurodox biosynthesis was converting kirromycin, to aurodox via a SAM dependent *O*-methyltransferase on the pyridone moeity, catalysed by the BGC encoded AurM*. Cloning and expressing AurM* in the kirromycin producer, *S. collinus*, resulted in aurodox production. Aurodox resistance genes at distinct genetic loci were also identified during sequencing. A Major-Facilitator Superfamily protein (AurT), present within the aurodox BGC, was thought to confer self-resistance to the producer, through aurodox efflux. However, heterologous expression indicated that *aurT* alone was insufficient for resistance. An additional, Elfamycin-resistant, copy of EF-Tu (*tuf2*) was identified in the genome. Cloning *tuf2* and expression in *S. coelicolor* M1152_AurI indicated that both *tuf2* and *aurT* are required for aurodox immunity in the producing strain. Elucidation of *aurM**, *aurT* and *tuf2's* role has allowed dissection of aurodox biosynthesis. Further genetic manipulation of the BGC could lead to production of novel derivatives.

P474

Chemical genomics reveals conditional essentiality of genes in Klebsiella pneumoniae

Micheal Alao ORCID iD[1], Hugo Blandford[1], Danesh Moradigaravand[2], Manuel Banzhaf[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Abstract

Chemical genomics is a screening technique that identifies conditionally essential genes in bacteria. To understand the genotype-phenotype relationship in K. pneumoniae, a transposon knockout mutant library was probed against different chemical and environmental perturbations. Fitness of the mutants in all tested conditions were quantified with an image analysing software called Iris. Overall, the methodology developed here allows the high throughput screening of knockout mutant libraries against diverse conditions to yield genotype-phenotype relationships important in understanding gene functionality and metabolic network behaviours.

P475

Structural characterisation of a TAXI TRAP transporter substrate binding protein from *Vibrio cholerae*.

Joseph Davies[1], Nick Massouh[1], Andrew Daab[1], Gavin Thomas[2], Christopher Mulligan[1]

[1]University of Kent, Canterbury, United Kingdom. [2]University of York, York, United Kingdom

Abstract

Transport proteins are vital for the import of nutrients and signalling molecules and their function is essential for bacterial survival, antimicrobial resistance, and virulence. Tripartite ATP-independent periplasmic (TRAP) transporters are a large family of binding protein-dependent secondary-active transporters found widespread in prokaryotes. TRAP transporters are composed of a substrate binding protein (SBP) and a membrane component that can consist of 2 unequally sized integral membrane proteins or a fusion of these 2 proteins into a single polypeptide. TRAP transporters can be further divided into 2 subfamilies, the DctP- and TAXI-type subfamilies; the primary difference between these subfamilies is that the SBPs are completely unrelated at the amino acid level. While the range of substrates and binding determinants have been described for dozens of DctP-type SBPs, TAXI-TRAP SBPs have been neglected and a basic understanding of this subfamily is lacking.

To develop a better understanding of the structure and specificity determinants of TAXI TRAP transporters, we have characterised a TAXI SBP from *Vibrio cholerae*, VcGluP. Our analysis reveals that VcGluP can bind a range of related compounds, but preferentially binds L-glutamate with a Kd of ~80 nM. Analysis of L-glutamate bound crystal structure and alanine scanning of binding site reveals the nature of substrate binding, which is substantially different to the well-characterised DctP-type TRAP SBPs.

P476

Expanding the Microbial Phosphorus Cycle

Jack Nicholls ORCID iD[1], Jason Chin[1], Timothy Lenton[2], Tom Williams[3], Vincent O'Flaherty[4], John McGrath[1]

[1]Queen's Unviersity Belfast, Belfast, United Kingdom. [2]University of Exeter, Exeter, United Kingdom. [3]University of Bristol, Bristol, United Kingdom. [4]National University of Ireland, Galway, Galway, Ireland

Abstract

Biochemistry relies on phosphorus (P), mainly in the form of phosphate. Yet, microorganisms can utilize a variety of other P-containing compounds, the metabolism of which remains largely unexplored. Such compounds include polyphosphate and phosphite. The former is a biopolymer of phosphate residues metabolized intracellularly by all organisms as a store of P and energy. The latter is a reduced form of phosphate involved in a recently discovered microbial P redox cycle where phosphite is oxidized for energy or biomass. Collectively, polyphosphate and phosphite may represent a significant source of environmental P for microorganisms, effecting primary productivity and nutrient cycling throughout many ecosystems.

The microbial diversity of phosphite oxidation and polyphosphate metabolism was initially investigated via bioinformatic analysis. Firstly, homologs of known phosphite oxidation genes were identified, then refined using sequence similarity networks and the resultant homologous groups were searched for partial or complete gene clusters. Numerous potentially novel phosphite oxidizing organisms across the Proteobacteria and Actinobacteria phyla were identified this way. Secondly, scanning of microbial genomes also highlighted haloarchaea as unable to degrade polyphosphate using any of the recognised bacterial or eukaryotic pathways. Subsequently, a culture-based approach found several haloarchaeal isolates not only metabolized polyphosphate internally, but also utilized polyphosphate as their sole source of external P for growth. As a result, a previously unrecognised polyphosphate-degrading pathway and enzyme is being characterised. The diversity, prevalence and evolution of both metabolic traits could have significant implications for microbial phosphorus cycling throughout oligotrophic and extreme environments.

P477

The transcriptional activator of an RNA operon directly regulates the enzymatic activity of its associated RNA ligase

Angel (Jingjing) Xia, Danai Athina Irakleidi, Ioly Kotta-Loizou, Martin Buck

Imperial college London, London, United Kingdom

Abstract

In the model organism Escherichia coli, the Rtc RNA repair system comprises the RNA cyclase RtcA and the RNA ligase RtcB, together with their transcriptional activator RtcR, an enhancer-binding protein with a central AAA+ domain and a N-terminal CRISPR associated Rossmann fold (CARF) domain for ligand recognition. The Rtc system plays a central role in maintaining cell fitness, recycling ribosomal RNAs during stress, and antibiotic resistance. Here we show that RtcR not only effect transcription, but also regulates the activity of the Rtc RNA repair enzymes. Time and dose dependent RNA ligation assays with RtcB and fluorescently labelled tRNA fragments were carried out in the presence and absence of full length RtcR or the RtcR CARF domain on its own and visualised using denaturing urea polyacrylamide gel electrophoresis. The presence of the RtcR protein noncompetitively inhibited the enzymatic activity of RtcB, as demonstrated by its ability to ligate the fluorescent tRNA fragments. The RtcR CARF domain, known to physically interact with RtcB, is at least partially responsible for the observed inhibition. In parallel, the ability of potential ligands to activate RtcR via it's CARF domain was assessed through in vitro ATPase activity assays. In the future, we will investigate the effect of ligand-bound, transcriptionally active RtcR on the RtcB liagse. Together, our results advance the current understanding of RtcR's role in regulating the E.coli Rtc RNA repair operon and provide another possible target for combatting antibiotic resistance in the future.

P478

Molecular analysis of how iron regime affects the turnover of ferritin A of *E. coli*

Abdallah Al Hrazi[1,2], Afrah Salman[1,3], Simon C. Andrews[1]

[1]University of Reading, Reading, United Kingdom. [2]Jazan University, Jazan, Saudi Arabia. [3]Mustansiriyah University, Baghdad, Iraq

Abstract

Iron-storage proteins (ferritins) are key component of iron homeostasis. In *Escherichia coli*, iron is mainly stored by ferritin A (FtnA). Although the process of iron uptake is well understood, mechanisms governing iron release remain unclear. Thus, in this study, the role of FtnA proteolytic degradation in the iron release process is explored. An inducible promoter was used to control FtnA production in *E. coli* mutants that were devoid of all other iron-storage genes. Results showed surprisingly low levels of cellular FtnA, suggesting that FtnA is subject to a rapid turnover. Mutations in a range of protease showed that absence of FtsH resulted in a major increase in FtnA levels, whereas loss of other proteases had little effect. In addition, substitution of the highly-conserved 2nd amino acid residue (Leu2) of FtnA for Lys or Ala resulted in a substantially increased level of FtnA. This suggested that FtnA may be subject to the 'N-end rule' that governs protein turnover. The L2A/K-*ftnA* strains were found to exhibit a ~50% increase growth under iron restriction (compared to L2-*ftnA* controls), but not under iron sufficiency. In addition, the L2A/K-*ftnA* strains displayed a 23% increase in cellular iron content. These results are consistent with an increase in cellular iron-stores as a result of raised FtnA levels arising from the L2A/K substitutions. Current experiments are aimed at determining whether FtsH is responsible for the apparent N-end rule dependence of FtnA levels and whether the rate of FtnA turnover is impacted by relevant environmental factors.

P479

The Rtc RNA repair system is linked to antibiotic tolerance in *Escherichia coli*.

Danai Athina Irakleidi ORCID iD, Martin Buck, Ioly Kotta-Loizou

Imperial College London, London, United Kingdom

Abstract

Antibiotic resistance is one of the biggest public health challenges of the 21st century. The Rtc RNA repair system, encoding the RNA end-modifying enzyme RtcA and the RNA ligase RtcB, is present in many pathogenic bacteria, including the model organism and putative pathogen *Escherichia coli*, and may be involved in antibiotic resistance. Pathogenicity assays were performed using the infection model *Galleria mellonella*, to study phenotypes and survival rates following larvae infection with *E. coli* wildtype and variants lacking the *rtc* genes. Larvae infected with wildtype *E. coli* die significantly faster than those infected with *rtc*-lacking mutants. Complementation of *rtc* gene deletions with wildtype Rtc proteins restores the infection phenotype to that of the wildtype. Larvae infected with wildtype bacteria or *rtc*-lacking mutants were then treated, post-infection, with the minimal inhibitory concentration (MIC) of antibiotics that inhibit ribosomal activity. Antibiotic treatment, either with chloramphenicol or tetracycline, has no effect on the survival of larvae infected with wildtype bacteria. Conversely, larvae infected with *rtc*-lacking mutants, and subsequently treated with either antibiotic, display significantly increased survival as compared to the untreated groups. Bacterial burden assays indicate that the survival rate differences observed cannot be explained by changes in bacterial proliferation. Present results suggest that *E. coli* bacteria with a fully functional Rtc system are more aggressive *in vivo* and display characteristics of tolerance against certain RNA-targeting antibiotics. Current findings open a window for further research to understand the ways in which Rtc contributes to virulence through RNA repair and antibiotic resistance, with potential for broader health benefit.

P480

First things first, primary metabolism in Streptomyces: directing carbon flux for specialised metabolite production

Molly Keith-Baker ORCID iD[1], John Munnoch[1], Steve Kendrew[2], Andrew Collis[2], Benjamin Huckle[2], Iain Hunter[1], Paul Hoskisson[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]GSK, Worthing, United Kingdom

Abstract

To combat the antimicrobial resistance crisis there is a need to develop new antibiotics and increase the production of existing antimicrobials. Pyruvate kinase (Pyk) has been shown to be a good target for metabolic engineering to increase antibiotic production. Pyruvatephosphate dikinase (PPDK) usually catalyses the opposite reaction to Pyk and in contrast is reversible and occurs in a three-step process converting pyruvate, ATP and inorganic phosphate into AMP, PEP and diphosphate. This enzyme is unusual in bacteria and enables carbon flux in both the glycolytic and gluconeogenic direction. There are two copies of Pyk in the majority of Streptomyces and two copies of PpdK, indicating there are four potential biochemical routes to converting pyruvate to PEP. There are currently no studies involving the role of PPDK in actinobacteria, however previous work has shown that one of the Pyk enzymes in S. coelicolor is upregulated 30-fold preceding antibiotic production.

Here we investigate the role of PPDK in Streptomyces by creating knockdown mutants using CRISPRi /dCas9 technology and over-expressing of the proteins in WT strains. These strains exhibit changes in growth, developmental phenotypes, and antibiotic titre. We hope this enables us to better understand the role of PPKD in the primary metabolism of streptomycetes and the flow of metabolites around the pyruvate-phosphoenolpyruvate-oxaloacetate node of central metabolism under a range of conditions and to understand how this impacts the availability of precursor molecules for specialised metabolite production.

P481

Polymyxin Resistance and Heteroresistance Are Common in Clinical Isolates of *Achromobacter* Species and Correlate withModifications of the Lipid A Moiety of Lipopolysaccharide

Lewis Macdonald ORCID iD[1], Sean Keenan[1], Flaviana Di Lorenzo[2], Nana Adade[3,1], Dervla Kenna[4], Beverley Millar[1,5], John Moore[1,5], Jose Ramos Vivas[6,7], Antonio Molinaro[2,8], Miguel Valvano[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]University of Naples Federico II, Naples, Italy. [3]University of Ghana, Accra, Ghana. [4]UK Health Security Agency, London, United Kingdom. [5]Northern Ireland Public Health Laboratory, Belfast, United Kingdom. [6]Universidad Europea del Atlantico, Santander, Spain. [7]Instituto de Salud Carlos III, Madrid, Spain. [8]Osaka University, Osaka, Japan

Abstract

The *Achromobacter* genus includes opportunistic pathogens that can cause chronic infections especially in people with cystic fibrosis (PWCF), treatment of which is complicated by antimicrobial resistance. Work from our group has shown that *Achromobacter* survives within phagocytes, causing pyroptosis. In this study, a collection of *Achromobacter* clinical isolates, from CF and non-CF sources, were investigated for polymyxin B (PmB) resistance. Additionally, the effect of PmB challenge in a subset of isolates was examined and the presence of resistant sub-populations within the isolates was described. Further, chemical and mass spectrometry analyses of the lipid A of *Achromobacter* isolates enabled the determination of common structures and showed that PmB challenge was associated with lipid A modifications including the addition of glucosamine and palmitoylation, and the loss of the free phosphate at the C1 position. This study demonstrates that lipid A modifications associated with PmB resistance are prevalent in *Achromobacter*, reflecting the high levels of resistance seen within our isolate collection (66% of isolates with an MIC ≥4μg/mL). We show that sub-resistant populations displaying gain of positively charged residues and additional acyl chains to lipid A can be selected for and isolated from PmB-sensitive *Achromobacter* clinical isolates. The lipid A modifications following PmB challenge and the level of heteroresistance within our collection are particularly troubling due to the increasing use of polymyxins to treat chronic infections of PWCF. This highlights the clinical relevance of our findings, with the potential for infections to re-emerge despite apparently successful treatment as seen in other Gram-negative pathogens.

P482

Analytical approach for regulation of reshuffling of gene encoding enzymes in microbes by hydrogen peroxide

MISBAH Jamil

University of the punjab, lahore, Pakistan

Abstract

Analytical approach in scientific research considering as a basic implementation towards embezzlement retraining in world . Objective of present work constitute gene encoding enzymes interpretation by presumptuous avenue. Constitution of methodology in microbiology based on series of seclusion of behaviour of general pattern in microbes. As different species belong to various families show control by input of chemicals Constitution. Like water activity ,growth factors mechanics with time intervals. In this assays hydrogen peroxide considering as mode of renovation as hydrogen peroxide plays pivotal role in controlling behaviour of gene that was diversified with comparison of different strains with exposure. Na2S2o3 ,KH2PO4 ,NH4CL, MgCL2.4H2O, Ampiciiln, leucine, glycine, tryptophan , glucose , water , biotin, thiamine Hcl , citrate,Na2H2O , CaCl2, EDTA , K2HPO4, KH2PO4 was imposed with various concentration for analysing output protractans. Regulation of perspective assays was control by optimization of general condition with elution of protraction with factors of representation. As catalytic applications in ecoli or bacillus in circulation of mechanics approach certain parameter was taken into consideration by imposing comparison like chemicals activity arise from carbon, glucose, acetate ammonia, sulfate, inorganic phosphate, like hydrolysis of p nitophenyl in pseudomonas , as behaviour of morphology of bacillus show restrictions as a inhibitory factors on peptidoglygen hydrolysis as compatiliblity of ca,cl, k,Na or mg present in organic matter as repression in ecoli . The aspirations of present deliberation confiscated towards evolution of enzymatic Regulation by reshuffling of ions by elaboration of sense of new bioactive pharmacological compounds for compensation of drugs complement.

P483

Identification of a hypervariable genomic locus involved in cell surface modification across the genus Clostridium

Orlagh Anderson ORCID iD, Emily Flack, Tessa Keenan, Martin Fascione, James Chong, Gavin Thomas

University of York, York, United Kingdom

Abstract

Although *O*-linked modification of flagellin is well characterised in Gram-negative bacteria, with established roles in flagellar assembly, motility and virulence, reports of this process in Gram-positive bacteria are limited. During a comparative genomic analysis of *Clostridium butyricum* strains, a hypervariable region (HVR) of the genome was identified within the flagellar biosynthetic locus. Although the size and genetic content of this region differs considerably across strains, the presence of genes encoding several putative glycosyltransferases and sugar modification enzymes is a common feature of this region, suggesting a role in flagellar glycosylation. In several pathogenic strains this region is predicted to encode genes for the biosynthesis of the nonulosonic acids pseudaminic and legionaminic acid, based on homology to the well characterised components of these pathways in *Campylobacter jejuni*. Expansion of this study revealed that the HVR observed in *C. butyricum* is a conserved feature of the genus *Clostridium*. Moreover, in more than one third of the 50 *Clostridium* species included in this analysis, the HVR encodes putative pseudaminic acid biosynthesis genes, suggesting widespread use of these molecules in Gram +ve pathogens in addition to the established Gram –ve systems.

P484

Investigating the functional redundant peptidoglycan synthases PBP1A and PBP1B, their preferred major peptidoglycan hydrolases in Escherichia coli.

Xuyu Ma ORCID iD, Gabriela Boelter ORCID iD, Manuel Banzhaf ORCID iD, Dema Alodaini ORCID iD, Hannah Doherty ORCID iD, Micheal Alao ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Peptidoglycan (PG) is an essential, mesh-like structure that can be found in bacteria. It consists of polysaccharide strands which are crosslinked by short peptides. It surrounds and encases the cytoplasmic space to maintain bacterial cell shape and protects bacterial cells from environmental stresses. In Escherichia coli, PG synthesis needs to be precisely regulated to prevent lysis by PG hydrolases. Either PG synthases penicillin-binding protein 1A (PBP1A) or PBP1B is essential for PG cell wall assembly during cell elongation and cell division. PG endopeptidases and amidases are pivotal for peptide stem cleavage during PG cell wall expansion and daughter cell separation. Despite their importance for the bacterial cell cycle, it is still unclear how PG synthases and hydrolases cooperate with each other during growth. It is not known if PG hydrolases have a preferred major PBP synthase. In this study, we try to answer this question by probing the genetic interactions PBP1A and PBP1B with the PG endopeptidases (MepH, MepS, MepM, PBP4, PBP7) and PG amidases (AmiA, AmiB, AmiC) and their regulators (EnvC, NlpD) under envelope stress conditions. Here, we reported that under no salt condition, the gene mepS (encoded PG endopeptidase MepS) and mrcB (encoded PG synthase PBP1B) are sick. This results hints, that PBP1A requires the present of MepS in no salt condition to maintain optimal growth. Moreover, the double deletion mutant of amidases regulator envC and mrcB show synthetic lethality by CRISPRi gene knockdown. This indicates that PBP1A functionality requires amidases regulator EnvC.

P485

Community-level metabolic functionality of the preterm gut microbiome and associations with necrotising enterocolitis

Jonathan Chapman ORCID iD[1], Toby Brown[2], Andrea Masi ORCID iD[1], Nicholas Embleton ORCID iD[3], Janet Berrington ORCID iD[3], Christopher Stewart ORCID iD[1]

[1]Newcastle University, Newcastle Upon Tyne, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]Newcastle Hospitals NHS Trust, Royal Victoria Infirmary, Newcastle Upon Tyne, United Kingdom

Abstract

Preterm infants born <32 weeks gestation are 'immature' physiologically, anatomically and immunologically. Around 10% will develop necrotising enterocolitis (NEC), an inflammatory mediated bowel disease. NEC causes more deaths in childhood than leukaemia and lymphoma in the UK. Abnormal gut microbiome development is integral to NEC pathogenesis. NEC infants have reduced microbial diversity, increased Proteobacteria (gram-negative), and reduced Bifidobacterium (gram-positive). Metagenomic and 16S amplicon sequencing approaches can identify the bacterial species present within an infant's gut microbiota, allowing differences in taxonomic abundances and diversities between NEC and non-NEC to be identified. However, these approaches do not allow for functional differences between microbiomes to be assessed.

To advance this understudied area, in on-going work we are investigating the metabolic functionality of gut microbiome communities present in neonatal stool samples using Biolog AN Microplate assays. These assays allowed us to observe and quantify the utilisation of a range of 95 carbon sources by the gut microbiota and compare metabolic activity between infants with NEC and matched controls. Such work may detect associations between gut microbiome functionality and disease development that completement and expand upon sequencing-based approaches. Compared to molecular biomarkers, bacterial metabolic pathways found to be associated with NEC could be developed more readily into point-of-care biomarkers. Accurate diagnosis of NEC is difficult and the disease progresses rapidly, meaning there is an urgent need for novel biomarkers for the disease and potential assays to inform precision medicine therapeutics.

P486

Unfolding the molecular chaperone function of the atypical Mycobacterial chaperonins

Bakul Piplani ORCID iD[1], C. M. Santosh Kumar ORCID iD[2,3], Peter Lund ORCID iD[2,3], Tapan Chaudhuri ORCID iD[1]

[1]Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi, India. [2]School of Biosciences, University of Birmingham, Birmingham, United Kingdom. [3]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

Chaperonins are ubiquitous oligomeric nanomachines vital for cellular proteostasis. Bacterial chaperonins are well known for their characteristic double-ring assembly central to their ATP-dependent protein-folding function. The pathogenic bacterium *Mycobacterium tuberculosis* encodes two paralogous chaperonin proteins, MtbCpn60.1 and MtbCpn60.2. Both the MtbCpn60 proteins share substantial sequence similarity with each other and with GroEL, the prototypical bacterial chaperonin from *Escherichia coli* that assists the folding of about 10-15% of the cellular proteome. However, their roles in cellular proteostasis are not well understood. While previous studies have shown that MtbCpn60.2 can functionally replace GroEL in *E. coli*, the function of MtbCpn60.1 remained an enigma. Here we investigate this conundrum using cell-based assays employing a GroEL-deficient *E. coli* model. We demonstrate that both MtbCpn60.1 and MtbCpn60.2 certainly function as molecular chaperones. We found that MtbCpn60.1 has a 'holdase-like' chaperone function, prevents client protein aggregation, and consequently supports cell survival and division. Furthermore, we found that MtbCpn60.2 is a true 'foldase' chaperonin that assists in client folding, and hence supports cell growth and proliferation. Our findings suggest distinct functional mechanisms for MtbCpn60.1 and MtbCpn60.2, with the latter presenting as a GroEL-like canonical chaperonin and the former offering augmented conformational protection. We hope that our study will serve as the starting point for in-depth research into the varied functional mechanisms of bacterial chaperonins. Moreover, our study may aid the design of simplified assays to evaluate the efficacy of small molecule chaperonin-inhibitors as antimicrobials against bacterial pathogens in general, and *M. tuberculosis* in particular.

P487

Discovery novel antimicrobial targets: Uncovering a multicomponent signalling network in *Burkholderia pseudomallei*

Matt Scurlock

University of Exeter, Exeter, United Kingdom

Abstract

Burkholderia pseudomallei causes melioidosis, an emerging tropical disease responsible for 90,000 deaths per year globally. New treatment strategies are needed to lower the mortality rates of melioidosis, which can exceed 40% even with antimicrobial treatment. One new antimicrobial approach is to target the molecular mechanisms by which bacteria sense and respond to their environment, with the rational being that disrupting appropriate signalling should leave them in a compromised disposition. Here, we have developed a bioinformatical method which can accurately predict complex signalling networks. These networks control the critical lifestyle choices of bacteria and therefore should make effective novel antimicrobial targets. In *B*. *pseudomallei* specifically, we reveal a novel signalling network comprised of 4 histidine kinases and 4 response regulators which link chemotaxis to c-di-GMP metabolism. Deletion of components of this network massively altered growth rates, biofilm formation, swimming motility and nitric oxide sensitivity. This network should therefore make for an effective antimicrobial target against *B*. *pseudomallei*. Our bioinformatical method can now be applied to predict novel signalling networks in other fully sequenced pathogens, and therefore also new antimicrobial targets against them.

P488

Differential expression of key systems underpins colony biofilm formation in *Clostridioides difficile*

Megan G. Taggart[1], Barry M. G. O'Hagan[1], William J. Snelling[1], Patrick J. Naughton[1], Roberto M. La Ragione[2], James S. G. Dooley[1], Nigel G. Ternan[1]

[1]Ulster University, Coleraine, United Kingdom. [2]University of Surrey, Coleraine, United Kingdom

Abstract

Clostridioides difficile is the most common bacterial healthcare-associated infection in the USA (CDC 2019) and disease recurrence has been linked to biofilm formation in the gut. In previous studies, disruption of the key chaperone gene, *dnaK,* by ClosTron mutagenesis yielded a DnaK deficient strain (630Δ*erm:dnaK*) which exhibited 50 % cell elongation, increased cell hydrophobicity, and increased biofilm production (Jain et al. 2011).

Here, we used a colony biofilm model to investigate gene expression in 24 h old *C. difficile* biofilms and in planktonically cultured cells using mutant strain (630Δ*erm:dnaK*), parent strain (630Δ*erm*) and wild-type (630). RNA sequencing revealed that the *dnaK* operon expression was significantly decreased (up to 14.5-fold) in 630 and 630Δ*erm* biofilms ($p < 0.001$). However, in the *dnaK* mutant (630Δ*erm:dnaK*) biofilm only very modest changes in gene expression were observed for the *dnaK* operon. Thus, unlike 630 and 630Δ*erm* biofilms, the chaperone genes in the dnaK mutant exhibit much less extreme expressional changes when the organism grows as a biofilm.

More generally, biofilm transcriptomes showed a significant decrease in motility gene expression while the majority of sporulation associated genes were increased in expression, some by >1000 fold. A large number of cell wall function related genes exhibited both increased and decreased expression, revealing a shift in cell wall architecture related to the biofilm mode of life.

Further studies will focus on elucidating the core genes involved in biofilm formation in *C. difficile*, and should facilitate for the identification of key biochemical pathways underpinning biofilm development.

P489

P. aeruginosa strains isolated from chronic wounds displayed elevated AaaA activity, reinforcing that this virulence factor is important for the formation of chronic biofilms.

Bethan Roberts ORCID iD[1], Claire Laxton[1], Ana Da Silva[2], Chris Penfold[1], Kim Hardie[1]

[1]BDI University of Nottingham, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Pseudomonas aeruginosa has intrinsic multi-drug resistance and forms chronic infections in immunocompromised individuals that are difficult to eradicate, resulting in high patient mortality and a global burden of disease from this versatile Gram-negative bacterium. *P. aeruginosa* is the leading cause of death following infection of the lungs of people with Cystic Fibrosis and is one of the main bacteria associated with chronic wound infections. This project aims to determine the role in pathogenesis of one of the virulence factors made by *P. aeruginosa* that is crucial for the development of chronic infections (AaaA). AaaA is an arginine specific aminopeptidase tethered to the surface of *P. aeruginosa*, but the function it has during the establishment of the coordinated biofilm communities that *P. aeruginosa* forms in chronic infections is unknown. This project recreates biofilms within a collagen-based synthetic chronic wound (SCW) model, using a variety of clinical isolates isolated from chronic wounds, to track AaaA activity and discern its contribution to *P. aeruginosa* infections. Biofilms grown in the SCW model are disrupted to remove the collagen-matrix, and the level of AaaA activity is quantified using our optimised assay to determine if there is a significant difference between WT and mutant strains. Results showed a range of AaaA activity within the different clinical isolates when grown in planktonic conditions. However, when the clinical isolates are grown within the SCW model levels of AaaA activity are significantly higher further supporting the theory that AaaA is important in chronic infections.

P490

The regulation of the phosphorous stress response in Burkholderia cenocepacia

Isabel Aberdeen[1], Holly Shropshire[1], Richard Guillonneau[1], Miguel A Valvano[2], David Scanlan[1], Yin Chen[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

Burkholderia cenocepacia is an opportunistic pathogen of the Burkholderia cepacia complex, which is highly successful in colonising the respiratory tract of immunocompromised individuals. Phosphorus is an essential nutrient required for membranes and multiple cellular processes. As an intracellular pathogen, B. cenocepacia likely encounters phosphorus limitation within innate immune cells during infection. The Pho regulon, which manages the phosphorus stress response, is not well understood in B. cenocepacia. This project focuses on the two-component regulator of the Pho regulon, PhoBR.

Wild type B. cenocepacia and an insertional inactivation mutant of the phoR component were challenged with phosphate replete (1 mM) or deplete (0 mM) conditions. Comparative proteomics identified that multiple proteins involved in the response to phosphate limitation were highly induced in the wild type. This included proteins directly involved in the phosphate stress response, such as PhoBR, and proteins associated with virulence. These protein expression changes were not observed in the phoR mutant. To investigate the role of PhoBR in virulence, Galleria mellonella were infected with either wild type or the phoR mutant. Unexpectedly, the phoR mutant was able to establish infection in the Galleria, like the wild type. This suggests that the regulation of the phosphate stress response may involve additional nutrient stress responses during infection, as part of a complex regulatory network. Current work focuses on investigating other proteins that may interact with PhoBR using lipidomics, comparative proteomics and macrophage infection models. This will help to further understand how B. cenocepacia can overcome nutrient limitation during infection.

P491

A genome wide analysis of heat survival mechanisms of *Escherichia coli*

Muhammad Yasir ORCID iD[1], Keith Turner[1], Megan Truong[2,3], Sarah Bastkowski[1], Mark Webber[1], Ian Charles[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Quadram Institute, Norwich, United Kingdom. [3]University of Sydney, Sydney, Australia

Abstract

Background

Escherichia coli is a cause of many food or waterborne infections and heat is a common method of protecting foodstuffs from being contaminated. We examined how *E. coli* can adapt to higher temperature and whether it can be 'trained' to develop increased temperature tolerance.

Methods

We used 'TraDIS-Xpress' which assays a large transposon library incorporating outward-transcribing inducible promoters allowing both gene inactivation and modulation of transcription to be scored. We tested responses to four different temperature levels ranging from 37°C to 50°C and we also assayed genes allowing growth at 47°C and 50°C after initially sensitising cells at 44°C.

Results

We identified genes encoding the cell envelope, DNA repair and chaperone proteins involved in protein misfolding and aggregation as important for survival at high temperatures. Besides known mechanisms, we also found mutations in metabolic genes that are not reported before for high temperature tolerance. We found genes with mutations in protein export, secretion system and nucleoside metabolism were important at 47°C and 50°C but after sensitisation, mutations in amino acid metabolism genes are important which were not otherwise identified.

Conclusion

This data shows multiple mechanisms of survival at high temperature including the known mechanisms and that how temperature stress is applied are both important for *E. coli* to adapt to heat stress.

P492

An Azobenzene G-quadruplex Ligand Exhibits Promising Antibacterial Activity against *Escherichia coli.*

Yuiko Takebayashi, Javier Ramos-Soriano, Jenny Samphire, Michael O'Hagan, Catherine Gurr, Kate Heesom, Philip Lewis, James Spencer, M Carmen Galan

University of Bristol, Bristol, United Kingdom

Abstract

With the rise in bacterial antimicrobial resistance (AMR) and decline in antibiotic discovery, global healthcare is at a point of jeopardy. There is great need for the development of novel antimicrobials that target bacteria that have become resistant to existing antibiotics, in particular Gram-negative species such as *Escherichia coli*. Here we demonstrate that a novel pyridinium-functionalised azobenzene scaffold L20, identified as a candidate ligand able to target G-quadruplex (G4) structures in bacterial genomes, shows promising antibacterial activity (MIC values ≤ 4 mg/ml) against multi-drug resistant *E. coli*. Tandem Mass Tag (TMT) proteomics applied to cultures of the *E. coli* type strain ATCC 25922 treated with sub-lethal concentrations of L20, identified G4-containing sequences as potential targets for L20. Fluorescence resonance energy transfer (FRET) stabilisation assays indicate L20 binds these selected sequences with variable and moderate affinity, in contrast to two comparator G4 ligands (stiff-stilbene L5 and pyridostatin (PDS)) that better stabilise G4 structures but exhibit a lower antimicrobial activity. However, proteomic experiments also reveal that, alongside its superior antibacterial activity, L20 treatment influences expression levels of more G4-associated proteins than either L5 or PDS, and upregulates multiple essential proteins involved in translation. These findings identify strategies discovering potential G4 ligands as approaches that can lead to antibacterial candidates active against priority targets such as multi-drug resistant *E. coli*, and that targeting G4 sequences, and ligands such as L20, warrant further exploration as potential novel therapeutics with G4-mediated mechanisms of action.

P493

Effects of fungal physiology and metabolism on lignocellulose composition and structure during its degradation.

Shuqi Shen[1], Thu Vuong[2], Paul Knox[3], Emma Master[2], Jolanda van Munster[1]

[1]Scotland's Rural College, Edinburgh, United Kingdom. [2]University of Toronto, Toronto, Canada. [3]University of Leeds, Leeds, United Kingdom

Abstract

Understanding effective biodegradation of lignocellulose is essential to advancing renewables-based biotechnology. Similarly, in livestock farming, lignocellulose degradation by the rumen microbiome is essential for feed digestion, and therefore critically impacts ruminant farming. As producers of effective carbohydrate active enzymes, fungi are highly important in both areas. Our insight in the fungal physiology and metabolism relating to lignocellulose degradation is accelerating. However, to fully understand fungal degradative mechanisms, we now need to combine this with knowledge of the effects that fungi and their enzymes have on the composition and structure of the actual complex lignocellulose substrates.

We previously investigated how industrial work-horse *Aspergillus niger* regulates gene expression and enzyme secretion in response to lignocellulose (PMID 32313551, 28184248). We then investigated how lignocellulose composition and structure changed after exposure to this fungus, to create a full picture of its degradative mechanism. We are now comparing the degradative activity of *A. niger* with that of anaerobe rumen fungi, as both fungi display stark differences in physiology (aerobe vs anaerobe) and degradative mechanism (soluble enzymes vs enzyme complexes). We employed complementary techniques to assess changes caused in the lignocellulose matrix, including fiber analysis and glycoprofiling to assess changes in polysaccharides, and mass spectrometry-based imaging to identify surface exposure of lignin and polysaccharides. We identified differences in lignocellulose degradation efficiency between fungal species as well as distinct patterns in degradation of hemicelluloses and cellulose. We will contrast the effects of the degradative activity of the different fungi, and highlight how these may be exploited.

P494

Charge Reversal Mutant in Periplasmic Domain of YidC suggests a role in OMP biogenesis

Sravanthi Veerla, Damon Huber, Max Wynne, Prizam Sandhu, Sarah Vael

University of Birmingham, Birmingham, United Kingdom

Abstract

YidC is an essential component of the bacterial Sec machinery, which is responsible for transporting a subset of newly synthesised proteins across (or inserting them into) the cytoplasmic membrane. In Escherichia coli, YidC contains two domains: an integral cytoplasmic membrane domain and a soluble periplasmic domain. The membrane domain is evolutionarily conserved in bacteria and bacterially derived organelles of eukaryotes and facilitates the insertion of a subset of proteins into the cytoplasmic membrane. However, the function of the periplasmic domain is not well understood. To gain insight into the potential function of the periplasmic domain, we determined the phylogenetic distribution and sequence conservation of the periplasmic domain. These studies suggested a role in outer membrane biogenesis and identified a conserved pocket located at the interface with the membrane domain. The incorporation of an unnatural amino acid that can produce covalent bonds with adjacent molecules into this pocket suggested resulted in adducts with many outer membrane proteins. Charge reversal substitutions in a conserved structural loop that protects this pocket disrupted the formation of these crosslinks and caused a defect in outer membrane biogenesis in vivo. These results are consistent with recent reports that YidC interacts with the β-barrel assembly machinery (BAM) and suggests that YidC plays an important role in outer membrane protein biogenesis.

P495

Understanding the Regulatory Mechanisms that Underpin Specialised Metabolite Production

Ainsley Beaton, Tom McLean, Barrie Wilkinson, Matt Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

Streptomyces bacteria are ubiquitous in nature and produce many specialised metabolites, but their functions are not well understood. Understanding the regulation of specialised metabolism in response to environmental signals could be key to the discovery of new molecules because these bacteria encode 10x more specialised metabolites than they make under laboratory growth conditions. Two-component systems (2CS) are common signal transduction pathways in bacteria that sense and respond to changing environmental conditions.

The CutRS 2CS is highly conserved in the genus Streptomyces and is known to affect the production of specialised metabolites, with deletion of the cutRS genes increasing the production of the redox-active antibiotic actinorhodin in S. coelicolor. Our ChIP-seq and proteomics data suggest a link between protein secretion stress and CutRS. The only conserved residues in the extracellular sensor domain of all CutS homologues are two cysteines which may be important in sensing disulphide bond formation and correct folding of secreted proteins. Substitution of these leads to a change in the function of the CutRS system that suggests they are essential for CutRS functionality. Our results suggest that CutS senses extracellular stress via conserved dual cysteine motif in the extracellular domain which leads to changes in the regulation of genes involved in secretion stress and specialised metabolite production. We hypothesise that loss of CutRS induces actinorhodin production because this redox active molecule can oxidise cysteine residues and rescue the defect in extracellular protein folding.

P496

A novel phage shock protein system in the genus *Clostridium*

Benjamin Willson ORCID iD, Reyme Herman ORCID iD, Gavin Thomas ORCID iD

University of York, York, United Kingdom

Abstract

The phage shock protein PspA and its homologs have been implicated in the maintenance of the cell envelope under stress conditions and appear to be involved in both membrane maintenance and remodelling. Although PspA homologs are widely distributed, different groups of bacteria have recruited different accessory proteins to modulate the activation and expression of PspA. From multi-omics analyses that we have carried out on the industrial solvent-producing bacterium *Clostridium saccharoperbutylacetonicum* grown in the presence of inhibitory compounds, we identified a PspA homolog that was upregulated in the presence of butanol and furfural, and which is cotranscribed as a three-gene operon that includes genes for a membrane protein ('PspX') and an unknown soluble protein ('PspY') that appear to comprise a novel clostridial Psp system. Subsequent genomic analyses have identified putative homologs of these proteins throughout the clostridia and related genera, including other solvent producers such as *Clostridium acetobutylicum*, gas-fermenting species like *Clostridium kluyveri*, and even pathogenic species such as *Clostridium botulinum* and *Clostridium perfringens*.

P497

The lipoprotein DolP affects cell separation in *Escherichia coli*, but not as an upstream regulator of NlpD

Gabriela Boelter[1], Jack A. Bryant[1], Hannah Doherty[1], Peter Wotherspoon[1], Dema Alodaini[1], Xuyu Ma[1], Micheal Alao[1], Patrick Moynihan[1], Danesh Moradigaravand[1], Monika Glinkowska[2], Timothy Knowles[1], Ian R. Henderson[3], Manuel Banzhaf[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Gdansk, Gdansk, Poland. [3]University of Queensland, Brisbane, Australia

Abstract

Bacterial amidases are essential to split the shared envelope of adjunct daughter cells to allow cell separation. Their activity needs to be precisely controlled to prevent cell lysis. In Escherichia coli, amidase activity is controlled by three regulatory proteins NlpD, EnvC and ActS. However, recent studies linked the outer membrane lipoprotein DolP (formerly YraP) as a potential upstream regulator of NlpD. In this study we explored this link in further detail. To our surprise DolP did not modulate amidase activity in vitro and was unable to interact with NlpD in pull-down and MST (MicroScale Thermophoresis) assays. Next, we excluded the hypothesis that Δ*dolP* phenocopied Δ*nlpD* in a range of envelope stresses. However, morphological analysis of double deletion mutants of amidases (AmiA, AmiB AmiC) and amidase regulators with *dolP* revealed that Δ*amiA*Δ*dolP* and Δ*envC*Δ*dolP* mutants display longer chain length compared to their parental strains indicating a role for DolP in cell division. Overall, we present evidence that DolP does not affect NlpD function *in vitro*, implying that DolP is not an upstream regulator of NlpD. However, DolP may impact daughter cell separation by interacting directly with AmiA and AmiC, or by a yet undiscovered mechanism.

P498

Bacteroides thetaiotaomicron Extracellular Vesicles: Biogenesis, Functionality and Engineering

Rokas Juodeikis, Emily Jones, Simon Carding, Martin Warren

QIB, Norwich, United Kingdom

Abstract

Our work revolves around extracellular vesicles produced by the commensal gut bacterium Bacteroides thetaiotaomicron. Recently we demonstrated that these extracellular vesicles function as chelating agents for vitamin B12 playing an active role in its uptake while sequestering it from other organisms. Significantly, this activity may be important in the bioavailability of the vitamin in the gut thereby having a direct impact on health.

We present further work on this topic, in particular the investigation into the specific proteins involved in this activity, both on the binding of the vitamin to the vesicles as well as the requirements for bacterial uptake of the bound vitamin. For this work we utilise comparative proteomics, generation of mutants and a variety of bioassays. Furthermore, we utilise fluorescently labelled vitamin B12 analogues to investigate vesicle binding capacity.

In addition, we discuss our work on B. thetaiotaomicron vesicle release dynamics and their bioengineering.

P499

Towards solving the mystery of the B12-dependent rSAM enzymes involved in vitamin B12 biosynthesis.

Amira Abood ORCID iD[1], Andrew Lawrence ORCID iD[2]

[1]University of Kent, kent, United Kingdom. [2]University of kent, kent, United Kingdom

Abstract

Vitamin B12 is a critical dietary cofactor that is required for human life. The presence of the lower ligand, 5,6-dimethylbenzimidazole (DMB), is required for its biological activity. However, only a few prokaryotes and archaea can synthesise this molecule. We have worked towards the identification of BzaD and BzaE enzymes that are responsible for DMB synthesis anaerobically. Bioinformatic analysis showed that they are cobalamin-dependent radical SAM enzymes. These enzymes are significant players in the biosynthesis of many important natural products including antibiotics and anticancer agents. To fully understand their mechanism of action, they have been recombinantly overexpressed in E. coli and purified anaerobically by IMAC. Both proteins were soluble and appeared brownish-yellow, indicating the presence of a Fe-S cluster. In vivo activity proved their involvement in DMB synthesis as the cells are able to produce vitamin B12. In vitro activity has been investigated against the proposed substrates or their cobamide derivative. These substrates were biosynthesised using an engineered E. coli strain and the product was purified using a B12 binding protein (BtuF) followed by reverse phase chromatography to yield a pure product. This was chemically verified by HPLC-MS. our initial results indicate that BzaD carries out non-radical-based SAM-dependent methylation while BzaE follows a radical SAM-based mechanism. Characterization of these enzymes will help in understanding the enigma of the apparent requirement for vitamin B12 in its own biosynthesis and the chemical versatility of rSAM enzymes that are crucial in the synthesis of natural products.

P500

LpqF is a low molecular weight penicillin binding protein that contributes to lateral wall homeostasis in mycobacteria.

Clare Thomas ORCID iD[1], Rudi Sullivan ORCID iD[1], Abigail Layton[1], Jiemin Chen[1], Ian Cadby ORCID iD[2], Gurdyal Besra ORCID iD[1], Apoorva Bhatt ORCID iD[1], Manuel Banzhaf ORCID iD[1], Andrew Lovering[1], Patrick Moynihan ORCID iD[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Species of mycobacteria, which kill over 1.5 million people annually, have a complex cell wall contributing to their resistance to many front-line antibiotics. The inner-most layer of the cell wall is made of peptidoglycan which is comprised of a repeating disaccharide of N-acetylglucosamine and N-acetylmuramic acid. These glycan chains are cross-linked by short peptides, creating a mesh-like sacculus. To investigate genes responsible for cell envelope biosynthesis, we conducted a transposon-mediated colony morphology screen in Mycobacterium marinum. One of the hits was mapped to a previously uncharacterised gene predicted to encode a penicillin-binding-protein (PBP) called LpqF. Here we show that a strain of Mycobacterium marinum lacking lpqF produces longer cells with increased septation. We have also solved the X-ray crystal structure of M. tuberculosis LpqF showing that it is comprised of a classical penicillin-binding-protein domain and an NTF2 domain which hints at an unusual mode of auto-inhibition. Given its structural similarity to penicillinases, we sought to clarify the biochemical function of LpqF. Our assays show that LpqF is not a beta-lactamase and that the protein binds peptidoglycan directly through its NTF2 domain, likely through a distinct mechanism from other peptidoglycan-binding NTF2 domains. Together, our data show that LpqF plays an important, but previously unknown role in mycobacterial peptidoglycan biogenesis.

P501

Avian pathogenic and Uropathogenic *Escherichia coli* are capable of cytotoxic activity and invasion of avian intestinal, human intestinal and human bladder cell lines

Sarah Johns, Diane Newell, Arnoud Van Vliet, Roberto La Ragione

University of Surrey, Guildford, United Kingdom

Abstract

In humans and canines, uropathogenic *E. coli* (UPEC) are important causes of urogenital infections. In contrast, avian pathogenic *E. coli* (APEC) can cause systemic infections and high morbidity and mortality in poultry. Although, the zoonotic potential of these *E. coli* pathotypes is not fully understood, the genomic similarities between UPEC and APEC suggest the potential for zoonotic infections. This risk has been investigated by comparing the bacterial-host cell interaction between genetically similar APEC and canine and human UPECs and the cell lines 8E11 (avian intestine), HT29 (human colon) and 5637 (human bladder).

Three human UPEC, three canine UPEC and three APEC isolates were selected from phylogroup B2 based on their genomic similarity. Quantitative adhesion, invasion and cytotoxicity cell culture assays were performed using the different cell lines. Electron microscopy was used to visualise bacterial interaction with the avian cell line (8E11).

There was no significant difference between the capacity of the bacterial isolates to adhere to, or invade, the different cell lines, supporting genomic evidence for the potential of cross-host infection. Nevertheless, some host-specific interactions were observed. For example, 8E11 cells appeared more susceptible to invading bacteria. However, all the *E. coli* isolates were more cytotoxic to the human cell lines (HT29 and 5637) than to the avian cell line (8E11), which might explain this observation. Interestingly, scanning electron microscopy showed that some bacteria could form small cell surface bound colonies. Such cell-bound extracellular colonies appeared to induce morphological changes, suggesting a host cell response to the bacteria.

P502

The role of the Gut Microbiota and Anti-Neutrophil Cytoplasmic Autoantibodies (ANCA) in the pathobiology of Canine Cutaneous and Renal Glomerular Vasculopathy

Jack Whitehouse[1], Mark Chambers[1], David Walker[2], Joy Leng[1], Diane Lee[1], Josh Walker[2], Naomi Creelman[2], Roberto La Ragione[1]

[1]University of Surrey, Guildford, United Kingdom. [2]Anderson Moores Veterinary Specialists, Winchester, United Kingdom

Abstract

Canine Cutaneous and Renal Glomerular Vasculopathy (CRGV) is an idiopathic, often fatal disease characterised by skin lesions, and in many cases, acute kidney injury (AKI). The aetiology of CRGV is currently unknown, hindering the development of effective interventions. However, a number of bacterial pathogens have been proposed to be associated with CRGV. Vasculitis of the glomerular arteries, reminiscent of anti-neutrophil cytoplasmic autoantibody (ANCA) associated vasculitis (AAV) is evident on examination of tissues post-mortem. In this study, the faecal microbiota composition of healthy dogs and those suffering from AKI and CRGV was examined. Serum was obtained from the same three cohorts to evaluate whether ANCA may play a role in CRGV. The serum was tested by capture enzyme-linked immunosorbent assays for the presence of c-ANCA or p-ANCA. The microbial community profile of dogs suffering from CRGV revealed differences in the structure when compared to healthy dogs, with increases in the bacterial families Enterococcaceae and Enterobacteriaceae in the diseased dogs. While the immunological profiles revealed a significant difference in the antibody titre of both c-ANCA and p-ANCA between healthy dogs, and those suffering from AKI and CRGV ($p < 0.05$). The findings from this study may offer a possible predictive biomarker that could contribute to the early and accurate diagnosis of CRGV and the development of an effective intervention, while also providing a potential explanation for the pathology observed in CRGV. This is the first study to characterise the gut microbiota and presence of ANCA in dogs suffering from CRGV.

P503

Production of acyl-homoserine lactones (AHLs) is conserved across members of the genus *Hafnia*

A. Kate Fala ORCID iD[1,2,3], Nigel Halliday ORCID iD[4], Miguel Cámara ORCID iD[4,5], Avelino Álvarez-Ordóñez ORCID iD[6], Alain Filloux ORCID iD[7], Cormac Gahan ORCID iD[8,2,9], Paul Cotter ORCID iD[10,11]

[1]APC Microbiome Ireland, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland. [3]Teagasc Food Research Centre, Fermoy, Ireland. [4]School of Life Sciences, University of Nottingham Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom. [5]The National Biofilms Innovation Centre, University of Nottingham, Nottingham, United Kingdom. [6]Department of Food Hygiene and Technology and Institute of Food Science and Technology, Universidad de León, León, Spain. [7]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore. [8]APC Microbiome Ireland, University College Cork, Cork, Ireland, Cork, Ireland. [9]School of Pharmacy, University College Cork, Cork, Ireland. [10]APC Microbiome Ireland, University College Cork, Cork, Ireland. [11]Food Bioscience Department, Teagasc Food Research Centre, Fermoy, Ireland

Abstract

The genus *Hafnia* comprises Gram-negative bacteria commonly isolated from the environment, particularly foods and the gastrointestinal tract; their proposed use as adjuvant cultures and probiotics draws contrast with their associations in the literature with food spoilage and opportunistic pathogenesis. Collective phenotypes, such as secretion of extracellular enzymes and virulence, are frequently regulated by quorum sensing and while some individual *Hafnia* strains have been characterised for their capacity to produce and detect acyl-homoserine lactone (AHL) quorum sensing molecules, a broad genus-wide screen has not been performed to date.

LC-MS analysis was performed to determine the types and concentrations of AHLs produced by a panel of 24 *Hafnia* strains, consisting of 14 *H. alvei*, 9 *H. paralvei* and 2 *H. proteus* strains. Comparative genomic analysis was performed on the whole genome sequencing data and using available metadata for the isolates. AHL production was found to be almost universally conserved amongst the isolates. Oxo-C6-HSL (homoserine lactone) was the major AHL, with smaller concentrations of oxo-C8-HSL, C4-HSL and C6-HSL also detected. *H. alvei* PCM1196 was the sole isolate which did not produce detectable levels of C4, oxo-C6 or oxo-C8-HSL. This isolate was found to have a 12kbp chromosomal deletion of a region containing an AHL synthase gene *halI* as well as its cognate transcriptional regulator *halR*.

Overall, AHL production is widespread and generally conserved amongst the genus *Hafnia*, with minor differences observed between species and environmental source. The impact and significance of QS-proficiency across the genus is identified as an area for future study.

P504

Structural basis for ABC transporter-regulated histidine kinase sensing of antibiotics

Martin McAndrew ORCID iD[1], Deborah Brotherton[1], Jonathan Cook ORCID iD[1], Susanne Gebhard[2], Phillip Stansfeld ORCID iD[1], Allister Crow ORCID iD[1]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Bath, Bath, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a growing challenge facing global healthcare systems and is at risk of undermining the many advances in modern medicine. Many Gram-positive bacteria use a multiprotein complex composed of an ABC transporter, histidine kinase and a DNA response regulator to sense, and provide resistance to, antibiotics such as vancomycin, nisin and bacitracin. In these systems, resistance is conferred by the activity of the ABC transporter which is thought to prise apart complexes formed between each antibiotic and their lipid targets. The ABC transporter also communicates its activity directly to the kinase which can further upregulate the expression of the transporter via activation of the response regulator. While structures of the ABC transporter are known, molecular details of the resistance mechanism and the process by which it communicates with the histidine kinase are incompletely understood. Here, we have used a combination of structural techniques and simulations to explore how these transporters facilitate signalling and resistance in the presence of antimicrobial agents.

P505

Using the Spycatcher-spytag system for determining surface exposure of proteins

Rachael Duodu, Jack Leo ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Understanding protein localization is important as it helps with the understanding of the function of individual proteins as well as cell organization. Reporter fluorescent proteins have been commonly used to label outer membrane proteins to study their expression, secretion, interactions, etc. However, their use is challenging when investigating secreted proteins as many reporter proteins unsuccessfully mature within the periplasm and may impede the secretion process. Therefore, we have employed the Spycatcher (SC) -Spytag (ST) system. This is a versatile and efficient bioconjugation system consisting of the 15 kDa SC protein which recognizes its cognate 13- amino acid ST (AHIVMVDAYKPTK) to form a covalently bonded isopeptide. The system started off as a method of protein ligation and has since gone on to be employed in protein labelling and in vaccine modular production among other applications.

We have used the SC-ST system to determine the surface exposure of proteins on the outer membrane of Gram-negative bacteria. To this end, we fused green fluorescent protein (GFP) to SC to enable easy detection. By employing a spytagged intimin as the model protein, we used SC-GFP system to determine surface exposure of the ST by fluorescence measurements and microscopy, as well as by in-gel fluorescence using semi-native SDS-PAGE to visualize the protein.

In a nutshell, Spycatcher – spytag system is a versatile and convenient technology that has proven useful in the production of stable multi-protein complexes, protein labelling and determining outer membrane protein localization and topology of surface proteins in bacteria.

P506

Uncovering O-antigen modulation of antibiotic biosynthesis in *Serratia*

Daniel Plaza ORCID iD, George Salmond

University of Cambridge, Cambridge, United Kingdom

Abstract

Antimicrobial resistance and the reduced new antibiotic discovery pipeline are endangering human and animal health worldwide. Controlled antibiotic biosynthesis requires a mechanistic understanding of the natural regulatory processes involved to be exploitable. Therefore, connecting the genetic and physiological factors that influence antibiotic biosynthesis is pivotal.

Serratia sp. ATCC 39006 (herein *Serratia*) is a useful Gram-negative model for studies on the biosynthesis of bioactive secondary metabolites, particularly two antibiotics - a carbapenem and prodigiosin. They are tightly regulated in response to various physiological and environmental signals, including quorum sensing.

We identified and characterised novel regulators of antibiotic production via random transposon mutagenesis, employing wild type (WT) as the parental strain and differences in prodigiosin production (a red compound) for mutant screening. Transposon insertion effects on phenotype were verified by transducing out the mutations into a WT genetic background.

Two hyperpigmented and rough colonies harboured insertions in two contiguous coding sequences that belong to an O-antigen operon. We chose the insertion in the putative transport system for further characterisation since it triggered enhanced prodigiosin production. This mutant showed elevated production of both antibiotics, controlled at transcriptional level, and reduced virulence, cell-wall-degrading enzyme synthesis, and transcription of the quorum-sensing-signal synthase. We next generated double mutants with a large set of known *Serratia* regulators, to infer connections to the wider regulatory network and elucidate mechanisms. Thus, the functional characterization and physiological effects of O-antigen transport in both antibiotic production modulation and wider pleiotropy were dissected.

P507

Stress adaptation drives evolution of virulence-traits and antimicrobial resistance phenotype in clinical and environmental strains of *Vibrio cholerae*

Nana Eghele Adade[1,2], Sam Duodu[1], Stephen Dela Ahator[3], Lydia Mosi[1], M.A Valvano[2]

[1]West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Accra, Ghana. [2]Queen's University, Belfast, United Kingdom. [3]Department of Medical Biology, Faculty of Health Sciences, UiT- The Arctic University of Norway, Tromsø, Norway

Abstract

Both environmental persistence and the ability to cause disease requires adaptation of bacterial pathogens to stress, potentially generating more virulent variants. We show here that exposure to stress drives changes in antimicrobial resistance (AMR) phenotypes to selected antibiotics. Employing experimental evolution, clinical and environmental strains of *V. cholerae* were exposed to oxidative, osmotic, pH and iron limitation stress for up to 200 days. Analysis for virulence-associated traits including colony morphotypes, biofilm formation, protease, haemolytic activities and pathogenicity using Galleria mellonella alongside phenotypic AMR testing were performed. The strains were whole genome sequenced and genetic variants were analyzed using a combination of SNIPPY, DNAdiff, MuMmer4, Gubbins, Phandango, UGENE, Clustal Omega and SMART. Irrespective of the stress condition, the *V. cholerae* strains evolved to yield variants with wrinkled colonies, increased biofilm biomass, haemolytic and protease activities, changes in AMR phenotypes, including susceptibility to selected antibiotics and increased pathogenicity to *G. mellonella*. Single nucleotide polymorphisms in genes encoding EAL and GYP domain-containing proteins were implicated in the prolonged survival, evolution of virulence-associated traits especially the increased biofilm biomass and pathogenicity of the evolved variants. The changes in AMR phenotypes did not seem genetic dependent, suggesting phenotype plasticitiy, epigenetic or translational regulation of gene expression. We conclude that, stress adaptation can select for virulence- traits that influence the pathogenic potential of *V. cholerae* as well as antimicrobial susceptibility.

P508

Investigating the effects of amino acids on molecular mechanisms of biofilm formation and pyocyanin production in the pathogen *Pseudomonas aeruginosa* PAO1

Safura Shaikh, Ayesha Rahman

University of Wolverhampton, Wolverhampton, United Kingdom

Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that infects immunocompromised patients and respiratory tract diseases such as Cystic Fibrosis and pneumonia. Non-toxic amino acids create disruption in major virulence factors such as biofilm formation and pyocyanin production of *P. aeruginosa* PAO1. Crystal violet staining of biofilm was used primarily to establish discrepancies of biofilm formation in presence of different amino acids in two distinguished growth stages such as initial attachment and dispersal of biofilm after maturation. The chloroform-hydrochloric acid method was used to derive the pyocyanin (blue-green) pigment from the bacteria at different intervals in presence of different amino acids. Aspartic acid demonstrates 60% to 69% inhibition of biofilm in both isomeric forms at the concentration range of 2.5mM to 40mM, glutamic acid ranges from 56% to 42% of inhibition, histidine shows 89% to 10% inhibition amongst both isomeric forms and asparagine ranges from 18% to 75% of inhibition. Similarly, for dispersal of biofilm in both isomers for aspartic acid, glutamic acid, histidine, and asparagine ranges from 9.5% to 35%, 24% to 75%, 19% to 80%, and 23% to 80% respectively. Pyocyanin production was reduced to 17% to 68% by aspartic acid, 9% to 68% for glutamic acid, and 9% to 98% for histidine when compared to control in both isomeric forms. Amino acids have the potential of being used as a novel treatment or in combination with antibiotics to combat virulence factors of *P. aeruginosa*. Extensive further research is required to examine in detail other virulence factors.

P509

Campylobacter jejuni multi- phase variable gene expression states and their contributions to chicken colonisation and extraintestinal spread

Michelle Jones, Christopher Bayliss

University of Leicester, Leicester, United Kingdom

Abstract

The mechanisms *C. jejuni* utilises to spread from the lower intestine of chickens (caeca and ilea) and colonise the liver and spleen have not yet been fully elucidated. Phase variation (PV) of homopolymeric G or C tracts in the A-T-rich genomes of *C. jejuni* has been identified as an important on/off gene-switching mechanism for adapting to changing, within-host selection pressures. This study analysed the multi- phase variable (PV) gene on/off expression states (phasotypes) of *C. jejuni* M1 and NCTC 11168 strains and ST353 and ST464 isolates from a large-scale broiler chicken experiment to identify sources of extraintestinal *C. jejuni* spread. Median-joining networks were calculated in Network v10.2 to visualise the distance between chicken-specific phasotypes observed in the caeca, ilea, liver and spleen relative to the starting inoculum. Principal component analysis (PCA) of the same data was also performed using RStudio. Network analysis and PCA revealed potential directional phasotype changes within individual chickens which may be a result of bottlenecks or selection acting on the tissue site populations. Overall, this study provides insights into how *C. jejuni* may be spreading within the chicken host and colonising extraintestinal tissues, identifying potential points of interference that could reduce the risk of chicken tissue handling and consumption being a source of *C. jejuni* gastrointestinal infections in humans.

P510

Enhancing a multipurpose artificial urine for culture and gene expression studies of uropathogenic *Escherichia coli* strains

Patricia Rimbi[1], Gillian Douce[1], Mariagrazia Pizza[2], Andrew Roe[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]GSK, Siena, Italy

Abstract

Uropathogenic *Escherichia coli* (UPEC) strains are the most common cause of urinary tract infections (UTIs), which pose a great burden on global health and the economy. Antimicrobial therapy is successful in treating UPEC-associated UTI in some individuals, however, the increase in antimicrobial resistance among some UPEC strains has resulted in the need for novel therapies and strategies. Development of more effective treatments requires a clearer understanding of gene expression by the bacteria during infection. Pooled human urine can be used as a growth medium for *in vitro* studies; however, its composition varies, even if collected from the same donors. Artificial urine offers an alternative growth medium to pooled human urine, although many formulations may contain components not found in healthy human urine. We have shown a previously reported multipurpose artificial urine to be unable to support the growth of UPEC strains CFT073 and UTI89. Comparative analysis, using liquid chromatography mass spectrometry, showed the multipurpose artificial urine to have a different metabolic profile compared to pooled human urine. We have modified the multipurpose artificial urine, using metabolites identified by LC-MS, to generate an improved artificial urine that now supports bacterial growth. Transcriptomic analysis of CFT073 and UTI89 cultured in our enhanced artificial urine and pooled human urine showed the gene expression profiles of selected antigens to be comparable. Further studies using our enhanced artificial urine are ongoing, with the aim of producing a reliable and reproducible alternative to pooled human urine in which gene expression of UPEC strains may be studied.

Session Topic: Environmental and applied microbiology Forum

P511

Searching for microorganisms as life signals for planetary missions

Yongda Li

Dublin City University, Dublin, Ireland

Abstract

One of the principal objectives of planetary exploration is the search for traces of past life and evidence of conditions that may have supported life. The chances of extra-terrestrial life, if it exists on the soil of a planet, is that it will most likely be of microbial origin and physiology. The research of Benjamin Diaz (2006) indicated that E. coli could survive when they were protected in a microhabitat more than 5cm below the surface at the simulative hostile Martian condition (low temperature (-35°C), low-pressure conditions (83.3 kPa), and ultraviolet (UV) irradiation (37 W/m2)).

Therefore, searching for microbial signals using simple-step, ultrasensitive microbial confirmatory tests which could identify specific areas of the planet under exploration as candidates for the presence and support of life are crucial to planetary missions. In this study, E. coli was introduced as a model organism and a miniaturized approach was developed and optimized to assess microbial existence on Martian soil simulants. These miniaturized culturing approaches could prove valuably transferrable to small mission instruments in the case of sampling and securing soil from a future mission and testing it for microbial life or for any possible inhibitory role on microbial growth.

P512

Evaluation of antibacterial potential of marine sediment - derived Actinomycetes from Southern coastal regions of the Arabian sea.

Deepa Mathew P, Dr.V.Robin Perinba Smith

Scott Christian College, Nagercoil, India

Abstract

The development of multi drug resistant uropathogens is a big threat to human race.Infections with multidrug-resistant bacteria are hard to treat. The present study 'Evaluation of antibacterial potential of marine sediment - derived actinomycetes from Southern coastal regions of the Arabian sea' aims to prove marine actinomycetes have some bioactive secondaty metabolites which are antagonistic to multidrug resistant uropathogens. Infections caused by multi drug resistant pathogens are hard to treat and is a big threat to human race. The in vitro antibacterial activity of marine actinomycetes isolates were evaluated against 5 prominent multidrug resistant uropathogens by agar well diffusion assay and disc diffusion assay. The ethyl acetate extracts of selected actinomycetes isolates showed excellent antibacterial activity against all the tested pathogens with zone of inhibition ranging from 12 to 18 mm in disc diffusion and 20-24mm in well diffusion assay. The result was consistent on both well diffusion and disc diffusion.This study concludes that the ethyl acetate extract of the marine sediment-derived actinomycetes can be taken forward as a promising candidate against multidrug resistant bacterial pathogens The initial in vitro experiment confirmed the efficacy of marine actinomycetes secondary metabolites as natural antimicrobial compounds thereby suggesting the possibility of employing them in drugs for the treatment of infectious diseases. Compound characterization and in vivo studies are highly warranted to prove its efficacy to take forward it in the frontline therapeutics treatment.

Keywords: Marine actinomycetes, Secondary metabolites, Uropathogens

P513

Understanding *Listeria monocytogenes* persistence through food industry disinfectants using a biofilm evolution model.

Oleksii Omelchenko[1], Ana Victoria Gutierrez[1], Maria Diaz[1], Mark Webber[1,2], Matthew Gilmour[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Listeria monocytogenes (*Lm*) is the third-leading cause of death from foodborne pathogens, and is increasingly linked to contamination of foods at production facilities. Biofilm formation is one of the mechanisms that *Lm* possess to survive food industry disinfectants and persist in manufacturing environments. Prolonged exposure to sub-inhibitory concentrations of the commonly used disinfectant benzalkonium chloride (BC) has been shown to result in emergence of BC tolerant *Lm* strains. However, there is limited understanding of how *Lm* evolve in the context of biofilms in response to sub-inhibitory concentrations of BC and other disinfectants. Our research combines a biofilm evolution model with short-term disinfectant efficacy testing to study how adaptation of *Lm* occurs and what alternative disinfectants could reduce the threat of *Lm* in food manufacturing. The biofilm evolution model involves constant exposure to sub-inhibitory BC concentrations for a prolonged period, which is paired with whole-genome sequencing to mutations in existing genes/pathways that improve the overall fitness of *Lm* in the context of biofilms and biofilm formation. At present, we adapted a *Salmonella* biofilm evolution model to *Lm* by identifying optimal sub-inhibitory concentrations of BC and confirming the conditions for sufficient cell transfer between the substrates. Additionally, commercially available disinfectants used in the food industry were tested for efficacy against *Lm* biofilms and we propose a potent alternative against *Lm*.

P514

Improving the Sensory Quality and Shelf Life of Bread Using Bacteriocinogenic *Pediococcus pentosaceus* IO1 and *Tetragenococcus halophilus* PO9 Strains as Adjunct Cultures

Isaac Adesina ORCID iD[1], Daniel Arotupin[2], Anthony Ojokoh[2]

[1]University of Medical Sciences, Ondo City, Nigeria. [2]The Federal University of Technology, Akure, Nigeria

Abstract

Bacteriocinogenic lactic acid bacteria are able to reduce microbiological spoilage and improve the safety of food products. The aim of this study was to determine the effect of mono and combined adjunct cultures of bacteriocinogenic *Pediococcus pentosaceus* IO1 and *Tetragenococcus halophilus* PO9 strains on the sensory quality and shelf life of bread. Bread samples were produced by using the adjunct cultures *P. pentosaceus* IO1 and *T. halophilus* PO9 singly and combined with the starter culture (yeast). No significant differences ($P > 0.05$) were found in the aroma, taste, and texture of treated bread samples compared with the control. Bread produced with a combination of bacteriocinogenic culture strains PO9 and IO1 was rated the best in crust colour, crumb colour, and overall acceptability. The addition of the bacteriocinogenic culture strains (PO9 and IO1) as adjunct cultures in bread production improved the quality and extended the shelf life of the bread by 4 days when compared to control (bread leavened by yeast, without calcium propionate). Hence, combined bacteriocinogenic *Pediococcus pentosaceus* IO1 and *Tetragenococcus halophilus* PO9 strains can be used as adjunct cultures to improve the quality and extend the shelf life of bread.

P515

The role of butyrate and serotonin on expression of the PrfA regulon under micro-aerobic effect

Lamis Alnakhli, Ian Roberts

University of Manchester, Manchester, United Kingdom

Abstract

Listeria monocytogenes is a food-borne pathogen which causes listeriosis. It is an intracellular parasite invading the epithelial cells where it escapes from the vacuole into the host cytoplasm to replicate, using actin-based motility to move within and between cells. The intracellular life cycle is well documented whereas the time spent in the lumen of the intestine is poorly understood. The aim of this study was to investigate the mechanism by which L. monocytogenes adapts to the environment of the small intestine prior to invasion. Specifically, to determine if the PrfA regulon, that encodes the virulence factors of L. monocytogenes, is switched on by signals within the intestinal lumen. Initially three signals were examined, butyrate, a short chain fatty acid molecule synthesised by bacteria within the gut microbiota, micro-aerobic (5%v/v oxygen) and serotonin (5-HT), a key neurotransmitter that modulates brain behaviour. 5-HT is secreted by enterochromaffin cells (EC) into the intestinal lumen where it acts to control gut motility, secretion and vasodilation. L. monocytogenes InlA strains with chromosomal pactA::egfp transcriptional fusions were grown aerobically or micro-aerobically in defined MD10 media with either glucose or glycerol as a carbon source with and without 5 mM butyrate or 100 µM 5-HT and Gfp expression monitored. There was significant induction of the pactA expression in micro-aerobic versus aerobic conditions. The addition of 5-HT had no effect while butyrate significantly lowered actA transcription. These data indicate that the PrfA regulon is responsive to signals likely to be encountered in the small intestine.

P516

Growth Inhibition of the Fish Pathogens *Yersinia ruckeri*, *Piscirickettsia salmonis*, *Vibrio anguillarum* and *Tenacibaculum maritimum* by Bacteria Isolated From the Skin of Atlantic Salmon (*Salmo salar*) and from Marine Sponges

Stephen A. Jackson ORCID iD[1,2], William Hurley[3], Jack Caplice[1], Sarah Liston[1], David Rogers[1], David J. Clarke ORCID iD[1,4], Alan D.W. Dobson ORCID iD[1,2]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]Environmental Research Institute, University College Cork, Cork, Ireland. [3]Mowi Ireland, Rinmore, Fanad, Co. Donegal, Ireland. [4]APC Microbiome Ireland, University College Cork, Cork, Ireland

Abstract

Aquaculture is increasingly being used to address sustainable food production and is one of the fastest growing food producing sectors. It is predicted that 62% of fish production will come from aquaculture by 2030. Antibiotic use is common in aquaculture to protect stocks from bacterial diseases and the associated economic costs, which can reach up to approximately 6 billion USD annually. New antimicrobial agents are needed to suppress bacterial fish diseases, to reduce the economic impacts of fish diseases in aquaculture, and to combat the spread of antimicrobial resistance globally.

Streptomyces spp. were isolated from shallow-water and deep-sea marine sponges and diverse heterotrophic bacteria were isolated from the skin of farmed Atlantic Salmon (*Salmo salar*). Sponge and fish-derived isolates were tested in deferred-antagonism assays against a panel of known fish pathogens. Selected isolates were fermented in liquid media, including both N- and P-limited media. Organic and aqueous extracts of fermentation broths were tested in disc diffusion assays against a range of fish pathogens.

Two fish-skin isolates, *Rahnella* sp. and *Galactobacter* sp., inhibited the growth of *Yersinia ruckeri* and *Piscirickettsia salmonis* respectively, in deferred-antagonism assays. Extracts from nutrient-limited fermentation media of 10 *Streptomyces* spp. isolates inhibited the growth of *Vibrio anguillarum* and *Tenacibaculum maritimum*, whereas extracts from non-nutrient-limited media displayed no such bioactivities.

The fish skin microbiota contains host-associated bacteria that are antagonistic to fish pathogens, while One Strain Many Compounds (OSMAC) strategies can induce production of anti-bacterial metabolites in *Streptomyces* spp., with activity against fish pathogens.

P517

Engineering myxobacterial super-predators to fight crop disease

Emily Radford, David Whitworth

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

The *Myxococcota* phylum (myxobacteria) comprises a group of diverse, gliding bacteria which are known to show multicellular cooperation and predatory activity. Predation mechanisms of myxobacteria include contact dependent killing as well as the secretion of antimicrobial secondary metabolites and lytic outer-membrane vesicles. Their prey range is varied and strain dependent but is uncorrelated with phylogeny, suggesting that horizontal gene transfer is important in conferring the genetic basis for successful predation. Gram-positive and negative bacteria as well as many fungal species are common prey for myxobacteria. Fungi such as *Zymoseptoria tritici* and *Fusarium spp.* infect wheat crops globally causing substantial losses in yield and nutritive value. As ubiquitous predators of fungi, myxobacteria hold the potential to be used as biocontrol agents in the field.

However, our *in vitro* predation assays have demonstrated variation in predatory activity across a panel of prey, even between two strains of the same species of myxobacteria. Subsequently, genomic interrogation by GWAS (genome-wide association study) methods has allowed identification of genes correlated with successful predation against specific prey. This allows us to use genetic manipulation to produce strains which are deliberately engineered to be proficient predators against specific wheat phytopathogens.

This poster presents the results of our predation assays and GWAS analyses, plus our strategy and progress in engineering myxobacterial strains with bespoke predatory activity. Ultimately, *in planta* assays will assess the ability of our engineered myxobacterial predators to reduce phytopathogen abundance, and resulting yield and crop health improvements.

P518

Exploring the Application of REIMS to Bovine Health Monitoring and Milk Quality Analysis

Rachel Murdock, Sharon Huws, Simon Cameron

Queen's University Belfast, Belfast, United Kingdom

Abstract

Due to limitations associated with time and cost, there is clear imperative to develop a rapid system that identifies the causative pathogen of Bovine Mastitis (BM). This will allow timely treatments to be delivered, thereby reducing the use of empirical prescribing practices and the consequent development of AMR. Rapid evaporative ionisation mass spectrometry (REIMS) has previously been used to provide a high-throughput platform which provides both accurate and rapid identification of bacterial and fungal species. This project is extending the current analytical potential of REIMS to the direct-from-milk identification of BM pathogens. With REIMS, a sample can be analysed in under 10 seconds and thus offers the potential to remove the time consuming and expensive steps of traditional agar culture methods. This project has collected monthly milk samples from the same 24 cows on four farms for one year. Of these, 371 samples were diagnosed as mastitic based on somatic cell counts and the causative pathogen isolated and identified using MALDI-ToF mass spectrometry. As well as collecting macronutrient composition data, we are currently conducting REIMS analysis in order to identify biomarkers that are specific to different taxonomies of pathogens. Uniquely, we are also able to look at longitudinal changes in individual cows that may identify pre-symptomatic markers of BM.

P519

Rate and efficiency of synthetic plastic degradation by microbes isolated from environments containing natural hydrophobic polymers.

Scott Coughlin, Stephen Kelly, Julianne Megaw

Queen's University, Belfast, United Kingdom

Abstract

Plastics and microplastics (<5 mm) are becoming increasingly ubiquitous pollutants in the environment. This is driven by their recalcitrance to environmental and biotic degradation, and their tendency to accumulate through food webs across ecosystems. Many studies have isolated microbes from environments with high quantities of synthetic polymers. This project uses a culture-based approach to screen for plastic-degrading microbes in environments with high abundance of natural high molecular weight polymers (e.g., cellulose, chitin, or lignin). Bacterial isolates were identified by 16S rRNA gene sequencing and characterised based on phylogeny. Rates of plastic degradation were measured by mass loss of high-purity film (i.e., no additives) over a 28-day period. The bacterial adherence to hydrocarbons (BATH) assay as used to determine cell surface hydrophobicity and a staining method was used to determine biofilm formation under varying conditions. Environmental samples included compost, peat, and reed bed sediment. From these, 42 bacterial species were isolated as candidate plastic degraders, including Gordonia terrae which was shown to degrade polypropylene by up to 22.7% over a 28-day period. Whole genome sequencing of this isolate revealed an open reading frame with high percentage identity (75.06%) to alkane-1-monooxygenase from Rhodococcus ruber, a known to depolymerise polyethylene. Experiments to determine the effects of additional carbon sources to the rate and efficiency of degradation show a range of impacts. Addition of 1% v/v heptadecane increased degradation efficiency, possibly due to differential expression of the alkane-1-monooxygenase enzyme. These findings give evidence of plastic degradation by Gordonia terrae alkane-1-monoxygenase.

P520

The prototypical integrative and conjugative element *ICEnahCSV86* encodes naphthalene degradation in *Pseudomonas bharatica* CSV86^{T}

Harshit Malhotra[1], Balaram Mohapatra[1,2], Prashant Phale[1]

[1]Indian Institute of Technology, Bombay, Mumbai, India. [2]Gujarat Biotechnology University, Gandhinagar, India

Abstract

Pseudomonas bharatica CSV86^{T} degrades a wide range of aromatic compounds, including naphthalene, prior to simple carbon sources like glucose. The advanced draft genome revealed the naphthalene-degradation property to be localized on the prototypical integrative-conjugative element *ICEnahCSV86*. The strain transferred the naphthalene degradation property to *Stenotrophomonas maltophilia* CSV89 at a conjugation frequency of 7×10^{-8}. The naphthalene degradation genes were found be localized as *nah* and *sal* operons on *ICEnahCSV86*. The modular arrangement of *ICEnahCSV86* showed high synteny with the prototypical *ICEclc* family elements *ICEclcB13* and *ICEXTD*, encoding chlorocatechol and xylene/toluene degradation, respectively. Presence of type-4 secretion system (T4SS) core modules, tRNA-Gly^{CCC}, integrase, integrase regulator, multiple repeats and transposases implied its acquisition from other organisms *via* horizontal gene transfer. *In-silico* promoter analysis of *nah* and *sal* operons revealed the presence of strong promoters P*nah* and P*sal* that drive the transcription of the respective operons. Additionally, the LysR-type regulator, NahR, was found to be encoded on the complementary strand upstream of *sal* operon. Co-transcription analysis confirmed the polycistronic nature of these gene clusters. Further, 1-naphthol 2-hydroxylase was used as a reporter enzyme for expression from various promoter systems (P*trc*/*lacI*^{q}, P*nah*, P*sal* and P*sal*/*nah*R), which revealed P*nah* to be a strong leaky promoter, which was upregulated upon induction with salicylate, while P*sal* was tightly regulated and expressed only in the presence of NahR regulator and salicylate effector. This regulatory mechanism implied a strategy where leaky expression from P*nah* is essential to generate the salicylate effector, which upregulates both *nah* and *sal* operons.

P521

Controlled and Continuous Production of 3-Hydroxypropanoic Acid Using Hydrogel Beads Compartmentalized Saccharomyces Cerevisiae Strains

Haneef Rehman ORCID iD

Department of Natural and Basic Sciences, Turbat, Pakistan. Imperial College London, London, Pakistan

Abstract

3-Hydroxypropionic acid (3HP) is an important bio-based molecule and can be used for the conversion of acrylic acid, acrylic ester and amides. In current study, alginate hydrogel beads compartmentalized microbial cells consortia of mono and co-cultures S. cerevisiae strains system has been designed for 3-HP production using encapsulation technology. The compartmentalized S. cerevisiae strains within alginate hydrogel beads maintained 100% 3-HP production. The hydrogel beads prepared using 3.0% alginate polymer and 2.0% calcium chloride as a crosslinking agent encapsulated maximum S. cerevisiae cells in term of higher 3-HP production. The co-culture encapsulated strains hydrogel beads showed higher production of 3-HP as compared to mono-culture strains encapsulated hydrogel beads system. The hydrogel beads with smaller beads size of 3.5 mm supported higher production of 3-HP as compared to hydrogel beads with larger beads sizes. The incubation period for 3-HP production was increased after encapsulation and encapsulated co and mono-culture strains produced maximum 3-HP after 48 hours of incubation as compared to free strains which produced maximum 3-HP after 24 hours of incubation. The co-encapsulation of S. cerevisiae strains within single bead improved the 3-HP production and co-encapsulated S. cerevisiae strains hydrogel beads with 1:2 ratio showed higher 3-HP production as compared to separated hydrogel beads. The encapsulated S. cerevisiae strains exhibited good reusability and storage stability properties, and retained 100% 3-HP production after 10 batch cycle fermentation and 30 days of storage, respectively.

P522

Important clinical *E. coli* strains can survive and retain their virulence on environmental plastic waste

Hannah White, Michael Ormsby, Rebecca Metcalf, David Oliver, Richard Quilliam

University of Stirling, Stirling, United Kingdom

Abstract

Plastic waste is ubiquitous in the environment and there are increasing reports of such waste being colonised by human pathogens. However, the ability of pathogens to persist on plastics for long periods of time, and the risk that they pose to human health, is unknown. Here, under simulated environmental conditions, we aimed to determine if pathogenic bacteria can retain their virulence following a prolonged period on the surface of polyethylene. Using luciferase expressing variants, we show that clinically important strains of *E. coli* (enterohaemorrhagic *E. coli* (EHEC); enterotoxigenic *E. coli* (ETEC); adherent-invasive *E. coli* (AIEC); and uropathogenic *E. coli* (UPEC)) can survive on plastic for at least 28-days. Furthermore, these pathogens were able to retain their virulence (determined by using a *Galleria mellonella* model as a surrogate for human infection) and in some cases, had enhanced virulence following their recovery from the plastisphere. This indicates that plastics in the environment can act as reservoirs for human pathogens and could facilitate their persistence for extended periods of time. Most importantly, human pathogens in the plastisphere are capable of retaining their pathogenicity. Pathogens colonising environmental plastic waste could therefore pose a heightened public health risk, particularly in areas where people are exposed to pollution, such as at bathing water beaches.

P523

Improving the fitness of *B. subtilis* as a protein production host by suppressing autolysis and optimising single cell energy levels

Maria Dakes Stavrakakis, Kenneth Seistrup, Colin Harwood, Henrik Strahl

Centre for Bacterial Cell Biology, Biosciences Institute, Newcastle upon Tyne, United Kingdom

Abstract

High cell density fermentation is a key technology in utilising bacteria for production of valuable commodities including enzymes. However, such culturing conditions are problematic for the frequently used production host *B. subtilis*, which undergoes autolysis when stressed or starved for oxygen or nutrients. The autolytic processes of *B. subtilis* may, thus, negatively influence industrial production yields.

B. subtilis demonstrates distinct cell-to-cell heterogeneity in energy levels when cultured to high density, with a subpopulation becoming depolarised and likely lysing. A potential explanation for this is the ability of *B. subtilis* to differentiate into distinct, co-existing cell types. *B. subtilis* deletion mutants that lack the ability to develop competence or enter sporulation exhibit energy and lysis levels that are comparable to wild type, thus suggesting that these pathways are not responsible for the observed heterogeneity. In contrast, abolishing the production of two cannibalism factors previously linked to sporulation (Sdp and Skf), significantly supresses autolysis, linking cannibalism to the reduced fitness of dense *B. subtilis* cultures.

Suppressing autolysis and improving the cell energisation levels is, thus, a promising approach to minimise futile biomass production, to increase the fraction of the cell population actively producing and, ultimately, to improve protein production yields.

P524

Optimization of culture conditions for phytase production by indigenous strain of *Aspergillus niger* PHY82 isolated from soil of livestock farms

Zahid Manzoor[1,2], Ali Ahmad[3], Zaib Ur-Rehman[2], Aayesha Riaz[2], Zia Ur Rehman[4], Syeda Maryam Hussain[2], Zishan Ahmad[2]

[1]Pir Mehr Ali Shah Arid Agriculture University, Dept. of Parasitology and Microbiology, Rawalpindi, Pakistan. [2]Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. [3]University of Veterinary and Animal Sciences, Narowal campus, Narowal, Pakistan. [4]The Islamia University of Bahawalpur, dept. of Physiology, Bahawalpur, Pakistan

Abstract

Phytase is a phosphatase enzyme involved in catalyzing the hydrolysis of phytic acid and responsible in releasing a usable form of inorganic phosphorus. This study was undertaken with main objectives of meeting the growing industrial demands of phytase by standardization of local production at low cost and here we focused on optimization of culture conditions for phytase production by indigenous strain of *Aspergillus niger* PHY82 (*A. niger*) , isolated from soil of livestock farms in Lahore district of Punjab, Pakistan. Best phytase producing fungi was selected depending on size of zone of hydrolysis on plate of phytase screening medium agar and identified as *A. niger* PHY82 by morphological methods. Using one variable approach, culture conditions were optimized for maximum production of phytase by *A. niger* PHY82. Temperature, pH, substrate type and concentration were optimized in this studied using submerged fermentation. Results revealed that maximum phytase production 3.87 U along with 15.51 g/L biomass of *A. niger* PHY82 was observed at temperature 350C, pH 5 and 5% rice bran as substrate. Phytase activity was increased in up to 4.3-fold and the biomass matter was increased in up to 4.2-fold. Under optimized conditions Agricultural bye-products such as rice bran can be used for cost effective phytase production. Hence, to meet the phytase requirements in feed industries *A. niger* PHY82 can be used for large scale phytase production.

P525

Dissemination of Carbapenemase-producing *Enterobacteriaceae* in natural water sources in Central part of Thailand

wipawee songsaeng[1], Nuvee Prapasarakul[2,3], Thidathip Wongsurawat[4], Wandee Sirichokchatchawan[1,3]

[1]College of Public Health Science, Chulalongkorn University, Bangkok, Thailand. [2]Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. [3]Center of Excellence in Diagnosis and Monitoring of Animal Pathogens (DMAP), Bangkok, Thailand. [4]Division of Bioinformatics and Data Management for Research, Research Group and Research Network Division, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract

The dissemination of antibiotic resistance (AR) in the aquatic environments is a worldwide public health threat. Carbapenemase-producing *Enterobacteriaceae* (CPE) are particularly in great concern due to the resistance of carbapenems, the last resource drug for treating multi-drug resistance infections. This study aims to examine the dissemination of CPE in different water bodies located in the Central part of Thailand, and to characterize isolated strains. CPE were identified by using CHROMagar™ mSuperCARBA™ selective media, Carba NP test, and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility testing was done by using broth-microdilution method. Gene detection and genomic characteristic analysis were done by PCR and whole genome sequencing. From 30 water samples, 7 isolates including one *E. coli*, two *K. pneumoniae,* and four *E. cloacae* complex, were detected to carry carbapenemase genes such as $bla_{\text{NDM-1}}$ (n=2), $bla_{\text{NDM-5}}$ (n=1) and $bla_{\text{IMI-1}}$ (n=4). All 7 isolates were multi-drug resistance, resisting to carbapenems, beta-lactams, and colistin. Three carbapenemase genes were found to be located on rare p0111 (n=1) and IncC (n=2) plasmids. Whereas resistance genes from *E. cloacae* complex were found to be chromosomal encoded carbapenemase genes. Our findings display environmental dissemination of CPE in water bodies in the Central part of Thailand, indicating the high possibility of spreading CPE throughout the areas. Consequently, this poses a major threat to public health.

P526

Investigation of biogeochemical cycle through microbial community analysis of tidal flats in the Yellow Sea of the Republic of Korea.

Yeonjae Yoo, Jae-Jin Kim

Korea University, Seoul, Korea, Republic of

Abstract

Tidal flat has developed along the Yellow Sea coastal line of South Korea due to the considerable difference with the tide. A tidal flat is a unique area that combines the physical shapes of the sea and land, and it is being explored as a location for blue carbon, which can reduce carbon dioxide in the atmosphere, which has lately emerged as an environmental issue. Therefore, in this study, the effect of microbial diversity on the biogeochemical cycle in the study area was investigated based on the analysis of the microbial community of tidal flats and salt marshes in the Yellow Sea coast of Korea.

The microbial community analysis showed that the most dominant bacterial phylum in most of the sediment samples in tidal falts was Proteobacteria. Because of the salinity of the tidal flats, the distribution of halophilic bacteria and anaerobic bacteria to grow in sediments or to adapt to the aquatic environment could also be identified. In particular, the discovery of nitrogen-fixing bacteria involved in the nitrogen cycle in the area of salt marshes was especially notable. The significance of the discovery of nitrogen-fixing bacteria in the sediments of tidal flats is that they take nitrogen from the atmosphere available to plants, helping them to flourish, contribute to plant carbon dioxide absorption, and contribute to the utilization of carbon by other microbes in sediments. This indicates that the tidal flat has the potential to be designated as a new blue carbon area.

P527

COPPER RECOVERY FROM LOW-GRADE ORE AT DIFFERENT RATIO OF OXIDE AND SULFIDE ORE SAMPLES

Anna Khachatryan ORCID iD[1], Arevik Vardanyan ORCID iD[1], Sabine Willscher[2], Andreas Kamradt[3], Narine Vardanyan ORCID iD[1]

[1]SPC Armbiotechnology of NAS of Armenia, Yerevan, Armenia. [2]Faculty of Natural Sciences I, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany. [3]Economic Geology and Petrology Research Unit, Institute for Geosciences and Geography, Martin Luther University Halle-Wittenberg, Halle, Germany

Abstract

Background

Bioleaching is mainly used for the treatment of low-grade ores as an efficient and an environmental friendly technology. The aim of the present work was to study the possibilities of bioleaching for the efficient processing of Kajaran low-grade copper-molybdenum ore (Armenia).

Methods

Ore samples of Kajaran copper-molybdenum ore were subjected for bioleaching.Leaching tests were performed in flasks under periodic mode on orbital shaker incubator (180 rpm) at 35°C. The leaching intensity of ore samples was estimated by the amount of Cu2+ ions and total iron released to the medium using ICP-OES.

Results

Sulfide and oxide ore samples in various ratios were tested for bioleaching in order to increase the process intensity. Indigenous communities of chemolithotrophic bacteria, isolated from the AMD of Kajaran ore, were used for processing of Kajaran ore samples. Studies have shown that the addition of 4% oxide ore promotes the biodegradation of sulfide ore and leads to an increase in the amount of copper in the solution up to 82-84 %. Notably, the addition of oxide ore inhibits pyrite oxidation. The results of the analysis of the distribution, grain size and liberation of chalcopyrite in the residues of sulfide and oxide ores are fully consistent with the results of experiments on bioleaching.

Conclusion

Studies have shown that the extraction of copper can be enhanced with an increase in PD at the expense of oxide ore. From the point of view of copper extraction, the optimal ratio of sulfide and oxide ores in the pulp was 10:14.

P528

Plastics, pathogens, and persistence: African *Salmonella* sp. can survive and retain virulence on environmental plastic waste

Michael Ormsby[1], Hannah White[1], Rebecca Metcalf[1], David Oliver[1], Nicholas Feasey[2], Richard Quilliam[1]

[1]University of Stirling, Stirling, United Kingdom. [2]Malawi-Liverpool Wellcome Trust Clinical Research Program, University of Malawi College of Medicine, Blantyre, Malawi

Abstract

In low- and middle-income countries (LMICs), almost one million people die annually due to diseases related to mismanaged waste. Plastic pollution has increased concurrently with economic development and rapid urbanisation and has amplified the effects of inadequate waste management and sanitation infrastructure. Distinct microbial populations can colonise environmental plastic debris in what is collectively known as the 'plastisphere', with increasing evidence that plastic waste can become colonised with human pathogens and pose an increased public health risk. Diverse serovariants of *Salmonella enterica* are cumulatively the most frequently isolated pathogens in patients presenting to hospital with community-onset blood stream infections (BSI) in Africa. *Salmonella* sp. can persist for extended periods of time on different materials, including plastics. Here, we have analysed the survival rate of the three *Salmonella* serovars most commonly isolated from blood across Africa; *S.* Typhi, *S.* Typhimurium and *S.* Enteritidis on plastics, under conditions simulating those of urban waste piles commonly identified in informal settlements in Africa. All three serovars persisted at 20°C and 30°C for up to 28-days; and for up to 14 days at 40°C. Importantly, we provide evidence that each serovar retains virulence using a *Galleria mellonella* model of infection and is pathogenic at the concentrations recovered at each time point. Therefore, plastics in the environment can act as a reservoir for human pathogens that retain their virulence. Plastics colonised by pathogens may therefore present a heightened risk to public health, particularly in LMICs where people are regularly exposed to environmental plastic pollution.

P529

Development of a 3D printed chamber for efficacy testing of non-porous antimicrobial surfaces where environmental conditions can be controlled.

Alexander Cunliffe ORCID iD, Joanna Verran ORCID iD, James Redfern ORCID iD

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Antimicrobial materials used to control survival of microorganisms are becoming increasingly popular due to the reduced efficacy of traditional control methods (e.g. antibiotics). The environmental conditions (e.g. temperature, humidity and airflow) used for efficacy testing of antimicrobial materials in standardised methods differ greatly from end-use scenarios, particularly with regards to the presence of moisture (which may be limited at point of use). To test materials in conditions more appropriate to end-use, a 3D printed chamber has been developed to test potential antimicrobial materials at a range of temperature, humidity, and airflow values. Development involved prototyping 3D printed chambers coupled with Arduino-based electronics to maximise the usability and reliability of the chamber, which assessed antimicrobial activity using an adapted version of ISO22196. The effects of airflow were tested via survival rates at different positions within the chamber and mapping airflow using computational fluid dynamics modelling. The chamber maintained 15%, 40%, and 95% relative humidity (RH) at 24°C and 15% and 75% RH at 37°C (maximum operatable temperature). Similar survival rates of *E.coli* were observed between the new chamber and the standard ISO22196 method in a static environment (no manually introduced airflow). However, modifying the airflow conditions altered the survival rates of *E.coli* depending on their positions within the chamber, thus highlighting the complexity of what might initially appear to be a relatively simple system. Further work is required to understand these relationships, and to develop a reliable, reproducible standardised test method for antimicrobial non-porous materials that is applicable to point-of-use.

P530

In vitro biofilm inhibition and prevention using phytic acid

Rania Nassar ORCID iD[1,2], Mohannad Nassar ORCID iD[3], Abiola Senok ORCID iD[1], David W Williams ORCID iD[2]

[1]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE.
[2]School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom.
[3]Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, UAE

Abstract

Background Root canal infections are biofilm-associated and are treated with chemical irrigants. However, currently used irrigants have notable disadvantages and alternatives are urgently required. The aim of this research was to evaluate the *in vitro* antibiofilm properties of a potential natural alternative, called phytic acid (IP6).

Methods Single and dual-species biofilms of *Enterococcus faecalis* and *Candida albicans* were developed in 12-well plate static fed-batch model and on hydroxyapatite coupons (HA) using CDC bioreactor. IP6's antibiofilm efficacy was assessed by Alamar blue assay, crystal violet staining, biofilm regrowth and colony forming unit measurements. Confocal laser scanning microscope (CLSM) was used to directly assess and quantify biofilm viability. Effects of IP6 on virulence genes expression was quantified along with C*andida* hyphal transformation. Inhibition of biofilm development on HA surfaces preconditioned with IP6 was also investigated using CLSM and scanning electron microscopy.

Results IP6 had both bactericidal effects and altered the metabolic activity of biofilm cells in the static model. IP6 (1.5%, 2.5% or 5% for 5 min) biofilm treatment resulted in 96.8%, 99.0% and 99.7% reduction in *E. faecalis* CFUs, respectively. CLSM revealed that IP6 treatment led to significant reduction in live biofilm biomass compared to untreated controls. IP6 at sub-lethal concentration was found to upregulate *C. albicans hwp1* gene expression, although this was not reflected by hyphal transformation. Pre-conditioning HA surfaces with IP6 resulted in extensive inhibition of dual-species biofilm formation.

Conclusion This study highlights IP6's antibiofilm properties and its potential for future exploitation in areas where biofilm management is necessitated.

P531

Animal-environment microbial associations in the swine production industry

Mégan Van Der Merwe ORCID iD, Jarishma Gokul, Lise Korsten

University of Pretoria, Pretoria, South Africa

Abstract

Monitoring of the microbial communities in the pig gut has highlighted the important role of the gut microbiome. Diet has also been hypothesised to influence the composition of the microbiome, thereby influencing gut and animal health. Since animal feed and water ingested in the commercial production system is rarely investigated, not much is known on the effect of these communities on the gut microbiome and downstream swine performance. This study therefore aims to determine the microbial community composition of pig feed, water, and faeces in commercial piggeries from a One Health perspective. Pig feed, drinking and irrigation water (borehole, municipal, dam), and pig faeces were collected from three commercial South African piggeries that produced their own feed. Samples were analysed for their bacterial (V4 hypervariable region of 16S rDNA) and fungal (ITS 1-4 rDNA) composition using paired-end Illumina MiSeq sequencing prior to processing using an in-house MR DNA freeware, QIIME2 and R statistical analysis pipeline. It is expected that drinking water will show lower diversity and abundance when compared to irrigation water. Further, we predict that feed microbial communities will be clearly distinguishable with a higher diversity in the fungal community, as the pig diets are relatively stable. Faecal sample community compositions are expected to be similar to that observed in other faecal microbiome studies suggesting a convergence of the gut microbiome in finisher pigs that is largely shaped by their nutrition.

P532

Microbial community responses to alterations in historical fire regimes in montane grasslands

Jarishma Gokul[1], Gwynneth Matcher[2], Joanna Dames[3], Kuhle Nkangala[3], Paul Gordijn[4], Nigel Barker[1]

[1]University of Pretoria, Pretoria, South Africa. [2]South African Institute for Aquatic Biodiversity, Makhanda, South Africa. [3]Rhodes University, Makhanda, South Africa. [4]South African Environmental Observation Network (SAEON), Grasslands-Forests-Wetlands Node, Pietermaritzburg, South Africa

Abstract

The soil microbiome plays a critical role in ecosystem services and plant productivity, while shaping above and below ground diversity. Despite this, the link between plant and soil microbial diversity in montane grasslands, particularly under the influence of fire regimes, are relatively unexplored areas of interest on soil ecosystems. In this pilot study, metabarcoding was used to unravel the bacterial community structure in South African montane grasslands that have been subjected to an intermediate (up to five years) term fire-return interval gradient. For the first time, we demonstrate unique season-specific burn patterns for bacterial communities in southern African montane grasslands. Bacterial communities exhibited a shift in composition in the soils subjected to annual and biennial fires, supporting preliminary analysis of the dominant fungal taxa and previously tested soil chemical properties. Our data further suggests that taxon-taxon interactions may act as drivers of the observed bacterial community structure, with carbon and nitrogen as potential chemical drivers.

P533

Towards an electrochemical biosensor for the detection of ampicillin residues in milk

Magdalena Raykova[1], Fiona Henriquez[2], Damion Corrigan[1], Andrew Ward[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of the West of Scotland, Paisley, United Kingdom

Abstract

Antibiotic residues in food are strictly controlled and monitored by national laws in many countries and territories. In dairy farms, contamination of the bulk tank with milk-containing residues, such as beta-lactam antibiotics, presents a threat to confidence in supply and results in financial losses to the farmer and dairy industry. The disposal of contaminated with residues milk imposes a risk to the environment by creating reservoirs of antimicrobial resistance (AMR) further in the farm field and beyond. Ampicillin (AMP) is a broad-spectrum antibiotic, active against a wide range of gram-positive and gram-negative bacteria and a leading choice for the treatment of dairy cows. In this work, electrochemical detection of AMP was achieved through a short thiolated (at the 5') oligo tagged with a methylene blue (MB) at the 3' as the reporter molecule immobilised on a gold electrode. The remaining active sites were backfilled with 6-mercapto-1-hexanol (MCH). Square wave voltammetry was utilised in target-free 100mM NaCl solution to indicate the performance of the aptasensor. The sensor was then tested in the same media containing AMP and an increase in the current was observed as a response. Measurements in commercial milk samples spiked with a range of concentrations of AMP were then performed. The proposed method is reliable, rapid and sensitive to AMP with a limit of detection of 10nM. Its main purpose is to be implemented in dairy farms to monitor residues in milk in real-time and thus limit the release of large quantities of contaminated milk into the environment.

P534

SCREENING AND IDENTIFICATION OF MICROORGANISMS FROM EARTHEN PONDS FOR FLOCCULANT PRODUCTION

Oluwafemi Adebayo Oyewole[1], Olabisi Peter Abioye[1], Olalekan David Adeniyi[2], Evans Chidi Egwim[3], Abdullahi Afolabi Abdulhammed[1]

[1]Department of Microbiology, Federal University of Technology, Minna, Nigeria. [2]Department of Chemical Engineering, Federal University of Technology, Minna, Nigeria. [3]Department of Biochemistry, Federal University of Technology, Minna, Nigeria

Abstract

Bioflocculants are biodegradable polymers produced by microorganisms and have gained applications in aggregating dissolved and suspended substances in water. This study was aimed at screening, isolation and identification of microorganisms from water sediments for flocculant production. Water sediment samples were collected from earthen pond sludge and screened for microorganisms with bioflocculating ability using kaolin suspension and selective medium containing (per liter) NaCl 0.1 g, MgSO47H2O 0.2 g, K2HPO4 5 g, agar 5.5 g, yeast extract 0.5 g, urea 0.5 g, KH2PO4 2 g, and glucose 10 g for bacteria, and MgSO47H2O 0.2 g, K2HPO4 5 g, agar 5.5 g, yeast extract 0.5 g, urea 0.5 g, KH2PO4 2 g, glucose 10 g for fungi. Of all the microorganisms isolated, Prestia megaterium (ON184360) gave the highest potential (30 %) for flocculant production. Tyrosine-protein kinase gene was detected to be responsible for the bioflocculant production. The lyophilized bioflocculant produced by the bacterium was characterized using thermogravimetric property analysis, FTIR and SEM. A total of 10.67 g of bioflocculant was produced from 500 ml of the medium. The thermogravimetric property, FTIR spectra and scanning electron micrograph exhibited typical characteristics of bioflocculant. The results of this study showed that Priestia megaterium (ON184360) isolated from earthen pond sludge is a potential alternative for flocculant production.

P535

Comparative genomic analysis of the monocyclic aromatic hydrocarbons degradation potential in genus *Defluviimonas* in marine saltern environment

Jong-Hwa Kim ORCID iD[1,2], Jihye Baek ORCID iD[1], Kiyoung Kim ORCID iD[1], Wonyong Kim ORCID iD[1,2]

[1]Chung-Ang University, Seoul, Korea, Republic of. [2]LuxBiome Ltd., Seoul, Korea, Republic of

Abstract

Background: Monocyclic aromatic hydrocarbons (MAHs) are major environmental pollutant including benzene, toluene, and xylene, it can cause skin irritation, dizziness, headaches, and blood disorders including anemia and leukemia in humans. Many marine bacteria have a variety of beneficial properties, such as degradation of organic compounds. The purpose of this study is to determine the taxonomic properties based on phenotypic and chemotaxonomic features and to identify its beneficial function based on genomic analyses.

Methods: The whole genome of *Defluviimonas marina* CAU 1641^T was determined using Hiseq Illumina platform. Genes were annotated using RAST and PATRIC server, and protein and their functions were predicted by eggNOG pipeline. The benzene degradation pathway was mapped based on KEEG.

Results: Whole genome size of *Defluviimonas marina* CAU 1641^T was 5.02 Mb with 4,885 protein-coding genes and 92 core house-keeping genes. Compared with the strain CAU 1641^T and 15 reference *Rhodobacteraceae* strains, 17,806 genes were classified as the accessory and unique genes. The genome of the strain CAU 1641^T contains genes encoding the degradation pathway of benzene and phenol and Defluviimonas marina CAU 1641^T was found to be able degrade MAHs based on the gene annotation and biosynthesis pathway analysis.

Conclusion: These results suggest that *Defluviimonas marina* CAU 1641^T, which is a strain that represents a novel species in genus *Defluviimonas*, is proposed that support for biodegradation of monoaromatic compounds.

P536

Field evaluation of a qPCR assay detecting oral *Streptococcus* spp. as a marker for contamination with respiratory secretions on indoor surfaces: results from a pilot study

Ailbhe Barry[1], Paz Aranega Bou ORCID iD[1], Patricia Barkoci[1], Nicola Yaxley[1], Phoebe French[1,2], Thomas Pottage ORCID iD[1], Allan Bennett ORCID iD[1], Ginny Moore ORCID iD[1]

[1]Biosafety, Air and Water Microbiology Group, UK Health Security Agency, Salisbury, United Kingdom.
[2]University of Reading, Reading, United Kingdom

Abstract

Viruses targeting the human respiratory tract can be highly transmissible, particularly in indoor environments, and cause significant morbidity, mortality and economic losses. Identifying suitable mitigation strategies for different settings is important to reduce the burden of disease. However, detection of respiratory viruses in the built environment can be challenging and identification of a suitable marker that could detect respiratory secretions is desirable. Oral streptococci are a group of bacteria forming part of the oral microbiome and are dispersed via droplets and aerosols during respiratory activities. A qPCR protocol specific for oral streptococci was designed and used to investigate contamination of a double-decker bus. Eight handholds and eight seat rests were randomly selected for sampling. Bleach and IPA wipes were used to remove residual DNA before “background” samples were collected using sterile sampling sponges. After a 1-hour trip, during which 46 passengers used the vehicle, “exposed” samples were collected from the same sites. DNA was extracted and duplicate aliquots were analysed by qPCR. 8/8 handholds and 4/8 head rests had more oral streptococci DNA on the exposed samples compared to the background samples (an average difference of -3.7 Cts or ~7400 copies/extraction) suggesting respiratory secretions were deposited during the trip. The difference between the “background” and the “exposed” samples taken from the 4 remaining head rests was < 1 Ct implying minimal contamination. These results suggest that this protocol could be used to investigate contamination of the indoor environment with respiratory fluids and to evaluate interventions that reduce it.

P537

Air microbiome diversity in the built environment across a rural – urban gradient is driven by farm type, room type and animal presence.

Beatriz Delgado-Corrales ORCID iD[1,2], Hannah Davis[3], Angela Sherry[1,2]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Hub for Biotechnology in the Built Environment, Newcastle upon Tyne, United Kingdom. [3]Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

Recent research has shown that those growing up in urban environments are exposed to a less diverse built environment microbiome, compared to growing up in rural dwellings. As farming environments are thought to have protective effects against respiratory diseases, analysing the impact of rural gradients on microbial composition is key to build healthier cities.

To study the biodiversity across degrees of urbanisation, air microbiome samples were collected from a city farm and a rural dairy farm over a 6-month period. Microbial DNA was extracted for whole-genome sequencing. Sequences were then analysed using a non-assembled approach against the RefSeq PlusPF database using Kraken2. Potentially contaminant species were removed using the decontam R package. Statistical analyses were carried out in R using centered log-ratio (CLR) transformations and Euclidean distances.

Preliminary diversity analyses showed that microbial composition was significantly associated with farm type and room type, but not with the season when the samples were taken. This implies that the whole microbial diversity was influenced by farm location, animal presence and room features, but it does not have a seasonal component. However, when analysed individually, fungal communities appeared to cluster by season, while bacterial and archaeal communities did not.

These results shed light on the BE microbiome composition and dynamics and will be the basis of future functional analyses of air microbiomes in rural and urban farms. This study has the potential to understand microbiome composition in relation to building design to prevent microbial diversity loss for the benefit of future generations.

P538

Metabolic Engineering of *Clostridium acetobutylicum* for Isopropanol-Butanol-Ethanol production

Bunmi Omorotionmwan[1,2], Hengzheng Wang[2], Jonathan Baker[2], Minyeong Yoo[2], Ying Zhang[2], Nigel Minton[2]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

[Abstract text is redacted at the request of the presenting author]

P539

Uncovering the bacterial microbiome of cave-dwelling Homoscleromorpha sponges from the Fernando de Noronha Archipelago, Brazil

Bruno Francesco Rodrigues de Oliveira ORCID iD[1], Danilo Souza Almeida[2], Guilherme Muricy ORCID iD[2], Marinella Silva Laport ORCID iD[3]

[1]Department of Microbiology and Parasitology, Biomedical Institute, Fluminense Federal University, Niterói, Brazil. [2]Department of Invertebrates, National Museum, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [3]Department of Medical Microbiology, Institute of Microbiology Paulo de Góes, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract

The smallest class of the Porifera phylum, Homoscleromorpha, shares an ecological preference for shadowed or dark areas, including submarine caves and their surroundings. There are few studies assessing the microbiome associated with cave-dwelling members of this sponge class, whose microbiology remains scantly investigated. This study aimed to characterize the microbial communities in four species of Homoscleromorpha sponges (*Plakinastrella microspiculifera*, *Plakortis insularis*, *Plakortis microrhabdifera* and *Oscarella* sp.) sampled in two submarine caves and their vicinity located in Fernando de Noronha archipelago, Brazil. A metataxonomic approach was adopted, with Illumina sequencing of the V4 region of the 16S rRNA gene and all-encompassing bioinformatic data analyses. A total of 1,860,148 reads were clustered into 951 amplicon sequence variants (ASVs), which belonged to 23 different bacterial phyla. Chloroflexi was the top dominant phylum in all sponges, followed by Pseudomonadota, Poribacteria, Acidobacteriota, Actinomycetota and the candidate phylum PAUC34f. Families from the Gammaproteobacteria and Alphaproteobacteria classes and the Myxococcota phylum were found in higher abundance in the only sponge species collected outside the cave, *P. microrhabdifera*. Verrucomicrobia reached up to one-tenth of the microbial composition in *Oscarella*, while representing less than 1.0% of the microbial composition of the counterparts. Sponge habitat clearly influenced the overall structure of symbiont microbial assemblages. A single Chloroflexi ASV, affiliated to the SAR202 clade, was the predominant member of the core microbiome in all specimens. All species can be considered high microbial abundance sponges, a relatively novel feature in this poriferan class, opening new avenues in Homoscleromorpha microbiology.

P540

Siderophore production by aquatic isolates of selected Gram-negative bacteria

Alazhar Colombowala ORCID iD[1], Bas Boots ORCID iD[1], David G E Smith[2], Lijiang Song[3], Caray A Walker ORCID iD[1]

[1]Anglia Ruskin Univeristy, Cambridge, United Kingdom. [2]Heriot-Watt University, Edinburgh, United Kingdom. [3]Warwick University, Coventry, United Kingdom

Abstract

The growing problem of bacterial antibiotic resistance makes the discovery and development of new antibiotics increasingly urgent. Current research in this field is focussed on mechanisms to use nutrient-import transporters to transport antibiotics into the bacterial cell. An example of this is exploiting siderophore-dependent pathways for the uptake of iron. In this study, we investigated aquatic environments for bacteria-producing siderophores, as the low iron content and iron complexed with organic ligands is a peculiar feature of the aquatic ecosystem. 110 bacterial isolates were acquired and assessed for siderophore production using the chrome azural S assay. Taxonomical identification using 16S rRNA and whole genome sequencing (WGS) identified an *Enterobacter* species, as a promising isolate, showing high siderophore production. The study also investigated the characterisation of the siderophore using high-performance liquid chromatography (RP-HPLC) and liquid chromatography-mass spectrometry (LC-MS). The results suggest the siderophore produced was aerobactin or a highly similar ligand. However, *Enterobacter* spp. possesses biosynthetic loci for two distinct metallophores namely hydroxamate (aerobactin) and catecholate (enterobactin), surprisingly, RP-HPLC-LCMS did not reveal the presence of enterobactin in the culture supernatant of the *Enterobacter* isolate. As a result, we are continuing to investigate siderophore production by purifying siderophores at different time points and growth phases. The study will also investigate the use of the siderophore as xenosiderophore on mutant *Escherichia coli* strain lacking the *ent*A gene, rendering it incapable of producing fully functional enterobactin. This work will help us understand the transport and synthesis of *Enterobacter* spp. siderophore(s) and their potential applications.

P541

Investigating *Bacillus subtilis* biofilm morphology under different environmental stressors.

Sara Drais[1], Gail McConnell[2], Vernon R. Pheonix[1]

[1]Department of Civil & Environmental Engineering, University of Strathclyde, Glasgow, United Kingdom.
[2]Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Abstract

Biofilm development is fundamental for survival in different environments. This study aims to understand biofilm development under different environmental stressors.

Fluorescent Microscopy. *gfp*-expressing *Bacillus subtilis* pellicle biofilms were grown at 37C at different concentrations of *Gingko biloba* extract. Biofilms under flow condition were developed in sterile flow cells. 2L of LB media at different LB strength were pumped through sterile tubing systems using a peristaltic pump. Systems were each inoculated with 250 µL of diluted culture (OD600 = 0.3) and run for 48 hours at 40 mL/h.

Bacterial Growth Curves. *B. subtilis* cultures were grown at 37C in a shaking incubator and OD_{600} measurements were taken every 30 minutes for 12 hours.

Motility Test. Agar plates with different agar concentrations and different *Gingko biloba* extract were inoculated and incubated overnight at 37C and 30C.

Pellicle biofilms showed a concentration-dependent effect of the extract on biofilm development and morphology, resulting in highly organized filaments. Biofilms formed under a flow displayed multiple multicellular filaments, with tertiary rope-like structures and cellular aggregates. In presence of the extract, bacterial cells showed a shift from rod shaped to spherical cells.

Growth curves showed a delay in bacterial replication compared to the controls. Furthermore, the extract and temperature influenced colony morphology and motility.

B. subtilis biofilms exhibit different spatial arrangements and cellular morphology depending on the environmental stressor, with some inducing filamentation. While biofilms remain a challenge, little is known about biofilm resilience. Findings suggest that *Gingko biloba* can be used as a safe antimicrobial alternative.

P542

RODENTGATE – A European project on future rodent management for pig and poultry health

Hayley Thompson[1], Steve Belmain[1], Judy Bettridge[1], Sophie Bouvaine[1], Daniel Bray[1], Bram Vanden Broecke[2], Katarzyna Domanska-Blicharz[3], Maciej Frant[3], Florian Huels[4], Jens Jacob[5], Malgorzata Juszkiewicz[3], Inge Milou Krijger[6], Herwig Leirs[2], Hope Cletus Okon[1], Goncalo Silva[1], Vincent Sluydts[2], Anna Szczotka-Bochniarz[3], Marek Walczak[3]

[1]Natural Resources Institute, University of Greenwich, Chatham, United Kingdom. [2]University of Antwerp, Antwerp, Belgium. [3]National Veterinary Research Institute, Pulawy, Poland. [4]Institute for Plant Protection in Horticulture and Urban Green, Julius Kühn-Institut (JKI), Münster, Germany. [5]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut (JKI), Münster, Germany. [6]Dutch Pest & Wildlife Expertise Centre (KAD), Wageningen, Netherlands

Abstract

Rodents are a considerable threat to animal health as they are known reservoirs for several pathogens that cause economically significant infectious diseases. The main tool to manage rodent pests is the use of anticoagulant rodenticides. However, these poisons are accumulating in the environment, and many jurisdictions are introducing regulations that increasingly restrict their use. This raises concerns about the potential increase in rodent-borne diseases in livestock and the possibility of the circulation and adaptation of new pathogens that may spill over to humans.

The main objective of RODENTGATE is to investigate the health risks to pigs and poultry from rodents and how this might change with alterations to rodent management practices. Within this we aim to investigate the role of rodents as reservoirs and/or vectors of pathogens and assess pathogen sharing between rodents and livestock.

Rodents on pig and poultry farms in different parts of the UK, Belgium, Netherlands, Germany, and Poland are being sampled twice per year. Blood, tissues and ectoparasites are collected from trapped rodents, along with non-invasive samples from pigs and poultry. Preliminary molecular screening using PCR of rodent samples from the UK, Germany, and the Netherlands, shows that rodents harbour a range of pathogens known to infect poultry and pigs, including *Brachyspira* spp., pathogenic *Leptospira* species and *Lawsonia intracellularis*, which could be spread to pigs and poultry on these farms. Samples will be further screened by metagenomic sequencing to reveal the diversity and genetic variability of the pathogens in rodents and livestock.

P543

Surveillance options for antimicrobial resistance in the wider environment – wildlife and potential sentinel species

Purvi Mali[1], Wiebke Schmidt[2], Alwyn Hart[1], Daniel Read[3], Holly Tipper[3]

[1]Environment Agency, Nottingham, United Kingdom. [2]Environment Agency, Bristol, United Kingdom. [3]UK Centre for Ecology & Hydrology, Wallingford, United Kingdom

Abstract

Antimicrobial resistance (AMR) is one of the most serious global health threats with an estimated 1.27 million deaths in 2019 from antibiotic resistant infections. International organisations have called for increasing surveillance of AMR not just in clinical and veterinary settings but also in environmental matrices and the United Nations Environment Programme recognised AMR as one of the most critical pollution issues for the environment. As a result, many authors such as O'Neill have called for surveillance of AMR in environmental settings.

However, there are many practical problems to overcome in adequately sampling and analysing environmental compartments to understand the AMR burden present. The use of indicator organisms such as ESBL Escherichia coli may provide timely data but not reflect the true diversity of organisms and genes present. Conversely sequencing techniques may provide more detail but take longer and at higher cost affecting the sample density that can be supported. A compromise may be to look for sentinel higher organisms within a food chain that can be used to report on environmental quality and AMR presence.

This poster will report on 2 projects carried out for the Environment Agency to evaluate a selection of existing wildlife surveillance efforts in England as a starting point for an environmental AMR surveillance programme. The report also reviews the current literature on AMR in wildlife (including wild animals and plants) to identify key AMR hosts and markers for surveillance.

P544

The transcriptomic landscape of lignocellulose degradation by anaerobic gut fungi.

Jessica Matthews[1,2], Stephen C. Fry ORCID iD[2], Jolanda van Munster ORCID iD[1]

[1]SRUC, Edinburgh, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom

Abstract

Anaerobic gut fungi (AGF) are powerful degraders of lignocellulose – a potentially renewable alternative to fossil fuels. To exploit AGF in renewable-based biotechnology, we must understand their degradative mechanisms. The colonisation of lignocellulose by AGF, although integral to both their lifecycle and degradative mechanisms, currently is poorly understood. Here we aim to understand how carbohydrate recognition and uptake drive the colonisation of plant biomass by AGF in the rumen.

The variability of how inducers affect the enzymatic activities generated by AGF during the degradation of plant biomass will be assessed. We will use time-course RNA-SEQ and untargeted proteomics to identify AGF gene expression and protein production during the colonisation of wheat straw, a major biofuel feedstock. We will assess if AGF activity is constitutive, or whether there are temporal changes in activity in response to changing composition and structure of wheat straw. To investigate this further, the transcriptomic response will be compared with that of AGF grown on individual plant components of potential industrial interest, e.g., cellulose, arabinoxylan, and galactomannan. Examples of degradative enzymes, such as glycoside hydrolases, carbohydrate esterases and polysaccharide lyases, that degrade specific polysaccharides of lignocellulose will be assayed via detection of their reaction products using thin-layer chromatography and HPLC.

Together, these studies enable the identification of enzymes that AGF employ as part of their degradative mechanisms and elucidate how their production is affected by lignocellulose-derived inducers. This knowledge will be exploited to create combinations of fungi and lignocellulose with increased potential in selective lignocellulose pre-treatments for bioprocessing.

P545

Characterization of communal sink drain communities of a university campus

Zoe Withey ORCID iD[1], Tim Goodall[2], Sheila MacIntyre[1], Alisha Awan[1], Naol Duguma[1], Elsie Fell[1], Naomi J. Martinez[1], Ed Neary[1], Hyun S. Gweon ORCID iD[1,2]

[1]University of Reading, Reading, United Kingdom. [2]UK Centre for Ecology & Hydrology, Wallingford, United Kingdom

Abstract

Microorganisms are widely distributed throughout the built environment and even those found in concealed environments such as sink P-traps can have an impact on our health. To date, most studies on sink microbial communities focused on those present in hospitals with no to little information regarding sinks in residential or communal settings.

We conducted studies characterizing microbial communities of over 250 communal restroom sinks across a university campus using 16S rRNA and ITS sequencing to investigate the diversity, prevalence, and abundances of the microorganisms that reside in this understudied environment.

Bacterial community compositions and structures were highly variable across individual sinks, while there were marginal differences between buildings. Proteobacteria were the most abundant phylum in the sink communities, and the families *Burkholderiaceae*, *Moraxellaceae*, and *Sphingomonadaceae* were found to be ubiquitous across all sinks. Notably, human skin was identified as a primary contributor to the sink bacterial community. Mycobial communities, however, were remarkably consistent in their composition across buildings, consistently dominated by Ascomycota. We found a core mycobiome independent of the building sampled, that included the genera *Exophiala*, *Saccharomyces* and *Fusarium*. Similarly, the frequent presence of *Malassezia*, a common skin commensal, further demonstrated the external influence of human activities as a potential source of microorganisms to sinks.

These studies provide novel insight into the sink bacterial and mycobial communities' constituents and highlights their importance as reservoirs of possible pathogens as well as, providing the foundation for subsequent studies that might explore community stability and resilience of in situ sink drains.

P546

Isolation of a Novel Welsh Myxobacterium, *Corallococcus psilocybin* sp. and Its Potential to Predate Against Clinically Important Bacteria and Yeasts

Benita Arakal, Michael McCarthy, Paul Livingstone

Cardiff Metropolitan University, Cardiff, United Kingdom

Abstract

Corallococcus spp. are common soil-dwelling organisms which kill and consume prey microbes through the secretion of antimicrobial substances. In this study, a novel myxobacterial strain RDP092 was isolated from a soil sample in Senghennydd, Wales using *C. albicans* as its prey-bait. Based on their morphological characteristics, this bacterial strain is a bacilli-shaped, Gram-negative organism that displayed the general features of *Corallococcus*, including orange-pigmented fruiting bodies and gliding motility on agar. Strain RDP092 was characterized as an aerobic and chemoheterotrophic bacterium resistant to many antibiotics. The strain showed the highest 16SrRNA gene sequence similarity to *Corallococcus* sp. EGB (99.37%) and *Corallococcus exiguus* NCCRE002 (99.13%), and formed a novel branch, both in the 16SrRNA gene sequence and phylogenomic tree. Furthermore, genomic comparison of strain RDP092 against all *Corallococcus* genomes revealed that these genomes shared an average nucleotide identity below 95% and digital DNA-DNA hybridization scores of less than 70%, indicating that they belong to distinct species. The size of the genome was 9,387,726 bp, with a DNA G+C content of 70.6 mol%. Interestingly, strain RDP092 exhibited a wide range of predatory activity against bacteria such as *E. coli, P. aeruginosa, C. freundii, S. aureus* as well as fifteen fungal pathogens which include *C. glabrata* and *C. parapsilosis*. In reference to the breadth of antimicrobial activities observed by the novel *Corallococcus* isolate as well as its polyphasic taxonomic characterization, this research proposes that strain RDP092 represents a novel species, *Corallococcus psilocybin* sp. highlights their antimicrobial potential in eradicating clinical bacterial and fungal biofilms.

P547

Prevalence assessment of a five months study of E.coli of the final effluent at the Dimbaza wastewater treatment works.

Olayinka Osuolale ORCID iD

Elizade University, Ilara Mokin, Nigeria

Abstract

The final effluents of Dimbaza wastewater treatment plants (WWTPs) were evaluated to determine their physiochemical quality and prevalence of E.coli. The selective media for E.coli was mostly negative for the organism. The mean total E.coli counts for the five months was 2.7×10^3 (cfu/100 mL), The final effluents were generally in compliant with the recommended limits for pH, temperature, total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity, phosphate, nitrite and nitrate. The study reflect the effectiveness of the wastewater treatment plant. Ensuring that the treated effluent returned to the environment is of a safe quality and does not lead to any grievous health risks.

P548

The utilisation of anammox for the treatment of landfill leachate using constructed wetlands

Cerys Maryan[1], Yueyue Si[2], Mark Trimmer[2], Victoria Hemsley[3], Marcela Hernández[1], J.Colin Murrell[1], Laura Lehtovirta-Morley[1]

[1]University of East Anglia, Norwich, United Kingdom. [2]Queen Mary University of London, London, United Kingdom. [3]Norfolk County Council, Norwich, United Kingdom

Abstract

Ammonium-rich leachate is a major issue in decommissioned landfill sites, with treatment and haulage costing £240,000 annually in Norfolk alone. Constructed wetlands provide a sustainable alternative by treating leachate in-situ using microbial anammox (anaerobic ammonia oxidation). Unlike conventional treatment, the anammox pathway does not emit the greenhouse gas N_2O and is more cost effective. The aims of this study were to assess the diversity and activity of anammox bacteria, in addition to the greenhouse gas potential of the vertical flow constructed wetland fed with landfill leachate.

Bacterial diversity of the wetland was assessed by 16S rRNA gene amplicon sequencing. Anammox species from the genera *Kuenenia* and *Brocadia* were detected in addition to an aerobic nitrifier, *Nitrospira* sp. and a potentially novel anammox species. Anammox activity was confirmed using the isotopic tracer $(^{15}NH_4)_2SO_4$ in anaerobic microcosms by measuring the $^{29}N_2$ in the headspace by GC-MS. The abundance of hydrazine oxidoreductase (*hzo*), a gene unique to anammox, was quantified using qPCR. The abundance of anammox bacteria present in the constructed wetlands was comparable to successful anammox-driven wastewater treatment facilities pioneered in the Netherlands.

The greenhouse gas potential of the system has been monitored to further the sustainability of this leachate management strategy. The use of nitrification inhibitors, including allylthiourea, have been trialled to attenuate the activity of AOBs such as *Nitrospira* sp. to limit the emissions of N_2O. Optimising the operation of anammox bacteria in constructed wetlands can provide a novel, cost-effective, environmentally sustainable leachate management strategy.

P549

Characterising bacterial communities in hospital sinks

Helen Rickard[1], Lena Ciric[1], Elaine Cloutman-Green[2,1], Faiza Asadi[2]

[1]UCL, London, United Kingdom. [2]Great Ormond Street, London, United Kingdom

Abstract

Hand washing is the most effective way of reducing healthcare associated infections (HCAI), however, hospital sinks are reservoirs of potential pathogens and have been implicated in several outbreaks. Sinks provide a unique habitat for microorganisms close to staff and patients, and provide a niche for biofilm formation. P. aeruginosa is a leading cause of HCAI acquired from plumbing, however, little is known about other Pseudomonas species colonising the same areas. This study aims to determine the effect of patients on the sink microbiota, and characterise the sink Pseudomonas population in terms of drug resistance and virulence factors. Sink swabs were taken before and after the opening of two new wards at Great Ormond Street Hospital. Ward A was a respiratory ward and Ward B was for post-operative care. Culturable bacteria were identified by MALDI-TOF, and antibiotic and disinfectant sensitivity profiles conducted. Pseudomonas species were tested for key virulence factors through phenotypic assays. 106 isolates were recovered from 45 sinks across the two wards. Prior to opening to patients Cupriavidus gilardii was the most common isolate. After opening, the most frequent isolates from Ward A were Pseudomonas species, and from Ward B Enterobacter species. 24 Pseudomonas isolates were recovered, including five P. aeruginosa. Of the 24 strains 25% produced pigment, 58% were haemolytic, 87.5% swam, 83.3% showed twitching motility, 33.3% produced alkaline protease and 8.3% gelatinase. This study demonstrates patients can influence microbial communities within sinks, and that several 'environmental' Pseudomonas species produce the same virulence factors as P. aeruginosa.

P550

The detection and potential survival of Legionella in saline environments.

Christina Scott, Joy E.M. Watts

University of Portsmouth, Portsmouth, United Kingdom

Abstract

Legionella are well known freshwater bacteria, the genus has a high public profile as opportunistic pathogens of man-made water services and spread of disease through aerosol production. Saltwater is not currently, a known reservoir for Legionella, however, it has been detected in a number of DNA based analyses of marine and estuarine environmental samples. Legionella can survive, and potentially persist in the environment via two routes as intracellular pathogens or entering a viable but not culturable state (VNBC), therefore, recovery of legionella in the laboratory using culture-based techniques can be limited.

Samples have been collected from the Solent and presumptive legionella colonies have been isolated using the international standard culture-based technique. To confirm identify DNA was extracted and Subjected to PCR amplification using universal 16S rRNA primers and specific Legionella primers that have confirmed Legionella species recovery and isolation.

Saltwater microcosms have been constructed to better understand the survival of Legionella in the environment using a range of culture based and molecular methods, the findings will be discussed.

It is critically important to understand the survivability of Legionella in seawater, to determine if Legionella could act as an opportunistic pathogen in environmental settings posing a risk to human health.

P551

“Ecological suicide” of *C. jejuni* via T6SS dependent functionality: a novel death-pathway of gut pathogens

Subhadeep Gupta, Bishnu Das, Parna Gupta, Dipjyoti Das, Amirul Islam Mallick

Indian Institute of Science Education and Research Kolkata, Mohanpur, India

Abstract

Indiscriminate antimicrobial use can alter microbial composition in the gut, destabilizing gut homeostasis and impairing gut barrier function. While most gut pathogens are armed with the intrinsic ability to thrive in the harsh gut environment, selective dysbiosis of gut colonizing pathogens is often challenging. We demonstrated the unique attributes of Campylobacter jejuni (C. jejuni), which naturally inhabits chicken ceca and is the single largest source of human infection. Though the presence of T6SS, can facilitate bacterial predation and host pathogenesis, how C. jejuni harboring functional T6SS coexists with other resident microbes is unknown. It is also unclear whether they have any role in the perturbation of gut homeostasis during their residence. Here, we demonstrate a novel ecological consequence of T6SS functionality as an example of “Ecological suicide” in an altered environment. Using bile salt as a gut stressor and E. coli as a T6SS target, we showed a significant reduction in T6SS-positive cells compared to T6SS-negative. Further, we elucidate the underlying mechanism by tracking Iridium-conjugated bile salt, which suggests T6SS-mediated stressor influx in C. jejuni, leading to enhanced oxidative stress and subsequent cell death. Using chickens as primary hosts, we also show that prey-driven activity of T6SS can perturb in vivo cecal colonization of C. jejuni in chickens administered with bile salt solution. Together, we illuminate how T6SS-dependent predation can lead to self-killing under an altered gut environment and highlight the prospect of using this unique feature of T6SS-dependent "predation cost" as an "antibiotic alternative" approach for improving gut health.

P552

Identification and potential use of arsenic resistant bacteria in bioremediation of contaminated soil through biosequestration

Soma Ghosh[1,2], Tarit Roychowdhury[3]

[1]Adamas University, Barasat, India. [2]Jadavpur University, Kolkata, India. [3]School of Environmental Studies, Jadavpur University, Kolkata, India

Abstract

Eight potential As resistant bacterial strains isolated from arsenic (As) contaminated paddy fields of West Bengal have been used in consortium to study the biosequestration of As by ad-/absorption mechanism in microcosm set ups. The consortium showed maximum tolerance to 600mM As(V) and 10mM As(III). Ad-/Absorption kinetics indicated that isolates could accumulate As(III) and As(V) in the range 0.1 – 0.8%; 0.28- 0.58% and 1.6 – 43%; 17.25 - 57.06% through adsorption and absorption, respectively. Microcosms were set under eight conditions: sterile/ non sterile, medium/ highly As contaminated paddy soil under flooded/ non-flooded condition with consortium. Major growth of the consortium occurred between 15-60 days of incubation. Estimation of As revealed substantial decrease (0.67–0.80 fold) in As concentration of the soil from 0- 90 days. Increase of As concentration (1.92-8.65 fold) in soil washed water and that in aqueous phase from anaerobic incubations (1.33–10.81 fold) from 0th-90th day indicated the mobilization efficiency of the consortium applied. Efficient bioaccumulation by the consortium was also found which increased consistently throughout the 90 days showing a maximum of 8.27 fold increase in non-flooded incubation with non-sterile highly contaminated soil. However, biomass harvested from aqueous phase of flooded incubations showed 3-4 fold higher accumulation than that found in biomass from their respective soils. Phylogenetic identification confirmed that they belonged to genus Bacillus, Paenibacillus, Staphylococcus and Ralstonia. The study provides a novel insight for potential use of these bacteria in bioremediating As contaminated paddy fields through biosequestration approach.

P553

Surveillance of SARS-CoV-2 RNA in open water sewage canals in India

Paramita Basu ORCID iD[1], Sandeepan Choudhury ORCID iD[2], Varsha Shridhar ORCID iD[3], Samrat Roychoudhury ORCID iD[4], Indranil Nandi[5], Angela Chaudhuri[6], Arindam Mitra ORCID iD[7]

[1]New York College of Podiatric Medicine, New York, USA. [2]Independent Water and Wastewater Consultant, Bengaluru, India. [3]Molecular Solutions Care Health, Bengaluru, India. [4]Indian Academy Degree College, Bengaluru, India. [5]Jubilant Pharma, Philadelphia, USA. [6]Swasti Health Catalyst, Bengaluru, India. [7]Adamas University, Kolkata, India

Abstract

Background

A sensitive, robust, and economical method of SARS-CoV-2 RNA detection from open waste-contaminated water bodies in resource-constrained regions is not in place. A protocol was employed in Bangalore, India, where SARS-COV-2 RNA levels were evaluated using two open canal systems during the first and second waves in the present study.

Method

SARS-CoV2-RNA was measured using two strategies: a modified TrueNAT™ microchip-based rapid method and traditional Real-time reverse transcription-polymerase chain reaction (rRT-PCR), which were compared for analytical sensitivity, cost, and relative ease of use. SARS-CoV2-RNA was measured using two strategies: a modified TrueNAT™ microchip-based rapid method and traditional Real-time reverse transcription-polymerase chain reaction (rRT-PCR), which were compared for analytical sensitivity, cost, and relative ease of use.

Results

SARS-CoV-2 RNA levels were detected at lower levels during the earlier half compared to the latter half of the first wave in 2020. The opposite trend was seen in the second wave in 2021. Interestingly, the change in RNA levels corresponded with the community burden of COVID-19 at both sites. The modified TrueNAT™ method yielded concordant results to traditional rRT-PCR regarding sensitivity, specificity, and cost.

Conclusions

This study provides a simple, cost-effective method for detecting and estimating SARS-CoV-2 viral RNA from open-water sewage canals contaminated with human excreta and industrial waste. This strategy can be adopted in regions or countries that lack structured sewage systems.

P554

Assessment of the risk to consumers as a result of disruption to the cold chain during direct supply of Qurbani meat and offal

Katy Moran, Iulia Gherman ORCID iD, Erica Kintz ORCID iD, Paul Cook, Anthony Wilson ORCID iD

Food Standards Agency, York, United Kingdom

Abstract

Background

Qurbani is a religious practice during Eid al-Adha. People practicing Qurbani normally wish to collect meat quickly after slaughter, meaning these products cannot complete normal chilling processes before leaving the slaughterhouse.

Methods

We assessed the risk to consumers from non-typhoidal *Salmonella enterica*, Shiga toxin-producing *Escherichia coli*, and *Clostridium perfringens*, considering prevalence and growth characteristics as well as the results of several surveys targeting food business operators, official veterinarians and consumers to understand factors such as chilling achieved post-slaughter, conditions of transportation to consumers and consumer storage and cooking behaviours. We also considered two scenarios: a baseline and a reasonably-foreseeable worst case.

Results

Our assessment suggests that under the baseline scenario the risk to consumers is not significantly higher than normal (*Very Low*: “very rare, but cannot be excluded”), but, in a reasonably foreseeable worst-case scenario, the risk to the consumer is higher (*Low*: “rare but does occur”). Significant uncertainties remain, including carcase and offal temperature when it reaches consumers, epidemiological data linking cases of illness to consumption of Qurbani meat and offal, and the prevalence and enumeration levels of the three pathogens in meat, especially goat, and offal.

Conclusion

Our risk assessment has now been published to support a public consultation which will be used to inform FSA policy on recommending mitigations to support Qurbani practices while maintaining consumer safety.

P555

Bacterial contamination of boar semen affects their quality and storability

NUTTHEE AM-IN ORCID iD[1,2], Phoo Pwint Pwint Thu[1]

[1]Department of Obstetrics, Gynecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. [2]Center of Excellence in Swine Reproduction, Chulalongkorn University, Bangkok, Thailand

Abstract

Bacterial contamination in boar semen can happen both during semen processing and at time of semen collection, which may be harmful to the semen quality. The aim of this study is to investigate on the boar semen contamination by bacteria and the correlation between the presence of *Escherichia coli*, sperm agglutination, and their storability. 102 semen samples were collected from sexually mature boars from 4 farms in Thailand. The evaluation of the semen sample was assessed, complied with international standards in terms of volume, pH, color, and sperm motility. Additionally, the semen samples were examined for bacterial contamination and the presence of agglutinated sperm cells. All samples were extended BTS and stored at 18°C for 3 days. The sperm motility and viability were evaluated every day. The results show that *E. coli* was the most common contamination, found in 71.56% of the semen samples (n= 73), and 75% of the ejaculates were contaminated with at least one kind of bacterium. *Enterobacter* (n = 26), *Klebsiella* (n = 12), *Proteus* (n = 42), *Pseudomonas* (n = 12), *Serratia* (n = 37), *Staphylococcus* (n = 21) and *Streptococcus* (n = 23) were among the other contaminating bacterial genera. Pearson's analysis of the data present that sperm agglutination and *E. coli* presence are positively correlated, while sperm agglutination and motility and viability of day 1, 2 and 3 of storage are negatively correlated. The extended semen was significantly reduced of storability when contaminated with more than 3.87×10^3 CFU/mL ($P<0.05$).

P556

Isolation of bioactive compounds from low-cost agricultural resources and its utilization in daily life.

Arpita Das ORCID iD, Anirban Debnath ORCID iD

Adamas University, Kolkata, India

Abstract

Background – *Oxalis corniculata Linn*. Also known as creeping woodsorrel it has phytochemical compounds, including flavonoids, tannins, phytosterols, phenol, glycosides, fatty acids, and volatile oil. Flavonoids can be employed as ingredients in cosmetics and medicinal goods because of their characteristics. We aimed to create a new innovative therapeutic soap with added benefits obtained from low-cost agricultural resources, which can be commercialized, and the same should be under the best-fit budget of middle-income countries.

Methods – Antioxidant, and antimicrobial activity was measured in the case of crude extraction and final product. A survey on benefits people expect from soap is listed also monthly expenditure on soap has been estimated.

Results – In the quantitative analysis of antioxidative components, crude samples had the highest amount of ascorbic acid and chlorophyll. In the analysis of 2,2-diphenyl-1-picrylhydrazyl scavenging ability, the final soap formulation exhibited the highest activity among the samples. The antibacterial capacity of the final product has shown significant variation from the crude product as well as from standard soap brought from market. Cost calculation was done for the final soap formulation.

Conclusion – We have created an organic soap from *Oxalis corniculata,* or creeping woodsorrel. Bioactive compounds present in its leaves have been found to contain antioxidant, antimicrobial, and antifungal properties. Based on the results, we find that an appropriate price for our prepared fortified soap would be approximately Rs. 25-30. Therefore, the fortified soap prepared by us is both beneficial to the skin and less expensive compared to other commercial soaps.

P557

Incidence of *Babesia* in ticks at National Parks and Areas of Outstanding Natural Beauty in England and Wales

Sanam Sewgobind ORCID iD[1], Sophie Harding[1], Insiyah Parekh ORCID iD[1], Karen Mansfield ORCID iD[1], Luis Hernández-Triana[1], Sara Gandy[2], Kayleigh Hansford[2], Jolyon Medlock[2], Nicholas Johnson[1]

[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]UK Health Security Agency, Porton, United Kingdom

Abstract

Babesia species are haemoparasites that cause haemolytic anaemia. Depending on the *Babesia* species and the host's immune system, the disease severity might result in chronic infection or acute illness. They are mainly transmitted by tick vectors and have zoonotic potential. The purpose of this study was to determine the incidence of *Babesia* species found in ticks at National Parks and Areas of Outstanding Natural Beauty (AONB) between 2014 and 2019.

A total of 3,932 *Ixodes ricinus* nymphs were collected from 21 locations across England and Wales. DNA was extracted and individual ticks tested for the presence of *Babesia*, using a pan-piroplasm PCR, which targets the 18S rDNA sequence. Amplicons were confirmed by agarose gel electrophoresis, and Sanger sequencing. Sequences were then compared against those deposited in the NCBI database.

The overall prevalence of *Babesia* species was low at 0.38% ± 0.19. In southern England, the following species were detected: *B. venatorum* (n = 9), *B. capreoli* (n = 4), *B. odocoilei*-like (n =1) and *B. divergens* (n = 1). There was a persistent presence of *B. venatorum* at the Cotswold site between 2017 and 2019. Moreover, maximum diversity of *Babesia* species occurred in 2019.

Within England and Wales, *Babesia* species were present at a low prevalence. *B. venatorum* was the most frequently detected *Babesia* species and one with zoonotic potential. However, the low prevalence within tick populations in England and Wales suggest that the risk of human infection is equally low.

P558

Isolation and characterisation of *Burkholderia* species causing onion rot

Rebecca Weiser ORCID iD[1], Kiri Mack[2], Louise Disbury[2], Peyton Goddard[2], Andy Richardson[3], Sam Rix[4], Ed Pissarro[4], Eshwar Mahenthiralingam ORCID iD[1], Alison Blackwell[2]

[1]Cardiff University, Cardiff, United Kingdom. [2]APS Biocontrol Ltd., Dundee, United Kingdom. [3]Allium and Brassica Agronomy Ltd., Lincoln, United Kingdom. [4]P. G. Rix Farms Ltd., Colchester, United Kingdom

Abstract

Burkholderia is a diverse genus containing species that are pathogenic to crop plants including rice and onions. In the UK, bacterial onion rot can cause up to £9M losses per annum and *B. gladioli* has been identified as the primary rot pathogen. However, this identification has been based on disease symptoms and basic culture microbiology which is often unreliable for differentiation of *Burkholderia* species. Our study systematically investigated *Burkholderia* as onion rot pathogens using a combination of phenotypic and genetic methods for improved identification and characterisation.

Burkholderia were isolated from different varieties of onion seeds, sets (immature onion bulbs) and mature onion bulbs using selective media. Isolates were confirmed as *Burkholderia* or *B. gladioli* using genus- and species-specific PCRs and a PCR fingerprinting technique used to differentiate strains. Sequencing and analysis of 16S rRNA and *recA* genes was performed for species identification. An *in vitro* onion tissue maceration assay was used to assess strain virulence.

Over 350 samples were tested and 192 *Burkholderia* isolates cultured from 15 brown and 42 red mature onion bulbs. The majority of isolates were *B. gladioli* (181/192), other species identified included the closely related *B. perserverans*. Multiple (>15) *B. gladioli* strain types were found and differed in their ability to induce onion rotting *in vitro*.

Our results correspond with agricultural industry findings that *B. gladioli* is the most common *Burkholderia* species in UK onion rot. Further investigations correlating onion rotting properties with *B. gladioli* phylogenomic clades will improve our understanding of this agriculturally important disease.

P559

The Necrobiome: Analyzing Microbes Associated with Human Decomposition for Post-mortem Interval Estimation

Nengi Ogbanga ORCID iD[1], Andrew Nelson[1], Sarah Gino[2,1], Hayley Mickleburgh[3,4,5], Noemi Procopio[6,2,4]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]University of Eastern Piedmont, Novara, Italy. [3]University of Amsterdam, Amsterdam, Netherlands. [4]Texas State University, Texas, USA. [5]Linnaeus University, Växjö, Sweden. [6]University of Central Lancashire, Preston, United Kingdom

Abstract

Microorganisms are essential to the decomposition of organic matter, including human remains. Forensic research on the necrobiome aims to understand the shifts in microbial community structure and composition during decomposition, particularly for post-mortem interval (PMI) estimation. However, much remains unknown regarding utilising the necrobiome for PMI estimation, especially in extended decomposition periods. Furthermore, these microbial shifts are also unexplored in the context of mass graves.

Hence, this project aims to analyse microbial communities on human cadavers at various anatomical sites – and burial soil – during decomposition and to evaluate the better predictor of PMI among the sites analysed. It also compares microbial succession in single and mass burials to explore the effect of the presence of multiple bodies on PMI prediction. Finally, the project will explore spatial distribution of microbes within the graves throughout the decomposition period.

For this, nine human cadavers donated to Texas State University's Forensic Anthropology Research Facility (FARF) were included in this study. Three cadavers were buried individually and the remaining six were placed together in a mass grave. Human microbial samples were collected by swabbing oral cavity, rectum, hand, foot and neck of each cadaver before burial, and upon excavation 18 months later, where available. Soil samples were also collected at regular intervals throughout the experimental period.

Using 16S rRNA gene metabarcoding, this research will add to existing knowledge on the implementation of the microbiome for PMI estimation and introduce a novel aspect on the behavior of microbial communities within a mass grave.

P560

Effectiveness of Ultraviolet-C mobile phone sanitizing device in the eradication of adhered bacterial cells

Rania Nassar[1,2], Lobna Mohamed[1], Syrine Boucherabine[1], Rashed Alghafri[3,4,5,6], Lotti Tajouri[3,4,6], Abiola Senok[1]

[1]College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE. [2]School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom. [3]General Department of Forensic Sciences and Criminology, Dubai Police, Dubai, UAE. [4]Dubai Police Scientists Council, Dubai, UAE. [5]Harry Butler Institute, Murdoch University, Murdoch WA 6150, Australia. [6]Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia

Abstract

Background: Mobile phones (MPs) exhibit microbial contamination and are fomites for pathogen dissemination. Ultraviolet-C devices (UV-C-device) have been introduced for MPs sanitization. The aim of this study was to evaluate the efficacy of a UV-C-device in eradicating surface adhered bacterial cells.

Methods: *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus warneri* and methicillin resistant *Staphylococcus aureus* (MRSA), isolated from healthcare workers' MPs and *S. aureus* ATCC 25923 and *E. faecalis* ATCC 51299 were used. To assess UV-C-device effectiveness, an in-house single-species experimental model for all isolates and dual-species model utilizing *S. warneri*, *S. aureus* and the two reference strains was developed. Briefly, 3-ml of bacterial suspension was prepared and placed in 35mm sterile petri-dishes and left to adhere for 1hr and 2hr. Adherent cells were treated in UV-C-devices for 20, 40 and 60s and colony forming units (CFUs) measurements were used to assess effectiveness.

Results: Treatment for 20s caused 100% reduction in 1hr-adhered bacterial cells except for the dual-species *S. aureus+E. faecalis*. Treatment resulted in a mean $\log_{10}$CFU/ml reduction ≥ 5 in 2hr-adhered bacterial cells, except in *E. faecalis* with mean $\log_{10}$CFU/ml reduction of 3.7, 4.5 and 6.2, after 20, 40 and 60s, respectively. For 2hr-adhered MRSA, *E. faecalis* and dual-species *S. warneri*+MRSA, 100% reduction occurred at 40s-treatment. For dual-species *S. aureus*+*E. faecalis*, 40 and 60s-treatment resulted in *S. aureus* 100% reduction and $\log_{10}$CFU/ml reduction of 7.3 and 7.9 in *E. faecalis*, respectively.

Conclusion: UV-C-devices showed a time-dependent bactericidal effect highlighting the potential usefulness of MP sanitization particularly in healthcare settings.

P561

Utilising 16S ribosomal RNA gene sequencing, metagenomics and Whole Genome Sequencing on UK bathing waters.

Aliyah Debbonaire, Arwyn Edwards

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

UK bathing waters have been a subject of interest in 2022 given the pumping of raw sewage into coastal waters. Currently, microbial monitoring of designated bathing waters rely on plate counts of *E. coli* and Intestinal Enterococci, though efforts are being made to utilise and standardise molecular biology. qPCR using primers specific to hosts whose faecal pollution may end up in the waters in one example of Microbial Source Tracking. In Wales, water samples collected and filtered over the 2022 summer bathing season have resulted in >800 samples ready to be analysed. Genomic DNA has been extracted from all samples and subject to PCR according to their 16S rRNA gene and the meta-barcoded libraries have been sequenced using the MinION mk1C. This has enabled the generation of bacterial community profiles for each sample collection site and date, including specific 'wet weather' events. By seeing how the bacterial community changes over the season and in response to wet weather events- events more common in response to climate change at a higher resolution than before, we can propose effective mitigating strategies. Furthermore, it is hoped these profiles will inform development of novel qPCR primers that may show higher sensitivity and specificity than their predecessors. Additionally, a subset of samples will undergo true metagenomic sequencing, and any Metagenome Assembled Genomes arising from these libraries may also inform novel qPCR primer development. Furthermore, coliforms used for plate counts have been captured and some will undergo WGS, capturing strain level differences in Faecal Indicator Bacteria.

P562

Combined *in vivo* and *in situ* genome-resolved metagenomics reveals novel symbiotic nitrogen fixing interactions between non-cyanobacterial diazotrophs and microalgae

Udita Chandola[1], Camille Trottier[1], Marinna Gaudin[2,3], Erik Manirakiza[1], Samuel Menicot[1], Isabelle Louvet[4], Thomas Lacour[5], Timothée Chaumier[1], Atsuko Tanaka[6], Samuel Chaffron[2,3], Leila Tirichine[1]

[1]Nantes Université, CNRS, US2B, UMR 6286, Nantes, France. [2]Nantes Université, École Centrale Nantes, CNRS, LS2N, UMR 6004, Nantes, France. [3]Research Federation for the Study of Global Ocean Systems Ecology and Evolution, FR2022/Tara Oceans GOSEE, F-75016, Paris, France. [4]Nantes Université, CNRS, CEISAM, UMR 6230, F-44000, Nantes, France. [5]fremer, PHYTOX, PHYSALG, Rue de l'Ile d'Yeu, 44311, Nantes, France. [6]Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, 903-0213, Okinawa, Japan

Abstract

Biological nitrogen fixation (BNF) is a predominant source of nitrogen in low-nutrient surface sun-lit oceans, supporting primary productivity. BNF is accomplished by some bacteria and archaea referred to as diazotrophs, which, in marine system, are conventionally considered to be mainly cyanobacteria. However, non-cyanobacteria diazotrophs (NCDs) were shown to dominate in surface waters, raising fundamental question on how they contribute to the marine BNF. We used a combination of metagenomics, microbiology isolation techniques, bioassays and bioinformatics to conduct this study. In our research, we identified a xenic accession of *Phaedactylum tricronutum* (*Pt*), able to survive in absence of bioavailable nitrogen source. We conducted metagenomic analysis of the bacterial community associated with the model diatom in nitrate- deplete (-N) and -replete (+N) conditions and identified different NCDs of mainly Sphingomonadaceae and Rhizobiaceae. We isolated several NCDs from *Pt15* and demonstrated their nitrogen fixation using a combination of different bioassays and *in silico* analysis. Our data indicated a clear taxonomic and functional shift of NCDs in -N in comparison to +N. Additionally, we showed the prevalence of NCDs in other microalgae and in the environment supporting further the ecological relevance of NCDs symbiosis with microalgae. Overall, this study provides evidence and new perspectives on an earlier overlooked aquatic symbiosis using an integrative model-based approach which will aid in further understanding the different facets contributing to marine BNF.

P563

COMPARATIVE METABOLOMIC ANALYSIS OF PHYLOGENETICALLY DIVERSE ACTINOMYCETES FROM SCOTLAND

Marla Itzel Macias Contreras, Katherine Duncan

Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

Abstract

Bacteria within the order Actinomycetales (often called actinomycetes) are a rich source of novel bioactive metabolites, yet the correlation of ecological niche to both biological and chemical diversity is not well understood. Rare actinomycetes, are all genera within this order except for the genus Streptomyces and are named due to their less-frequent study. As such, rare actinomycetes from underexplored habitats represent an exciting opportunity to focus on natural product discovery. Here, we have established a rare actinomycetes culture collection from Scotland sediment. Isolated actinomycetes were compared taxonomically (16S rRNA gene sequencing) and phylogenetically and profiled for their ability to inhibit the ESKAPE (E. coli, S. aureus and B. subtilis) pathogens. Strains showing antibacterial activity were cultured under temperatures associated with the environment they were isolated from and at 30°C. After 7-days, their metabolites were extracted and profiled using Liquid-Chromatography Mass Spectrometry and compared across taxa using molecular networking. We hypothesize that culturing strains under environmentally relevant conditions will influence microbial chemistry and the knowledge from this work will aid future biodiscovery efforts from diverse ecological niches.

P564

Investigating the antimicrobial efficacy of plasma activated water against isolated spoilage organisms from vacuum packaged meat products and a meat processing environment.

Laura Mc Clenaghan

Queen's university, Belfast, United Kingdom

Abstract

Spoilage organisms are a major concern for the meat industry. Their ability to survive and grow under processing and storage conditions followed by continuous adaptation and increasing tolerance of microbial population to antibiotics and disinfectants has made it even more difficult to control. Although they rarely cause human disease, they can affect the quality of the final product and cause premature spoilage during storage resulting in food waste. Cold atmospheric plasma is a novel and promising alternative technology for microbial inhibition in food processing environments where the antibacterial effects are a result of the low pH and reactive oxygen and nitrogen species. This study aims to identify the spoilage organisms in vacuum packaged meat using Nanopore DNA sequencing and to determine the antibiofilm efficacy of plasma activated water (PAW) generated using a novel plasma bubble reactor.

Sequencing results have shown that lactic acid bacteria including Carnobacterium divergens, Lactococcus piscium, Lactococcus cremoris and Leuconostoc gelidium were the dominant species. Complete inactivation with PAW was obtained in under 60 seconds for all the isolated spoilage organisms. Chemical scavengers were used to specifically quench reactive species generated in the PAW to investigate their roles in antimicrobial activity and showed the short-lived species superoxide and singlet oxygen are responsible for the bacterial inactivation. The impact of PAW on the quality of meat samples during storage was also assessed with no negative sensory changes. Further studies will include biofilm susceptibility with preliminary results showing a high antimicrobial effect against biofilms after a short exposure time.

P565

Glyphosate induces changes to cellular metabolic pathways and selects for antimicrobial resistance in *Klebsiella pneumoniae*

Leonard Koolman, Katie Wall, Guerrino Macori, Séamus Fanning

University College Dublin, Dublin, Ireland

Abstract

Klebsiella pneumoniae is associated with nitrogen fixation in plant roots and is characterized by its hypervirulence and multidrug resistance in clinical settings. Transient exposure to glyphosate in the environment could lead to changes in susceptibility to antimicrobial compounds in *K. pneumoniae* and these changes could lead to more severe community acquired infections that have an impact on public health.

In this study, two *K. pneumoniae* strains (a type strain MGH78578 and field isolate CFS3421) were exposed to a sub-inhibitory concentration of glyphosate (0.25X of the MIC) and antimicrobial susceptibility changes against a panel of antibiotics were monitored using Sensititre plates. In particular glyphosate effects on cellular metabolism (carbon, nitrogen, phosphorous and sulphur utilisation), chemical sensitivity and physiology (pH, osmosis and ionic effects) were studied using phenotype microarrays. Finally, biomass/biofilm formation in the presence of glyphosate and these substrates was determined using crystal violet assays.

When *K. pneumoniae* was exposed to glyphosate it expressed a significant ($P < 0.05$) increase in resistance to imipenem and tigecycline in both study isolates while MGH78578 expressed additional resistance to levofloxacin and nitrofurantoin. PM results showed that metabolic pathways are affected upon glyphosate exposure leading to reduced carbon and nitrogen metabolism potentially affecting its ability to fix nitrogen in roots. Increased biofilm formation to glyphosate exposed strains were observed in pH ranges (pH 3.5 to pH 10).

This study shows that herbicides such as glyphosate can exacerbate antimicrobial resistance, unmasking resistance to tigecycline and carbapenems, as well as inducing virulence traits such as biofilm formation.

P566

Phenotypic studies reveal the potential of the opportunistic pathogen *Klebsiella pneumoniae* to form biofilms on microplastics

Leonard Koolman[1], Stéphanie Devineau[2], Séamus Fanning[1]

[1]University College Dublin, Dublin, Ireland. [2]Université de Paris, Paris, France

Abstract

Plastic debris is ubiquitous in the environment yet our understanding of how microorganisms interact with these pollutants is lacking. This study aimed to elucidate this interaction through various biofilm methods using the opportunistic pathogen Klebsiella pneumoniae. Bacteria were incubated in Mueller-Hinton (MH) broth (nutrient-rich media) or M9 minimal media (nutrient-poor media) in the presence or absence of polyethylene (PE) or polypropylene (PP) microplastics at 25°C and 37°C. Crystal violet assays demonstrated that bacteria had reduced biofilm formation on PE and PP microplastics (at a concentration of 9.5 mg/ml) in M9 media yet increased biofilm formation in MH broth at both 25°C and 37°C. Strains CFS0367 and CFS0368, which demonstrate a weak biofilm phenotype under wild type conditions, exhibited increased biofilm formation on PE and PP microplastics. Resazurin assays showed that all cells were metabolizable in MH broth but metabolism was strain-dependent in M9 broth when incubated with PE and PP. Congo Red agar, with 1 mg/ml PE and PP embedded in the agar, demonstrated a red and smooth (RAS) phenotype for all strains with the exception of CFS0367 and CFS0368 which demonstrated a smooth and white (SAW) phenotype. Pellicle formation was consistently observed when strains were exposed to 1 mg/ml PE and PP in MH broth and M9 broth but inconsistent formation occurred in MH broth and was absent in M9 broth under wild type conditions. This study demonstrated that K. pneumoniae is able to use microplastics as a substrate for biofilm formation.

P567

Repurposed anti-cancer drug Titanocene as a novel antibacterial and antifungal agent

Antony Scimone ORCID iD, Afolarin Otusile

University of Bradford, Bradford, United Kingdom

Abstract

With the growing issue of antimicrobial resistance, novel antimicrobial compounds are needed to combat infections. A key barrier in the drug discovery pipeline is the translation from fundamental research to clinical trials. Repurposing existing drugs and compounds that have had clinical success may help to expedite this process.

The anti-cancer compound Titanocene dichloride was screened against *P. aeruginosa, S. aureus,* and *C. albicans* for antimicrobial and antibiofilm activities. Titanocene dichloride was dissolved in DMSO, and the MIC and MBC determined via broth microdilution assay. The effects on both early-stage and established biofilms were determined using the crystal violet biofilm assay. Biofilms were observed via SEM. The cytotoxicity of Titanocene against HDFs and HaCaT cells were assessed.

Titanocene effectively inhibited all organisms (MIC *P. aeruginosa* and *C. albicans* 0.5mg/mL, *S. aureus* 0.25mg/mL). However, it was not effectively bactericidal or fungicidal (MBC/MFC 4mg/mL). MBICs showed Titanocene was effective against early stage biofilms of *C. albicans* (0.5mg/mL) and *S. aureus* (0.25mg/mL). It was ineffective against established biofilms and instead increased the biofilm biomass, with a maximum increase of 300% for *S. aureus* at 0.25mg/mL. SEM confirmed this. where treated biofilms had a mat-like structure. No decrease in viability was observed for HaCaT or HDF cells.

These results indicate that in planktonic or early-biofilm stages, Titanocene may represent a novel treatment avenue for staphylococcal and candidal infections. The increase in biofilm biomass may have implications for industries where biofilms are desirable, such as microbial fuel cells, microbial biofactories and biorefineries.

P568

Methane production from dimethylsulfide in anoxic sediments: Old enzymes, new functions

Ozge Eyice[1], Stephania Tsola[1], Yizhu Zhu[1], Volker Brüchert[2]

[1]Queen Mary University of London, London, United Kingdom. [2]Stockholm University, Stockholm, Sweden

Abstract

Dimethylsulfide (DMS) is an abundant methylated compound with influence on the atmospheric chemistry and the Earth's climate. Additionally, microbial degradation of DMS in anoxic sediments is a source of methane, a highly potent greenhouse gas. However, our knowledge on this process relies on early, cultivation-based studies. We addressed this knowledge gap by studying the diversity and metabolism of DMS degraders across seven sediment layers (1–65 cm) from the Baltic Sea through sediment incubations with DMS, amplicon sequencing, genome-resolved metagenomics and metatranscriptomics.

We found that Methanolobus were the dominant methanogen, which assimilated DMS and produced methane in all sediment layers from the Baltic Sea. Interestingly, in the DMS-amended sediments, expression of the *mts* genes encoding for DMS-methyltransferases was undetectable. However, the genes encoding for enzymes related to trimethylamine (TMA)- and methanol-methyltransferases (*mtt*, *mtb*, *mta*) showed much higher levels of gene transcription. Furthermore, we found these genes in the four Methanolobus metagenome-assembled genomes we constructed from the metagenome sequences from the sediment DMS incubations. However, these MAGs did not contain the genes encoding for DMS-methyltransferases (*mts*).

Our results provide the first evidence that DMS is degraded by Methanolobus via the activity of TMA- and methanol-methyltransferases in anoxic sediments and challenges the long-standing view that substrate-specific methyltransferases are used for microbial degradation of methylated compounds to methane. These findings show the versatility of the key enzymes involved in methane production from methylated compounds and reemphasise the need for the understanding of the regulation of methane production pathways under changing environmental conditions.

P569

Archaeal Antimicrobial Resistance: Uncovering the Mechanisms in Halorubrum saccharovorum

Thomas Thompson, Asma Fakhoury, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistance is a major global health concern that has been tradtioanlly studied in bacteria. However recent research suggests that archaea may also be involved in human infections such as periodontitis and Parkinson's disease.

In this study, the haloarchaea *Halorubrum saccharovorum* was exposed to both constant and increasing subinhibitory concentrations of the antibiotic ciprofloxacin to assess its ability to develop resistance. The results showed that exposure to increasing concentrations of ciprofloxacin in both low- and high-nutrient media supported the development of resistance, with a more than three-fold increase in the minimum inhibitory concentration (MIC). Comparative genomics and transcriptomic analysis revealed a single nucleotide polymorphism in a gene associated with resistance in bacteria, leading to a change in protein structure and acquired resistance to ciprofloxacin.

In addition to this genetic mutation, the study also found that the presence of efflux pumps in the cytoplasmic membrane of *H. saccharovorum* may contribute to the development of antimicrobial resistance. This efflux system was inhibited by a wide range of compounds including CCCP, thioridazine, chlorpromazine, fluoxetine and verapamil.

These findings suggest that archaea, like bacteria, can develop resistance to antimicrobials following repeated sub-lethal exposures and may act as an additional reservoir for AMR genes in the environment. However, the limited knowledge of the archaeome in most environmental niches makes it difficult to predict the potential impact of this previously unrecognized AMR reservoir.

P571

The next-generation tools for risk assessment and precision food safety: use of shotgun metagenomics sequencing for characterisation of food and investigation of metagenome-assembled genomes

Guerrino Macori ORCID iD, Leonard Koolman, Siobhan McCarthy, Seamus Fanning

University College Dublin-Centre for Food Safety, Dublin, Ireland

Abstract

Next Generation Sequencing (NGS) technologies and the application of bioinformatic approaches are redesigning microbiology and their applications, covering aspects of food quality and precision food safety. Use of NGS protocols includes, among others, a deep understanding of the genomes of microorganisms in pure culture using whole genome sequencing and importantly, metagenomics has allowed the extensive comprehension of the microbiota and microbiome of food. Microbial communities along food chains have increasingly been studied for describing the genetic diversity, functionality, and succession of spoilage microflora, foodborne pathogens and for studying functional microorganisms used for producing food by fermentation. In this study, water kefir was used as a model for evaluating the performances of different sequencing approaches for the detailed description of complex microbial communities within foods. Several isolates were retrieved through culture-dependent techniques and were also identified as high-quality metagenome-assembled genomes (MAGs), including prominent probiotic species of the genus *Gluconobacter*, *Liquorilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus* and *Lacticaseibacillus*. Nanopore technology applications were used for describing the samples, through full-length 16S rRNA gene sequencing and shotgun metagenomic approach, coupled with software-controlled enrichment of the species isolated with culturing. Finally, the novel-identified species were sequenced from pure culture, providing a detailed characterisation of their genomes. These next generation of tools are going to change radically the risk assessment approaches and methods for better pinpointing the origins of contamination events, antimicrobial resistance spread and describing potential unknown pathogens, such as using metagenomics for resolving the pathogens present in food at the strain level using metagenome-assembled genomes.

P572

Environmental and Genetic Factors Controlling Biofilm Growth in the Domain Archaea

Bethan Johnson, Julianne Megaw, Brendan Gilmore

Queens University Belfast, Belfast, United Kingdom

Abstract

Biofilms are complex multicellular structures that are found in a multitude of environments. To date, little is known about the formation, dispersal, and advantages of archaeal biofilms compared to their bacterial counterparts. To further understand biofilms within archaea, the model species Haloferax volcanii has been utilized to determine the effect of varying salt concentrations on biofilm formation using an MIC protocol combined with the crystal violet staining method. Experiments on archaeal motility have also been undertaken using a H. volcanii transposon insertion mutant library. Mutants were stabbed into soft agar and visibly non-motile mutants picked, followed by amplification of the transposon insertion site via a nested PCR method, and Sanger sequencing to identify genes controlling motility. The salt concentration experiments showed high biofilm formation at high and low salt concentrations for the salts MgSO4 and MnCl2; NaCl showed high biofilm formation at low and high concentrations (up to 300g/L) after which it decreased. Sodium citrate produced large biofilms at 1g/L before tapering off at higher concentrations, and KCl showed both an increase in biofilm formation at higher and lower concentrations. Varying these salt concentrations allowed insight into how changes in environmental conditions can affect biofilm formation. The mutant experiments identified several genes affecting motility, including HVO_3001, encoding for an ABC transporter permease, and cheF2, a chemotaxis protein. Motility screenings uncovering non-motile mutants will create a clearer picture of genes involved in motility and further experiments will be done to determine their effects on biofilm formation.

P572

Microbiome surveillance of indoor air for environmental health and occupational hygiene.

Angela Sherry ORCID iD[1], Beatriz Delgado Corrales[1], Kevin Stott[1], Garreth White[2], Johnathon Acaster[2], Andrew Brabbs[2], Blythe Crawford[2]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Royal Air Force, Northallerton, United Kingdom

Abstract

The biological component of air is typically overlooked in environmental health risk assessment procedures, however in light of the Covid-19 pandemic it is imperative to consider the impact of the biological fraction of air on human health. Evidence compiled by DEFRA determined build-up of virus in aerosols in poorly ventilated indoor rooms[1], yet much remains to be investigated on the biological content of air and interactions between all components (e.g. bacteria, fungi, viruses).

A longitudinal air microbiome study of 11 sites was performed to investigate the composition of microbiomes within indoor air in relation to ventilation systems and levels of room occupancy. Concomitant with microbiological sampling, air quality parameters were monitored including volatile organic compounds (VOC), temperature, humidity and CO_2.

Bacterial communities were representative of the type of environment being tested, with taxa similar to those found in other air microbiome studies. Relative abundance of bacterial communities across the sites differed, with an obvious contribution to the microbiome from site occupants. Fungal communities included plant pathogens and spore-forming taxa ubiquitous to both indoor and outdoor air. Taxa were identified which were amongst the most abundant airborne species of fungi detected in indoor air in a previous study of 28 homes[2].

Ultimately the research could lead to the incorporation of microbiological profiling of indoor air into environmental health and occupational hygiene risk assessment, and holds potential societal impact related to improving workforce health.

[1]DEFRA Air Quality Expert Group, Rapid Evidence Review, 2020.

[2]Madsen *et al*., 2016. *Appl Environ Microbiol* 82:2479–2493.

P574

Examining the impact of taxonomic profilers on soil shotgun data analysis

Niranjana Rose Edwin[1,2], Florence Araham[3], Fiona Brennan ORCID iD[4], Orla O'Sullivan ORCID iD[1]

[1]Teagasc, Moorepark Food Research Centre, Moorepark, Fermoy, Cork, Ireland. [2]University of Galway, Galway, Ireland. [3]Functional Environmental Microbiology, University of Galway, Galway, Ireland. [4]Teagasc, Soils, Environment and Landuse Department, Johnstown Castle, Wexford, Ireland

Abstract

Background: Soil is recognized as one of the most diverse microbial habitats on earth. The soil microbiome also remains a major repository for antimicrobial resistance genes. With shotgun metagenomic sequencing it is now possible to detect and characterize microbial species in unprecedented ways. However, interpreting soil metagenomic data with existing incomplete sequence databases is challenging. The need to develop a soil microbiome database containing both culturable and nonculturable fractions including the high-quality MAGs available in NCBI still remains.

Methods: In this study, we classified shotgun metagenome data from 133 soil samples using major taxonomic classifiers like Kaiju and Kraken and a custom Kraken database derived from GTDB-TK genomes.

Results: Upon taxonomic analysis of the 133 soil shotgun samples, we found that creating custom databases increased the percentage of reads classified per sample to 34%, compared to 17% and 19% for Kraken and Kaiju, respectively. We hypothesize that this discrepancy is the result of differences in the underlying databases used to classify the reads. To test this hypothesis, we curated the largest repository of soil-associated genomes, which we will use to create an *in silico* mock sequence database to determine its impact on metagenomic reads classification.

Conclusions: A fine-tuning of pipelines for soil metagenomics can help us gain a better understanding of the soil microbiome.

P575

Metagenomics for the healthy biotransformation of milk

Meghana Srinivas ORCID iD[1,2], Calum Walsh ORCID iD[3], William McCarthy ORCID iD[1], Orla O'Sullivan ORCID iD[1,2,4], Paul Cotter ORCID iD[1,2,4], Douwe van Sinderen ORCID iD[2,5], John Kenny ORCID iD[1,2,4]

[1]Teagasc Food Research Centre, Moorepark, Fermoy, Ireland. [2]APC Microbiome Ireland, Cork, Ireland. [3]Department of Microbiology & Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia. [4]VistaMilk SFI Research Centres, Cork, Ireland. [5]School of Microbiology, University College Cork, Cork, Ireland

Abstract

Chlorine-based detergents are commonly used along the dairy supply chain to ensure microbiological safety. However, chlorine-based detergents often breakdown to chlorate ions which gain entry into milk through clean in place practices from processing, transport and storage equipment. The consumption of chlorate is known to be detrimental to human health, specifically that of an infant, and hence needs to be removed from milk and milk products. Our aim is to identify and apply chlorate-reducing bacteria naturally present in raw milk for this purpose. Preliminary tests have shown drastic reductions of chlorate in chlorate-spiked raw milk when stored at 4^0C for 14 days, or at 25^0C for 24 hours. To determine the potential effect of geographical location on raw milk microbial composition and its effect on chlorate reduction, raw milk was collected from 12 farms across Ireland and tested under the two conditions. The microbial composition of these samples is being studied through the development of a new amplicon sequencing approach involving long read sequencing of the 16S-ITS-23S (RRN) operon along with the construction of a publicly available database (FANGORN). The ability of RRN sequencing to provide improved resolution of microbial communities at strain level was tested using mock communities consisting of closely-related strains and will be tested for natural food-derived microbes such as the raw milk samples mentioned. Through high-throughput sequencing approaches, chlorate-reducing bacteria will be screened for to improve the quality and safety of milk and milk products.

P576

Antibiosis and cold adaptation in a novel member of the *Cryobacterium* genus

Aliyah Debbonaire, Arwyn Edwards

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

The *Cryobacterium* genus is commonly found within the cryosphere. Strains have been isolated in China, Svalbard, Greenland and the Antarctic. Strains isolated from both the High and Sub Arctic were sequenced using both short and long read sequencing technology. Hybrid assemblies representing high quality genomes were annotated and compared to publicly available *Cryobacterium* genomes. ANI comparisons indicated the uncovering of a new species within the genus (manuscript in prep). Furthermore, a pangenome was constructed of all sequenced strains and publicly available genomes with output showing 84-89% of genes as homologous. Exploration into the antimicrobial potential of these new species was undertaken with both phenotypic assays and bioinformatics. Assays testing 47 *Cryobacterium* isolates showed even members of the same species within the genus to have either growth promoting and inhibiting effects, with a slight correlation to geographical location. antiSMASH was used to probe genome sequences representing both publicly available genomes and the isolated novel member species. A core group of biosynthetic gene clusters was uncovered. In particular, a carotenoid-like cluster with low similarity to any known clusters was discovered to be ubiquitous to the genus. Given the extreme environments these bacteria inhabit, this pigment may play a key role in establishment and adaptation to the cryosphere. Pigment production is demonstrably down-regulated at higher temperature in similar genera, illustrating the scope for cold-adapted microbes to respond to environmental changes. Consolidating our understanding of the interactions between cold-adapted microbes and their rapidly-warming host environments is therefore timely.

P577

Valorisation of underutilised dairy waste residues: production of lactic acid through microbial fermentation

Chatan Rai Surana ORCID iD[1,2,3], Eoin Byrne ORCID iD[1], Michael Callanan ORCID iD[2,3], Olivia McAuliffe ORCID iD[1,2]

[1]Teagasc Food Research Center, Fermoy, Ireland. [2]VistaMilk SFI Research Center, Fermoy, Ireland. [3]Munster Technological University, Cork, Ireland

Abstract

Globally, 160-190 million tonnes of nutrient-rich waste streams are generated annually by the dairy processing sector, many of which are underutilised. This gives scope to biotransform such wastes into economically valuable products.

In this work, acid whey and salty whey were evaluated as feedstocks for production of lactic acid. A total of 466 lactic acid bacteria strains were screened on differential agar for homofermentative production of lactate, which was observed in 263 strains. HPLC quantification revealed a lactate yield of > 18 g/l for 40 of these strains in MRS, with the highest yield (26.48 g/l) observed for Lacticaseibacillus paracasei DPC6583. Eleven high-lactate producing strains grew well (OD600 > 1) in salty whey but lactate yields were substantially lower in salty whey (7.29 g/l) compared to MRS (24.44 g/l) in a controlled batch fermentation. Preliminary evaluation of acid whey suggests it is better substrate for growth than salty whey and investigation is ongoing to assess the lactate yield. LC-MS analysis of fermentates will reveal other potential high-value end-products.

Harnessing the power of microorganisms is an approach that shows great potential for biotransformation of waste streams and co-products. Such an approach can contribute to the circular economy by making food production systems more sustainable by reducing the burden of these waste streams on the environment.

P578

Production, partial purification and characterisation of potent bacteriocins from Lactococcus lactis isolated from cheese

Christian Anumudu ORCID iD, Taghi Miri ORCID iD, Helen Onyeaka ORCID iD

School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom

Abstract

Microbial contamination of foods can cause food spoilage and possible foodborne diseases with attendant public health risks to consumers. In recent years, more natural/biological approaches are being investigated for possible use to ensure food safety and protect public health. Accordingly, we investigated the ability of Lactococcus lactis isolated from cheese to produce bacteriocins in modified deMann Rogosa and Sharpe media (MRS) and investigated the biological and chemical properties of the produced bacteriocins. The results show that Lactococcus lactis isolates produced active biopeptides with inhibitory activity against food borne pathogens including the spore former Bacillus cereus and remained stable following exposure to high temperatures (70°C - 110°C), low and high pH ranges (pH2-5 and 9-11). The produced bacteriocins were stable in the presence of lysozyme and catalase but were degraded by proteinase K, confirming their peptide nature. Partial purification of the bacteriocins were achieved by 80% ammonium sulphate precipitation, followed by reverse-phase high performance liquid chromatography. Molecular evaluation of the producer organism reveals presence of nisin and lactococcin encoding genes. Mass spectrometric analysis shows similar daughter ions to nisin following fragmentation and analysis by the dynamic multiple reaction monitoring (DMRM) acquisition mode, confirming the presence of nisin-like bacteriocins. The production of bacteriocins and antimicrobial activity of the synthesized peptides can be enhanced by the addition of Mg2+. The results obtained from this study suggests a novel bacteriocin effective against a persistent food spoilage bacterium, indicating the potential application of this biopeptide in the food industry.

P580

Archaeal interactions with Carbonate Tufa.

Bethany M Farrington, Paul N Humphreys, Simon P Rout

University of Huddersfield, Huddersfield, United Kingdom

Abstract

Historic operation of lime kilns has resulted in significant quantities of CaO bearing waste, which have been disposed to landfill. The percolation of water through these deposits results in a highly alkaline leachate generation (pH > 12). Calcium hydroxide- dominated leachates increase solubility and subsequent precipitation atmospheric carbon dioxide as carbonates, referred to as tufa. Tufa formation can have significant impact on the local habitat through preventing gas exchange between atmosphere and water sources and enhancing chemical degradation of plant material. The generation of and subsequent fermentation of cellulose degradation products in anaerobic conditions provides substrates for methanogenesis, with research recently suggesting that carbonates may provide an additional carbon source for hydrogenotrophic methanogenesis.

An example of a lime kiln landfill can be found at Brook Bottom Valley within the Harpur Hill site, Derbyshire. The present study obtained samples of tufa and underlying soil from five sites and Archaeal specific 16S community profiling used to understand the distribution of methanogens in-situ. Methanogenic enrichments were then prepared from the soil (H2:CO2 headspace) and tufa (H2 only) to determine the ability of these communities to generate methane. Methane was produced in both soil (167.27mmoles in 49 days) and tufa (0.87mmoles in 114 days) enrichment microcosms, indicating archaeal taxa may be capable of hydrogenotrophic methanogenesis from both carbon dioxide and mineral carbonates. Future studies will focus on stable isotope probing to elucidate the genetic mechanisms and taxa responsible for methane generation from carbonates.

P581

Collective protection against the type VI secretion system in bacterial communities

William Smith ORCID iD[1,2], Elisa Granato ORCID iD[2], Kevin Foster ORCID iD[2]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

Abstract

The bacterial type 6 secretion system (T6SS) is a molecular speargun used to stab and intoxicate other cells. Among plant-associated bacteria, T6SSs mediate crucial ecological interactions between commensals and pathogens, in turn altering hosts' disease susceptibility. The physiological factors that make bacteria susceptible or resistant to such attacks are therefore of central interest, but remain poorly understood. We sought to test whether a key feature of natural communities—the extracellular matrix—could shield bacteria from competitors' T6SS attacks. Using agent-based simulations of community growth, we found that bacteria secreting extracellular polymeric substances (EPSs) could physically block T6SS toxin injection, with sensitives' survival improving as more cells engaged in EPS production. This resulted in a novel form of collective T6SS protection, with EPS pockets shielding not only EPS-producing cells, but also nearby non-producers. We tested our model empirically using engineered strains representing the three key strategies in our simulations: a T6SS-wielding attacker (the soil bacterium *Acinetobacter baylyi*), and two T6SS-sensitive target strains (*Escherichia coli*) that either secreted EPS or not. By studying microscale T6SS interactions via fluorescence microscopy, we confirmed the presence of collective protection, which occurred in part because secreted EPS shielded nearby non-producer cells from T6SS attacks. Our work identifies a key mechanism by which groups of bacteria can work together to defend themselves from attackers, through the production of a shared matrix.

P582

Evolution of native biofilm communities on waste plastic to enhance polyester degrading activity

Sophie Howard ORCID iD[1], Habteab Isaack Sbahtu[1], Uchechukwu Onwukwe[1], Maria J López[2], Ronan McCarthy ORCID iD[1]

[1]Brunel University, Uxbridge, United Kingdom. [2]University of Almería, Almeria, Spain

Abstract

Plastic waste is a growing worldwide problem desperately requiring a solution. Only 9% of all plastic waste has been recycled, and although recycling rates are increasing, the cost of recycling and limited downstream uses of recycled plastic mean an alternative method that removes plastic is needed. Biodegradation of plastic by bacteria is a promising approach to tackle plastic waste, however natural biodegradative enzymes are often poorly expressed or have low activity. In this study, we tested bacterial biofilm formation on several plastics and found that expanded polystyrene (EPS) promoted the highest levels of biofilm, this could be due to its floating at the air-liquid interface and many structural pockets. We therefore collected environmentally discarded EPS to characterise its native bacterial communities. The attached communities initially had limited plastic degrading activity. Using a strong selection pressure on these communities by limiting the carbon source to only that of the waste plastic, we performed a long-term evolution experiment. More than half of the resulting evolved bacterial communities had improved polyester degrading activity compared to the initial communities and the strongest degrader identified in the evolved communities was *Pseudomonas stutzeri*. Whilst prevalence of plastic-degrading genes are linked with prevalence of plastic waste, not all genes will be expressed or functional. Native plastic associated biofilm communities can therefore be a source of bacteria with plastic-degrading potential, which can be unlocked through selective pressures and *in vitro* evolution experiments, resulting in biodegradative communities that are better than nature.

P583

Ability of microorganisms from smoldering coal waste dumps to grow in polyextreme conditions in connection to astrobiology

Agnieszka Bylina[1], Piotr Siupka ORCID iD[2]

[1]Institute od Earth Sciences, University of Silesia in Katowice, Katowice, Poland. [2]Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Katowice, Poland

Abstract

Self-heating (smoldering) coal-waste dumps are anthropogenic geological formation resulting from the coal mining activity in which pyrite underwent oxidation generating heat that results in self-combustion of the organic matter in the shale. There are a number of them in Upper Silesian Coal Basin, Poland. The geochemistry of the dumps creates extreme environment for microorganisms were not only high temperature are present, but also high salinity, extreme pH condition, presence of polycyclic aromatic hydrocarbons, haloorganics as well as high concentration of heavy metals including mercury. We got interested in the microorganisms present in these environments and their ability to survive poly extreme condition with scope to astrobiology research. Because we had not available chamber for anoxygenic work, we decided to develop budget friendly chamber using commonly available resources, sensors and Arduino platform for the chamber controller. Additionally, we have isolated a number of microorganisms strain from the dumps, including 70 strains of extremophiles. We are investigating their ability to grow in poly extreme environment with low temperature, hypobaric and oxygen deprived conditions. Initial results showed that 21 strains are able to grow in anoxic, CO2 enriched atmosphere under <50 mbar pressure. The strains that will be able to grow in such environment will be then identified molecularly and they adaptations to the conditions will be tested. This ongoing research will provide insights into adaptation ability of microorganisms to polyextreme environments which is crucial to the Astrobiology field.

P584

Evolution of aerotolerance in environmental strains of *Campylocater jejuni.*

Shani Ali

University of Bath, Bath, United Kingdom

Abstract

Campylobacter jejuni (*C. jejuni*) is an enteric, zoonotic pathogen that is one of the most common causes of gastroenteritis worldwide. *C. jejuni* colonises the guts of animals including chickens, cattle and wild birds and is transmitted via consumption of undercooked meats and contaminated water sources. Whilst *C. jejuni* infection is typically self-limiting in more affluent countries, it induces a more significant burden in lower to middle income countries. In these countries infection typically peaks in childhood and can have long term impacts on growth. *C. jejuni* is well adapted to a micro aerophilic environment and the mechanisms that allow survival outside the host are not well understood. My project aims to begin to understand how aerotolerance in a series of *C. jejuni e*volves which may provide insight into transmission. The initial aerotolerance of the strains were assessed via growth curve assays. These strains were then repeatedly exposed to oxygen at two time ranges and then passaged and incubated under microaerophilic conditions. These strains were then sequenced and compared to the original ancestral strains as well as controls that were passaged alongside the experiment without oxygen exposure. This may allow for identification of candidate stress resistance genes which can be compared to the genes identified in a genome wide association study carried out by Yahara et al. These mutations may confer resistance to other stressors such as antibiotic resistance. The MICs against ciprofloxacin will be determined to address this cross-resistance hypothesis.

P584

ANTIMICROBIAL POTENTIAL OF DEEP EUTECTIC SOLVENT

Taiwo Akinyele, Samantha McLean

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Over the years, the emergence of multidrug-resistant bacteria has sparked numerous concerns, and studies into alternative antimicrobial drugs are continuously being researched. These include plant-derived and other natural substances to create efficient substitute antibacterial agents. Natural deep eutectic solvents (NADES) are a novel class of environmentally friendly solvents that may solubilize both natural and synthetic compounds with poor water solubility. NADES are combinations of two or more chemicals that function as both donors and acceptors of hydrogen bonds. NADES are thought to have low cytotoxicity and frequently demonstrate antibacterial action.

Antimicrobial assays were performed on numerous candidate NADES to determine the antimicrobial activity against clinically isolated bacterial pathogens. Antimicrobial assays showed the NADES' antimicrobial activity against these common bacterial pathogens.

NADES produced from chemical compounds such as amino acids or organic acids, having demonstrated pharmacological and antimicrobial effects, which may have similar qualities, suggesting that the solvents' biological activity and physicochemical properties can be adjusted. However, NADES' antioxidant activity is currently understudied, which could help characterize and apply its features. NADES could solve major pharmaceutical and nutraceutical formulation issues. After proving NADES is a safe and environmentally acceptable solvent, the toxicity and recovery of the chemical compounds in the NADES extract must be studied. These solvents need more research before they may be employed in the food, pharmaceutical, and nutraceutical industries.

P586

Biomining Atlantic Salmon Skin Microbiome in Search of Eco-friendly Bioprotective Strains

Fairouz S. H. Gzara, Stephen A. Jackson, Alan D. W. Dobson, David J. Clarke

School of Microbiology, University College Cork, Cork, Ireland

Abstract

The identification of novel antimicrobial compounds has become a priority with the emergence of multi-resistant microbial strains. Bioprospecting is the exploration of natural sources in search of new bioactive compounds and this approach can provide solutions to the problem. In the context of MARBLES, an EU project involving 14 partners across Europe, we have speculated that commensal microorganisms isolated from marine ecosystems may have a key role in their hosts' defence against pathogens. Therefore, we have focussed on bioprospecting the skin of healthy Atlantic salmon (*Salmon salar*) as a potential source of novel antimicrobial compounds that might be useful in sustainable aquaculture. Swabs taken from the skin of 3 healthy salmon in Donegal, Ireland, were cultured on LB agar and Marine Agar incubated at various temperatures (4°C, 28°C, room temperature), and resulting microbial isolates were identified by 16S ribosomal DNA sequencing. In total, we identified 302 culturable bacterial isolates belonging to 38 separate genera from 4 different phyla (Actinomycetota, Bacillota, Bacteroidota, Pseudomonadota). Deferred antagonism assays were carried out against a panel of 8 commercially-relevant Gram-negative fish pathogens. While no antimicrobial activity was detected using this specific approach, in order to unravel the encoded potential for novel bioactive molecule production associated with bacteria from this environmental niche, 18 bacterial isolates were further selected for genome sequencing. The true potential of the healthy fish microbiota to provide a sustainable alternative to the high levels of antibiotics currently used in aquaculture has not yet been fully assessed, with further studies ongoing.

P587

Genomic and Phenotypic Characterization of Staphylococci Isolated From Non-Human Primates (NHPs)

Caitlin Wildsmith, Jonathan Thomas ORCID iD, David Negus ORCID iD

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Members of the genus *Staphylococcus* are frequently found as part of the normal skin microbiome of humans and animals. However, several species are opportunistic pathogens and can cause severe infections. Due to the difficulty of obtaining samples from NHPs, little is known about the characteristics of staphylococci which colonize these mammals. Moreover, contact between NHPs and human handlers may result in the transfer of potentially pathogenic or resistant isolates. Therefore, we genomically and phenotypically characterized staphylococci isolated from NHPs housed at a UK zoo.

Swabs from the palms of seven NHPs were plated on mannitol salt agar for the isolation of presumptive staphylococci. The 16S rRNA gene from 88 presumptive isolates was amplified and sequenced. Representative isolates were selected for further characterization and whole genome sequencing (WGS). Genomic analysis included prediction of antimicrobial resistance (AMR) and virulence genes. Phenotypic assays were performed to confirm bioinformatic predictions of antimicrobial resistance and biofilm-forming ability.

Twenty-eight isolates were identified as members of the genus *Staphylococcus* by 16S rRNA gene sequencing, fourteen of which were further characterized by WGS. Assembled draft genomes were of high quality as determined by CheckM. Staphylococcal species isolated included *aureus, pasteuri, saprophyticus, epidermidis* and *warneri*. Spider monkeys harboured the highest diversity of species while marmosets harboured the least. Sequenced strains were predicted to harbour a minimum of 4 and a maximum of 16 AMR genes. Crystal violet staining showed the isolates possess a range of biofilm-forming abilities.

In summary, this study highlights the diversity of staphylococci inhabiting NHPs.

P588

Identification of functional group for the bioaccumulation and effect of lead (Pb) and copper (Cu) on Escherichia coli cells using Fourier transform infrared spectroscopy.

Chinenye Ugwa, Helen Onyeaka, Taghi Miri

University of Birmingham, Birmingham, United Kingdom

Abstract

Escherichia coil is a bacteria genus well-known for its ability to bind metals and its resistance to various heavy metals. Lead (Pb) and Copper (Cu)are common heavy metals in aquatic ecosystems that are toxigenic to marine species, even at a lower concentration. In this present study, the ability of E.coli to accumulate heavy metals, the site of accumulation and the effects of heavy metal on the bacteria were investigated. E.coli was cultivated for 24hr incubation at 37°C in a mineral salt medium with Pb, Cu and control alone or in combination. The biosorption and bioaccumulation of Cu and Pb by strain were observed to be pH 7-dependent. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) was used to examine the surface topography alterations of the strain, following exposure to different concentrations of heavy metals(100,200,500&1000ppm) images obtained revealed that heavy metal binding occurred on the bacterial cell surface. FTIR spectroscopy was used to investigate the effects of the two heavy metals on Escherichia coli toxicity. The FTIR spectra revealed that carboxyl, hydroxyl, amino, and phosphate groups on the strain surface were potentially available for typical coordination bonding with Pb and Cu. The difference in spectral characteristics of FTIR bands for (control, E.coli loaded with Pb and Cu) suggests that both metals bind to hydroxyl, amino, carbonyl, and phosphoryl functionalities with different bands at 3278.04 cm(-1) and 3257.82 cm(-1) respectively. The work highlights the significance of metal-metal interaction and its toxicity on E. coli.

P589

Transition metals supplementation enhance resilience against sulfur-induced toxicity in anaerobic wastewater treatment

Marco Prevedello ORCID iD, Ciarán Wilson, Vincent O'Flaherty, Florence Abram

University of Galway, Galway, Ireland

Abstract

Introduction

Anaerobic digestion is an established technology for the upcycling of organic waste in wastewater to biogas, a renewable energy source. Sulfur contamination of wastewater, however, is often reported for its negative impact on biogas quality and yield.

Methods

Between January 25th and March 22nd, 2021 (excluding February 22nd), sludge samples were collected by-weekly from two PAQUES IC® full-scale reactors (R1 and R2) treating industrial wastewater. R1 and R2 treated the same wastewater stream, R2 was additionally supplemented with transition metals (Biopaq Micromix Forte® by PAQUES and PIX Ferric sulfate by Kemira). Sludge samples were analyzed for solid content, metal content, specific methanogenic activity (SMA), and sulfate-mediated inhibition.

Results

The data obtained from the SMA assay show that methylotrophic methanogenic activity increases after 6 weeks of transition metal supplementation. Additionally, the data obtained from the inhibition assay suggests that transition metal exposure increases resilience to sulfur toxicity, allowing for both higher methanogenic rate and yield. It should be noted that no transition metals were supplemented during the inhibition assay. This suggests that the observed resilience is mediated either by ecological changes in the microbial community, the accumulation of transition metals in the biofilm, or a combination of the two. RT-qPCR of genes involved in sulfur-reduction and methanogenesis are under development for further analysis.

Conclusions

This study suggests that transition metal supplementation could be used to increase the resilience of mixed microbial communities to sulfur-mediated toxicity; potentially leading to an increase in the waste-to-energy conversion of wastewater organic pollutants.

P590

Metagenomics based approach to explore the microbiome and resistome of the hospital environment for effective management of hospital-acquired infections (HAIs) and antimicrobial resistance (AMR)

Muhammad Umer Asghar[1,2], Noor Ul Ain Noor Ul Ain[3,4], Muhammad Tariq[1,2]

[1]National Institute for Biotechnology and Genetic Engineering (NIBGE) College-Pakistan institute of Engineering & Applied Sciences (PIEAS), National Probiotic Lab-HBD, Faisalabad, Pakistan. [2]Pakistan Institute of Engineering & Applied Sciences (PIEAS), Nilore, Islamabad, Pakistan. [3]Centre for Clinical Microbiology, UCL Royal Free Hospital, Rowland Hill, London, United Kingdom. [4]Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan

Abstract

Background

Hospital environments provide favourable conditions for the expansion of the microbiome and resistome. We hypothesized despite extensive disinfection and cleaning the hospital environment provides a favorable reservoir for the survival of hospital-acquired infection (HAIs) associated pathogens and responsible for increased antimicrobial resistance (AMR). The spread of this profound global health threat is also reduced by the identification of the microbiome, resistome and their source, interactions, and transmission pathways.

Methodology

We designed a study at cardiac care hospital intending to explore microbiome, their source contribution, resistome, microbe-microbe interaction in dissemination of AMR and associated mobile genetic elements (MGEs) among four different wards and Operation theatre room (OT). By using high throughput 16S rRNA gene sequencing targeting the V4 hypervariable region on the MiSeq platform and shotgun sequencing approach,

Results

we found this hospital had homogenous microbial community structure with high abundance of HAIs associated pathogens. These pathogens positively interact with other clinical important microbes and disseminate the biocide and MGEs relatable to beta lactam resistance genes. suggesting failure of sanitation and disinfection practices.

Conclusion

This study in favor of alternative biocidal compounds such as probiotic disinfectants, which could bypass disinfectant resistance.

P591

Assessing the impact of fever temperatures on bacteria-phage interactions

Samuel Greenrod, Emily Stevens, Craig MacLean, Kayla King

University of Oxford, Oxford, United Kingdom

Abstract

In an era of increasing antibiotic resistance, a promising alternative is the application of bacteriophages, viruses that target and kill bacterial pathogens. Phages collected from the environment and then used therapeutically in humans and livestock will experience broad temperature ranges, including from infection-induced fevers. Yet, the extent to which phage treatment efficacy is maintained, promoted, or restricted across body temperatures (and phage types) is unclear. My PhD thesis will investigate the impact of temperature variation on phage infectivity and bacterial resistance to phage across ecological and evolutionary time. We will use the opportunistic pathogen Pseudomonas aeruginosa and a diversity of lytic phages across a range of typical mammalian body temperatures, including low and high-grade fevers. Preliminary results indicate that environmentally collected phages include both temperature specialists, which have low infectivity at high-grade fever temperatures, and temperature generalists which have high infectivity across the mammalian body temperature range. This work will be extended by assessing the impact of temperature on individual phage life-history traits and by using experimental evolution to 'train' phages to improve their performance across temperatures. This work improves our understanding of how phage activity varies with infection-induced fevers and will inform the development and application of phages to treat bacterial infections in both medicine and agriculture.

P592

Identification and characterization of a novel BHET hydrolase from polyethylene terephthalate (PET)-degrading bacterium, *Ideonella sakaiensis*

Jia Fong Chong, Min Fey Chek, Sun-Yong Kim, Kazuma Yasuhara, Toshio Hakoshima, Shosuke Yoshida

NARA Institute of Science and Technology, Nara, Japan

Abstract

Polyethylene terephthalate (PET) is a petroleum-based thermoplastic extensively used in the production of packaging plastics and polyester fibers. Short service time of PET products and its non-biodegradability properties have triggered serious environmental problems. Recent discovery of *Ideonella sakaiensis* 201-F6 with the capability to degrade and metabolize PET signifies a promising solution to plastic pollution. Two PET-hydrolytic enzymes importantly involved in the PET metabolism were identified: PETase and MHETase. However, thus far, it has been shown that microbial degradation of PET by *I. sakaiensis* is more efficient than the enzymatic degradation with purified PETase, indicating the presence of other factors in promoting PET metabolism. From the quantitative proteomic analysis with data-independent acquisition mass spectrometry (DIA-MS), we have found that a novel esterase IS3653 is localized on the surface of PET. The gene knockout induced delayed growth on PET film and a decreased PET weight loss. In vitro biochemical analyses showed that IS3653 protein possesses hydrolytic activity towards several substrates, including a PET oligomer, bis(2-hydroxyethyl) terephthalate (BHET), a PET model substrate, bis-(benzoyloxyethyl) terephthalate (BETEB) and its derivative, 2-hydroxyethyl benzoate (HEB), which is a different substrate spectrum from MHETase and PETase. Whereas mutations into the catalytic triad abolished its activity, respectively. Collectively, these findings imply the involvement of IS3653 in the PET metabolism of *I. sakaiensis*.

P593

Analysis of Pharmaceutical and Heavy Metal Contamination in Wastewater to Assess Contributions to Antimicrobial Resistance

Deborah Reid ORCID iD, Ania Escadero, Colin Hunter, Janice Spencer

Glasgow Caledonian University, Glasgow, United Kingdom

Abstract

Deborah Reid; Dr Escadero; Dr Hunter; Dr Spencer

Background

Environmental pollutants are linked to an increase in the levels of antimicrobial resistance (AMR). An important route for antimicrobial resistance (AMR) in the environment is via sewerage networks where key control points are the wastewater treatment plants (WWTPs).

Methods

Qualitative and quantitative analysis of wastewater and biofilms from a one year sampling campaign using Kirby Bauer antibiotic disc diffusion analysis for confirmation of resistant bacteria. High-throughput real-time PCR (qPCR) for AMR genes (blaNDM, blaCTX M, blaTEM_1, ermF_1, nimE, Mcr1, qepA, Sul1_2, dfrA1_1, Aac (1b_2, tetX). Flame Atomic Absorption Spectrophotometry for heavy metals (Silver, Iron, Lead, Zinc, Chromium, Nickel, Magnesium, Copper and Manganese). High-Pressure Liquid Chromatography Mass Spectrometry for pharmaceuticals (Amoxicillin, Azithromycin, Ciprofloxacin, Clarithromycin, Erythromycin, Flucloxacillin, Metronidazole, Ofloxacin, Sulfamethoxazole and trimethoprim).

Results

Variable quantities of AMR genes were detected throughout the WWTP with pharmaceuticals and heavy metals also detected in wastewater and biofilms showing significant variance throughout key treatment stages. This statistically fluctuating trend, also observed through different treatment approaches, possibly indicates pharmaceutical and heavy metal accumulation in primary and secondary settling tanks. This accumulation may be significantly influencing which plasmid borne resistance genes are horizontally transferred and expelled into receiving environmental waters.

Conclusion

The bioaccumulation of heavy metals and pharmaceuticals by wastewater biofilms may influence the quantity of resistance genes found in the surrounding aquatic environment. This research will guide wastewater management to reduce AMR.

P594

Stable isotope probing of carbonyl sulfide and cyanate pathways in microbial thiocyanate degradation

Yu-Chen Ling[1], Mathew Watts[2], John Moreau ORCID iD[3]

[1]Geomar, Kiel, Germany. [2]EPA-Victoria, Melbourne, Australia. [3]University of Glasgow, Glasgow, United Kingdom

Abstract

While the process of SCN- biodegradation has been well studied, relative contributions from the two known enzymatic pathways are still poorly resolved, hindering our ability to optimize bioremediation systems treating SCN- contaminated wastewater. Also, as SCN- biodegradation in nature impacts the biogeochemical cycling of sulfur (as well as carbon and nitrogen), a more quantitative understanding of enzymatic SCN- biodegradation pathways will yield insights into how the reactivity of this compound influences marine, terrestrial and atmospheric sulfur chemistry. Here we used carbon and nitrogen stable isotope probing to assess contributions to SCN- biodegradation from known enzymatic pathways in a microbial consortium grown from SCN- -contaminated mine tailings. We found that the cyanate pathway dominates SCN- biodegradation initially, before being supplanted by the hydrolase pathway. We attribute this ordering to the greater amount of bioenergy conservable via the cyanate pathway during the earliest stages of biodegradation.

P595

A flair for phosphonates; *Roseovarius nubinhibens* obtains phosphorus from (R)-1-Hydroxy-2-aminoethylphosphonate via a novel PbfA-PhnYA metabolic pathway

Leanne Murray ORCID iD

Queens University, Belfast, United Kingdom

Leanne L. Murray1, Katharina Pallitsch2 John W. McGrath1, and Jason P. Chin1.

1School of Biological Sciences, The Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL

2Institute of Organic Chemistry, University of Vienna, A-1090 Vienna, Austria

Abstract

Phosphonate breakdown factor A (pbfA) is an ammonia lyase recently shown to expand the substrate scope of the characterized phosphonoacetaldehyde hydrolase (phnX) pathway. pbfA acts on (R)-1-Hydroxy-2-aminoethylphosphonate (OH2AEP), a derivative of the most common biogenic phosphonate 2-aminoethylphosphonate (2AEP), converting it to phosphonoacetaldehyde in which the carbon-phosphorus bond is cleaved by phnX releasing inorganic phosphate for assimilation. Here we conducted a sequence homology search and gene neighborhood analysis of 3090 bacterial type-strain organisms and determined the presence of pbfA in 39 bacterial gene clusters encoding phnWYA, a pathway for the Pi-independent degradation of 2AEP).

We now show that *Roseovarius nubinhibens* metabolises OH2AEP via a novel PbfA-PhnWYA pathway as the sole phosphorus source. Within cells of R. nubinhibens regulation of aminophosphonate metabolism is substrate inducible, rather than PHO regulated, being under control of a LysR transcriptional regulator with gene expression occurring under ambient Pi concentrations. *R. nubinhibens* taxonomically belongs to the major clade of marine bacteria Roseobacteraceae. This clade represents 20% and 3-5% of bacterial cells in coastal and oligotrophic surface waters respectively. The versatility of phosphonate metabolism pathways in R. nubinhibens suggests that microbial species accessing phosphonate phosphate under LysR regulation may have important implications to understanding of the marine phosphorus-cycle.

Keywords: Phosphonates, marine, bacteria, pbfA

P596

He ain't heavy, he's my plasmid - fitness effects of an *IncB/K/O/Z* plasmid conveying cefotaxime resistance on plasmid naïve *Escherichia coli* strains.

Kim Summers ORCID iD, Victoria Clark, Séverine Rangama, Elizabeth Wellington

University of Warwick, Coventry, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a serious threat to human health. Plasmids containing AMR genes are of particular concern as they can transfer between bacterial species, facilitating the spread of resistance. Most research into plasmid adaptation has been conducted on lab adapted bacteria, however these can perform very differently from clinical and environmental strains. We present here the effects of a novel AMR plasmid on plasmid naïve *Escherichia coli* strains. An *IncB/O/K/Z* plasmid conferring cefotaxime resistance was conjugated from an ST131 strain to two non-pathogenic strains, 119 and 129, all isolated from the River Sowe downstream of a wastewater treatment plant. Transconjugants were competed against donors and recipients via serial batch transfer. Strain 119 with and without plasmid was grown in a chemostat for approximately 500 generations, with and without cefotaxime. The plasmid was stably maintained even without selection and plasmid bearing transconjugants outcompeted their parents. The transconjugant rapidly developed SNPs, mainly in intergenic regions, but also in cell wall associated genes that did not manifest in the original recipient strain. These results show that even in the absence of a selection pressure plasmid carriage conferred a fitness benefit rather than a cost for these initially plasmid naïve strains.

P597

Evaluating the pathogenic potential of environmental *Vibrio parahaemolyticus*

Marion Soree[1], Solen Lozach[2], Jennifer Ritchie[3], Dominique Hervio Heath[2]

[1]Ifremer, Nantes, France. [2]Ifremer, Plouzane, France. [3]University of Surrey, Guildford, United Kingdom

Abstract

The marine organism *Vibrio parahaemolyticus* is a leading cause of gastroenteritis associated with the consumption of raw or improperly cooked shellfish worldwide. Recent years has seen warming temperatures contribute to increased *Vibrio* populations and a greater number of human diseases attributed to *Vibrio spp*. in European coastal waters. Consequently, the identification of species characteristics that correlate with pathogenicity remains a high priority to safeguard both the shellfish industry and the consumer.

In this study, we examined the pathogenic potential of environmental *V. parahaemolyticus* strains using two *in vivo* models of *V. parahaemolyticus*-induced disease to complement our genetic analyses. The kinetics of disease following direct injection of waxmoth larvae (*Galleria mellonella*) or orogastric inoculation of infant rabbits was compared for bacterial isolates harbouring the much-studied thermostable direct haemolysin (TDH-) and the TDH-related haemolysin (TRH).

We found that the two models provided contrasting outcomes with strains capable of inducing mortality in larvae less able to cause disease in the mammalian host. Infection in larvae with defined mutants suggested that the organism's type three secretion system located on chromosome one plays a main role in inducing the host response, whereas dual carriage of TDH/TRH induced more rapid disease onset in rabbits. When taken together alongside our genetic data, our findings indicate that these two infection models reflect on different aspects of the bacterium's virulence and that *V. parahaemolyticus* found in seawater and shellfish have the potential to cause disease.

Session Topic: Antimicrobial resistance: from molecules to clinic, where are we now?

P598

In an intestinal organoid model, the virulence of genes and the effect of geraniol on adherent and invasive E. coli and Shigella sonnei were studied.

Ryath Al-Hemedawi[1], Jun Yu[2]

[1]Medical City Hospital Campus, Baghdad, Iraq. [2]SIPBS, GLASGOW, United Kingdom

Abstract

Diverse gastrointestinal (GI) disorders are brought on by gram-negative pathogens such as Shigella, which causes dysentery, and adherent, invasive E. coli, which has been associated with Crohn's disease. Both microorganisms are intracellular, inflammatory, and genetically predisposed to infection and antibiotic resistance. Existing antimicrobials are ineffective against these bacteria, necessitating the development of novel antimicrobials as soon as possible. It is possible to combat bacterial resistance competitively by targeting the dsbA gene, a master regulator gene required for both virulence and pathogenesis, in both microorganisms with geraniol.

As a model system, an intestinal organoid was used to determine phenotypic differences between infected and uninfected organoids following direct infection with AIEC and Shigella sonnei, or as a co-culture with macrophages to determine the effect of geraniol on the organoid. Statistical differences between the dsbA mutant and the wild-type AIEC and Shigella strains were identified by directly infecting intestinal organoids. This study sets up a way to co-cultivate RAW cells with intestinal organoids so that the toxicity of bacteria, the innate immune response, and the effectiveness (ability to treat) of therapeutic agents can be tested.

P599

Burkholderia cepacia Bacteremia in a Neonatal Intensive Care Unit of a tertiary care facility in north western Pakistan

Maria Khan ORCID iD

Rehman Medical Institute, Peshawar, Pakistan

Abstract

Background: Burkholderia cepacia complex are ubiquitous organisms with high virulence potential and capability to cause serious infections especially in neonatal intensive care units (NICU). The aim of this study was to evaluate the B. cepacia complex (BCC) infections by determining antimicrobial susceptibility patterns of the isolates in neonates.

Methods: The study was conducted in NICU, Rehman Medical institute, Peshawar, Pakistan, from January 2021 to May 2022. The culture of blood samples were done by an automated method using Bact-alert 3d (Biomeriux). During the early outbreak, samples from possible environmental sources including equipment and medications were taken from neonatal intensive care unit (NICU).

Results: The study included 217 cases of blood culture-proven septicemia due to BCC, among patients admitted to NICU. The first clustering of cases was noted in neonates having clinical and biochemical evidence of sepsis. Moreover, the source or route of infection was unidentified despite repeated epidemiological investigations. The susceptibility pattern of majority of organisms were similar, co-trimoxazole (100%), meropenem (95%), chloramphenicol (92%), and minocycline (92%) were found to be the most effective drugs followed by levofloxacin (12%), and ceftazidime (8.5%).

Conclusions: In our study, the index case was exposed to infection due to a physiological state of low immunity (preterm, low birth weight, and mechanical ventilation). The rest of the cases were exposed to this organism due to inadequate hand hygiene/improper cleaning and disinfection practices. Timely reporting and implementation of infection control measures can play a substantial role in curtailing this infection.

P600

Emergence of Colistin-resistant Extended-Spectrum ß-Lactamase-producing Escherichia coli in Chickens in the Center Region of Cameroon

TAGNE KAMGA CELESTIN

Catholic University of Central Afica, Yaoundé, Cameroon. University of Buea, Buea, Cameroon

Abstract

The fight against multi-drug resistant bacteria to antibiotics is a major public health problem, as illustrated by the spread of ß-lactam resistance and the increasing use of colistin in the poultry production. Colistin is widely used in veterinary medicine for the treatment of Gram negative bacterial infections in farm animals.

From August to November 2021, 160 faecal specimens were collected from broilers and layers in Center Region. Using the API 20E gallery, E.coli was identified. Antimicrobial susceptibility testing was performed according to EUCAST & CLSI. The Kirby Bauer disc diffusion method was used to determine the antimicrobial resistance profile and the ESBL phenotype of E. coli. The liquid microdilution method allowed us to detect colistin resistance. Data analysis was done using SPSS 25.0 software.

E. coli was present in 43.1%(69/160) of the population. High resistance was recorded with amoxicillin (95.7%), tetracycline(91.3%), amoxicillin + clavulanic acid (88.4%) ticarcillin (88.4%), ofloxacin (72.5%), doxycycline (76.8%), ciprofloxacin (69.6%) and colistin (34.8%). ESBL had a proportion of 44.9% (31/69). ESBL producing and colistin resistant E. coli was present in 38.7%(12/31). Factors such as age range [60-80[days and administration of antibiotics in preventive and curative reasons were significantly associated (OR>1and P≤0.05) with the emergence of ESBL producing and colistin resistant E. coli strains.

It is urgent that farmers get into the habit of administering antibiotics to chickens only under the advice of a veterinary doctor, as this would prevent the spread of ESBL- producer and colistin-resistant bacteria to animals, humans and the environment.

P601

Methylglyoxal, an active compound derived from monofloral manuka honey, produces bactericidal effects in *Salmonella* Typhimurium

Aubrey Woodard, Pamela Harvey

University of Colorado, Boulder, USA

Abstract

The rapid development of antibiotic resistance and decreased antibiotic development contribute to an urgent need for novel antibiotics. Historically, compounds isolated from natural products have been important sources of antibiotics. Methods of discovery have included compound screens and the identification of active compounds in natural products. We combined these approaches to screening the active compounds in manuka honey, a monofloral honey produced by bees that pollinate Leptospermum scoparium in New Zealand and Australia. In Salmonella Typhimurium cultured in liquid media similar in composition to conditions in which the bacterium replicates in vivo, we found that compared to two other forms of honey, manuka honey limits bacterial growth, likely due to its relatively low sugar content. We then identified and tested each of the most abundant compounds in manuka honey using the agar diffusion test. Testing of fourteen compounds revealed that methylglyoxal (MGO) confers the greatest antimicrobial effects, and in subsequent experiments, demonstrated that MGO exhibits bactericidal effects on Salmonella Typhimurium. The exploitation of natural products continues to represent a valuable strategy for the identification of novel antimicrobials, and MGO represents a promising compound for antibiotic development.

P602

Ventilator Associated Events: Incidence, Microbiological profile and Outcome in the Intensive Care Unit in a tertiary hospital of Eastern Nepal

Dharnish Jha ORCID iD

Koshi Hospital, Biratnagar, Nepal

Abstract

INTRODUCTION: Ventilator associated events (VAE) refer to new surveillance definition developed by Centre for Disease Control (CDC)/ National Healthcare Safety Network (NHSN) is in use since the year 2013, switching the focus of surveillance from VAP to VAE. A number of studies have been conducted in the United States and other Western countries to evaluate its practicality. However, information on VAEs in Asian countries is scarce.

MATERIALS AND METHODS: Patients admitted in ICU on Mechanical Ventilation were evaluated daily using the VAE surveillance criteria. At least 2 days of stable or decreasing ventilator settings followed by at least 2 days of increased ventilator settings was used as definition of VAE.

RESULTS: Of the 313 patients admitted to the ICU over the period of one year, 52 patients received MV for ≥2 days and met baseline criteria for VAEs Surveillance. Out of 52 patients, 14(27%) developed VAC only, 13(25%) developed IVAC only and 25(48%) patients developed PVAP. Endotracheal aspirate culture was positive in 25 patients (48%). The organisms isolated were Acinetobacter baumanii complex 14(53.84%), Pseudomonas aeruginosa 7(26.92%), Klebsiella pneumoniae 4(15.38%), and Escherichia coli 1(3.84%). Overall mortality rate in patients with VAE was 36.5% with highest mortality rate in PVAP (44%).

CONCLUSION: VAE mostly being health care associated event and prevalence of multidrug resistance in as observed in this study warrant clinician to practices infection control measures and rationale use of antimicrobials as effective measures for infection control.

P603

Clinical Stringent Response-Activating Mutations Promote Conjugal Transfer of Resistance Plasmids in *Staphylococcus aureus*

Ashley Deventer[1], Claire Stevens[1], Joanne Hobbs ORCID iD[2,1]

[1]University of Victoria, Victoria, Canada. [2]University of St Andrews, St Andrews, United Kingdom

Abstract

Background. Conjugation plays a crucial role in the dissemination of antibiotic resistance genes. We have previously shown that clinical mutations in the stringent response (SR) control protein, Rel, confer elevated (p)ppGpp levels and "partial" SR activation in *S. aureus.* As the SR has been linked to the dissemination of integrons, we hypothesised that Rel mutations may affect plasmid conjugation rates in *S. aureus.*

Methods. Conjugation/mobilisation frequencies were determined via filter matings for the three known families of conjugative staphylococcal plasmid and mobilisable pC221. Gene mutations/deletions were introduced into *S. aureus* Newman by allelic exchange. Chemical induction of the SR was achieved with mupirocin. Gene expression was assessed using LacZ reporter constructs.

Results. Mutants bearing a clinical Rel mutation exhibited a 5-to-10-fold increase in the frequency of donation of all plasmids (conjugative and mobilisable) compared with the wildtype/complemented strains. The frequency of plasmid receipt, however, was unaffected. Elevating cellular (p)ppGpp levels with mupirocin also increased the frequency of plasmid donation, while decreasing the level through inactivation of the three (p)ppGpp synthetases of S. aureus reduced donation. LacZ reporter constructs revealed no change in the expression of plasmid transfer genes in the Rel mutant strains.

Conclusions. SR activation in *S. aureus* increases the transmission of conjugative and mobilisable plasmids from diverse families. Furthermore, conjugation frequencies correlate with cellular (p)ppGpp levels. Investigations into the molecular mechanism behind this relationship are ongoing. This study demonstrates that SR activation is an important and clinically-relevant contributor to the dissemination of resistance genes.

P604

A Cell-Free Tool Box for the Safe Study of Antimicrobial Resistance in Infectious Diseases

Kameshwari Chengan[1], Charlotte Hind[2], Mark Sutton[2], Christopher Smales[1], Simon Moore ORCID iD[3,1]

[1]University of Kent, Canterbury, United Kingdom. [2]UKHSA, Salisbury, United Kingdom. [3]Queen Mary University of London, London, United Kingdom

Abstract

Limitations exist in how we can study antimicrobial resistance (AMR) mechanisms in major bacterial infectious diseases.

We present a rapid and safe cell-free system for studying antibiotic susceptibility and resistance at the level of gene expression, all from inside a test tube. First, we screen a small molecule library for antimicrobials and compare potency to cell-based assays and reveal selective import for some antibiotic classes. Then, we develop a range of AMR cell-free systems, which include single antibiotic-resistant strains and multidrug resistant clinical isolates. This reveals how single genetic determinants alter gene expression resistance to common antibiotics, and untangles the complexities of intracellular and transport AMR mechanisms. As an example, an RNA polymerase variant (found in *Mycobacterium tuberculosis* isolates) provides a 60-fold increase in resistance to rifampicin. In summary, we provide a rapid, tractable, and non-living cell-free system to directly study antimicrobial susceptibility and resistance in a clinically important Gram-negative bacterial infectious disease. We propose this technology is generalisable for the study of priority antibiotic-resistant strains or novel AMR mechanisms, as well as providing a platform for novel antibiotic discovery.

P605

Impact of media and strain choice on the effectiveness of antibiotics against *P. aeruginosa* biofilms

Brogan Richards, Samuel Fenn, Miguel Camara, Luisa Martinez-Pomares, Shaun Robertson

The university of Nottingham, Nottingham, United Kingdom

Abstract

There are a range of in vitro biofilm models currently used to test the effectiveness of antibiotics, in the context of cystic fibrosis (CF), which do not take into consideration the influence of media and strain variations on the determination of the minimal biofilm inhibitory concentrations (MBICs). This can result in high rates of antibiotic failure. We have assessed the effect of *P. aeruginosa* strains and media choice on antimicrobial tolerance to the β-lactam antibiotic meropenem. We have used a colony biofilm model and measured colony forming units and metabolic activity using a resazurin-based assay as a readout to determine the MBIC50 and MBIC90. Results showed that, for *P. aeruginosa* PAO1-L, the MBIC50 and MBIC90 significantly increased with media complexity, with PAO1-L grown in LB showing the highest sensitivity whereas growth in synthetic sputum media (SCFM2) revealed the highest levels of tolerance to the antibiotic. SCFM2 also resulted in a 10% reduction in total colony biomass indicating less overall drug activity in this media. In contrast, *P. aeruginosa* LESB58 showed the opposite trend where an increase in media complexity from LB to synthetic sputum media (SCFM2) reduced the sensitivity to meropenem. This could be due the slow growing nature of LESB58. This work shows that media and strain choices should be taken into consideration for in-vitro antimicrobial testing against *P. aeruginosa* biofilms to maximise the success of treatment.

P606

Development and evaluation of meropenem encapsulated nanostructured lipid carriers as an antimicrobial treatment of *Pseudomonas aeruginosa*.

Charlotte Wilson, David Wareing, Kamalinder Singh

University of Central Lancashire, Preston, United Kingdom

Abstract

Pseudomonas aeruginosa (P.aeruginosa) is a Gram negative bacterium which most commonly causes lower respiratory infections within immunocompromised patients. Meropenem is a carbapenem antibiotic with a beta lactam structure which is recommended as a first line treatment for *P. aeruginosa* infections. *P.aeruginosa* has developed multiple resistance mechanisms including resistance to beta lactam antibiotics such as meropenem.

Meropenem encapsulated nanostructured lipid carriers (mNLC) are prepared using a hot homogenisation technique with solid and liquid lipids. Liquid lipids with antimicrobial properties will be used to further enhance the antimicrobial effect. Meropenem loaded NLC will be tested for appropriate particle size, poly dispersion index and zeta potential. High performance liquid chromatography (HPLC) will be used to confirm drug encapsulation.Reference isolates of *P.aeruginosa* will be tested for minimum inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) using the mNLC. Synergistic effect of meropenem with NLC lipids will be investigated.Interactions between mNLC and *P. aeruginosa* cells will be investigated to determine if the drug delivery takes place intracellularly or extracellularly with electron microscopy and might give insight into the potential to bypass some antibiotic drug resistance mechanisms.

The use of meropenem encapsulated NLCs may provide an alternative drug delivery system with the potential to optimise therapeutic dosage without increasing nephrotoxicity.

P607

Tazobactam selects for multidrug resistance

Emma Holden ORCID iD[1], Muhammad Yasir ORCID iD[1], Keith Turner ORCID iD[1], Ian Charles[1,2], Mark Webber ORCID iD[1,2]

[1]Quadram Institute Biosciences, Norwich, United Kingdom. [2]Norwich Medical School, UEA, Norwich, United Kingdom

Abstract

Piperacillin-Tazobactam (Pip-Tazo) is a beta-lactam/beta-lactamase inhibitor combination and among the most prescribed antimicrobials in hospital medicine, used to treat pneumonia, septicaemia and urinary tract infections. Piperacillin is inactivated by commonly carried resistance enzymes, but Tazobactam inhibits these allowing successful treatment. The effect of Piperacillin on Gram-negative bacteria has been widely studied, but less attention has been paid to the effects of Tazobactam. We used the massive transposon mutagenesis approach TraDIS-*Xpress* to determine the genes in *Escherichia coli* that affect survival when exposed to Piperacillin, Tazobactam and Pip-Tazo. We found significant differences in the selective pressure of the two drugs: a striking finding was that multiple efflux systems were essential for survival in the presence of Tazobactam and Pip-Tazo, but only one was crucial in the presence of Piperacillin. Evolution experiments supported this finding, where bacteria selected after repeated culture with Tazobactam and Pip-Tazo showed increased efflux activity relative to Piperacillin or no drug. Increased efflux activity is a common precursor to development of high-level antimicrobial resistance as efflux synergises with other mechanisms. Therefore demonstrating that Tazobactam can select for multidrug-resistant pathogens is a worrying concern. We also identified multiple pathways affecting susceptibility to both drugs separately and in conjunction, including genes involved in cell envelope biosynthesis, signalling systems, DNA repair, protein translation and export and acid stress responses. These findings could have consequences for antibiotic prescription and should inform the development of future beta-lactamase inhibitors which should consider selective potential of the inhibitor as well as the active drug.

P608

A Novel Route to Inhibit Biofilm Formation in Urinary Tract Infections

Ashraf Zarkan ORCID iD, Cameron Croft, Alicia Liu, David Summers

University of Cambridge, Cambridge, United Kingdom

Abstract

Biofilm formation is a key aspect of the antibiotic resistance crisis, providing structural protection against the immune system and antibiotic treatment. Biofilms are made of numerous living micro-organisms, such as bacteria, growing and evolving as a community. Biofilms are implicated in up to 80% of infections in the human body, and associated with treatment failure, complications, and repeated infections such as regularly seen with urinary tract infections (UTIs). There is an urgent need for the development of biofilm inhibitors to be used in combination with standard antibiotics (as antibiotic enhancers) in the treatment and management of biofilm-associated infection, such as UTIs.

UTIs are among the most frequent bacterial infections affecting 150 million people per year worldwide and 75% of infections are due to uropathogenic *E. coli*. Work in our laboratory and elsewhere highlighted an important role for the tryptophanase-mediated signalling pathways in the formation of *E. coli* biofilms. Tryptophanase is a pyridoxal 5'-phosphate (PLP)-dependent enzyme involved in the degradation of multiple amino acids, but best known for catalysing the hydrolytic β-elimination of L-tryptophan to indole, ammonia and pyruvate. Combining molecular, biochemical, biophysical and computational techniques, we analysed the role of tryptophanase in *E. coli* biofilms and identified a set of small-molecule inhibitors that are capable of inhibiting biofilm formation in clinical strains of uropathogenic *E. coli*. The ultimate aim is to develop effective biofilm inhibitors to be used in conjugation with antibiotics in the treatment and management of UTIs.

P609

Resistance is Mobile: Exogenous capture of mobile antimicrobial resistance in private drinking water supplies in Ireland.

Marwa Alawi ORCID iD[1,2], Cian Smyth[1], ThiThuy Do ORCID iD[1], Trinidad Velasco-Torrijos ORCID iD[3,2], Fiona Walsh ORCID iD[1,2]

[1]Biology Department, Maynooth University, County Kildare, Ireland. [2]Kathleen Lonsdale Institute for Human Health, Maynooth University, County Kildare, Ireland. [3]Chemistry Department, Maynooth University, County Kildare, Ireland

Abstract

Background: Drinking water has been identified as a reservoir for the persistence of mobile antimicrobial resistance genes (ARGs).

Aim: This study investigated the presence of mobile ARGs in Irish private drinking water supplies.

Methods: Drinking water samples were provided by citizens. Using the exogenous capture method, transconjugants were selected on LB agar supplemented with a combination of rifampicin and either amoxicillin, tetracycline, or ciprofloxacin. Plasmids were extracted and sequenced using MinION technology.

Results: Plasmids (n=11) were extracted and sequenced from three household samples. Mobile ARG *bla*TEM which confers resistance to beta-lactams was present in two of the household drinking water samples. Other mobile ARGs identified were the tetracycline-resistance gene *tetB*, phenicol resistance genes *cfrA, catI* and *floR*, as well as aminoglycoside and sulphonamide resistance genes *apha*-variants and *sul2*, respectively. Multidrug resistance (beta-lactam, chloramphenicol and tetracycline or ciprofloxacin) was found in two of three water samples. In addition, MDR plasmid from one household carried virulence factors genes including variants of the *iro, iuc, and iut* which are associated with iron-chelating siderophores. Replicon types identified included the IncFIA, IncFIB, IncFIC, IncQ1, Col(MGD2), Col(Ye4449) and ColRNAI. Three cases were found to contain mobility or conjugative machinery MOBF and/or MOBP.

Conclusion: Private drinking water supplies were reservoirs of ARGs and the mobile elements required for dissemination.

P610

Detecting phage transducible *ermB* in environmental *Clostridioides difficile* isolates

Wing Ki Charlotte Chan, Shan Goh ORCID iD

University of Hertfordshire, Hatfield, United Kingdom

Abstract

Mobile genetic elements harbouring antimicrobial resistance genes contribute significantly to the fitness of *Clostridiodes difficile*, an important gut pathogen. Investigating phage transduction of *ermB*, conferring erythromycin resistance in *C. difficile*, is challenging because indicator strains for phage infection are lacking. This study aimed to develop a quantitative qPCR assay to detect phage transducible *ermB* derived from *C. difficile* lysogens. Three environmental isolates predicted to contain transposons with *ermB* and 11 prophages were used. Phage endolysin genes were aligned and degenerate primers were designed to detect all phage particles. Phages were induced from lysogens with 3mg/mL mitomycin C (MC) or 64 mg/mL ciprofloxacin (CIP), bacterial nucleic acid was removed by DNase I and RNase A, and phage encapsulated DNA (PE-DNA) was extracted. By PCR, erm B was detected in PE-DNA of 2/3 and 3/3 lysogens treated with MC and CIP, respectively. However, endolysin was detected in PE-DNA of only one lysogen treated with MC and CIP. Efficiency of *ermB* and endolysin degenerate primers by qPCR were 72% and 51%, respectively. By qPCR *ermB* and endolysin were detected in PE-DNA of 3/3 and 1/3 lysogens, respectively, regardless of treatment. In conclusion, *ermB* is potentially transducible by new phages of environmental *C. difficile* but transduction frequency was not determined because the endolysin degenerate primers were inefficient. Finding a more conserved gene in diverse *C. difficile* phages is needed to develop a “universal primer” for a quantitative assay of phage transduction.

P611

Antimicrobial assessments of cationic surfactants against prevalent pathogens in CAUTIs

Xiao Teng, Shuai Zhang, Matthew Wylie, Caoimhe Clerkin, Colin McCoy

School of Pharmacy, Queens University Belfast, Belfast, United Kingdom

Abstract

Background

Invasion of bacteria from urethral meatus when catheterization is the main reason of catheter associated urinary tract infections (CAUTIs), leading to medical problems and increase the health care costs. In this work, the feasibility of two cationic surfactants, benzalkonium chloride (BAK) and didecyldimethylammonium bromide (DDAB) against common bacteria in CAUTIs was studied.

Methods

MIC and MBC tests were carried out to determine the susceptibility of S. aureus and E. coli. Synergistic effect was investigated by checkerboard assay. Bacteria adherence assay was performed to exam the anti-adherent ability.

Results

The MICs of DDAB and BAK ranged from 1 to 8 μg/mL, and the MBCs of DDAB and BAK ranged from 2 to 16 μg/mL. Both BAK and DDAB showed bactericidal effect because MBC/MIC ratio was below 4, but DDAB indicated stronger killing ability because of lower MBCs.

From the checkerboard assay, BAK and DDAB indicated addition effect due to ∑FICs is in between 0.5 and 2.

Above 3 log reduction of the adherent bacteria was observed on the p(HEMA) surfaces after being loaded with BAK and DDAB. Especially, on DDAB-loaded hydrogel surfaces, bacteria adherence was reduced below limit of detection.

Conclusion

BAK and DDAB can effectively kill S. aureus and E. coli, but compared to BAK, DDAB is more potent to be an antimicrobial agent against common uropathogens. In addition, combination use of two cationic surfactants can make antimicrobial effect stronger. In terms of the reduction of bacteria adherence, DDAB showed stronger anti-adherent ability than BAK.

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16s RNA sequencing using Oxford Nanopore Technology in a clinical diagnostic setting: a new approach.

David Carlisle[1], Edward Cunningham-Oakes[2,3], Jack Pilgrim[4], Aleksandra Rzeszutek[5], Christopher Parry[6,7], Andrew Frankland[1], Alistair Darby[2,8], Anna Smielewska ORCID iD[1,6,9]

[1]Liverpool Clinical Laboratories, CSSB, Liverpool University Hospitals Foundation Trust, Liverpool, United Kingdom. [2]Department of Infection Biology and Microbiomes, Institute of Infection, Institute of Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [3]NIHR Health Protection Research Unit in Gastrointestinal Infections, Liverpool, United Kingdom. [4]Department of Evolution, Ecology and Behaviour, Institute of Infection Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [5]School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom. [6]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom. [7]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [8]Center for Genomic Research, Liverpool, United Kingdom. [9]School of Medicine, University of Liverpool, Liverpool, United Kingdom

Abstract

In an era of increasing antibiotic resistance, it is essential to provide rapid and precise infection diagnostics leading to targeted antimicrobial treatment. 16S ribosomal RNA sequencing is a well-established method in specialist Microbiology laboratories for bacterial identification, where culture methods are unsuccessful or otherwise unapplicable.

The current gold standard involves diagnostic sample amplification followed by Sanger sequencing of a component of the 16S gene. This approach overlooks smaller populations of organisms within the sample as a consensus sequence of only a part of the genome is analysed.

Oxford Nanopore Technology (ONT) has been recognised as a promising prospect for rapid antimicrobial diagnostics and identification. We present a novel 16S workflow using ONT, successfully introduced as part of routine care within an NHS laboratory. The validation and verification process was carried out against the in-house Sanger sequencing assay incorporating the standard EQA (external quality assurance) scheme. The challenges inherent in clinical diagnostics such as workflow, logistics and cost efficiency; including a new bioinformatics pipeline and custom controls were successfully overcome and benchmarked against current laboratory standards. Overall, the process was shown to improve the limit of detection and yielded additional bacterial taxa identified in all samples tested. Expert Microbiology opinion was obtained to confirm the clinical impact of the additional data obtained.

In conclusion we have successfully demonstrated the advantages and challenges of introducing ONT into routine diagnostic use within the NHS including the potential clinical impact of the service on timely diagnostics, treatment and antimicrobial stewardship.

P612

Repurposing aurodox as an anti-virulence treatment for Enterohaemorrhagic *E. coli* infections.

Rebecca McHugh ORCID iD[1], Andrew Roe[1], Paul Hoskisson[2]

[1]School of Infection and Immunity, University ofGlasgow, Glasgow, United Kingdom. [2]Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

Abstract

Enterohaemorrhagic *E. coli* (EHEC) is an acute pathogen of the small intestine which is responsible for foodborne outbreaks of bloody diarrhoea. EHEC infections are often associated with high morbidity and mortality rates due to the production of Shiga toxins which can initiate Haemolytic Uremic Syndrome (HUS) which is a major cause of acute renal failure in children. Unlike most bacterial infections, EHEC cannot be treated with traditional antibiotics as they can lead to increased production of Shiga toxin, resulting in poorer patient outcomes. In these studies, we investigate aurodox- a natural product of *Streptomyces goldiniensis*- as a potential anti-virulence therapy for the treatment of EHEC. To understand its mechanism of action and assess the suitability of this molecule for repurposing as an anti-virulence compound, a multidisciplinary approach to understanding aurodox was used. Whole transcriptome analysis, cell infection and transcriptional reporter assays have been used to were used to demonstrate that aurodox transcriptionally downregulates the expression of the Type III Secretion System (T3SS)- an essential colonisation factor in EHEC. We have also observed these effects across other enteric pathogens carrying a homologous T3SS such as Enteropathogenic Escherichia coli (EPEC) and Salmonella species. Significantly, unlike traditional antibiotics, aurodox does not induce the production of Shiga toxin. Furthermore, we have developed a Citrobacter rodentium + Shiga toxin animal model in which we are able to test the efficacy of aurodox.

P613

Phage-encoded lysins: A promising approach to treat urinary tract infections caused by uropathogenic *Escherichia coli*

Sonia Nain[1], Sakshi Dhar[1], Utkarsh Gaharwar[1], Vaishali Gupta[1], Sarmad Hanif[1], Ritam Das[1], Bhakti Chavan[1], Sanket Shah[1], Deepa Sikriwal[1], Urmi Bajpai[2], Syed Ahmed[1]

[1]Techinvention Lifecare Pvt. Ltd, Mumbai, India. [2]Acharya Narendra Dev College, University of Delhi, Delhi, India

Abstract

Background: Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) affects 150 million people globally. The WHO has described antibiotic resistance in uropathogens as a key pressure point in growing AMR crisis. Therefore, we are exploring phage encoded-lysins as potential alternatives to antibiotics to treat UTIs. Our study involved an *in silico* strategy for the discovery and characterization of lysin sequences targeting *E. coli* cell wall and evaluating the bactericidal activity of these recombinant lysins using in vitro assays.

Methods: Novel lysin sequences were searched by BLAST homology and by screening *E. coli* prophages in the database (using PHASTER). Simultaneously their characterization and domain architecture was examined. Based on physicochemical properties, 7/16 were selected for cloning, expression, and purification as recombinant proteins for evaluating bactericidal activity.

Results: In silico analysis showed lysozyme-like domain in 9 /16 lysins and the predicted structure was either modular or globular. Using spot on lawn assay, most promising lysin seq5 showed lytic activity on *E.coli* ATCC 25922 reference strain, ATCC 700928 UPEC strain, and BL21 DE3 cells. Turbidity assay showed a 74.94% drop in OD_{600nm} in BL21 DE3 cells treated with lysin seq5 (15 µM) after 3 hrs of incubation at 37°C. Log killing assay showed 4 $\log_{10}$ reduction in the treated cells. Lysozyme assay demonstrated higher than / comparable lysozyme activity with positive control.

Conclusion: Lysin seq5 exhibited highest activity against different *E. coli* strains. Enzyme stability, kinetics studies, and screening against *E.coli* clinical isolates from UTI patients are underway.

P614

Dissemination of Multidrug and Extensively Drug-Resistant Salmonella Typhi inPediatrics' Septicemia Patients in Pakistan

Muhammad Usman Qamar[1], Aliya Batool[2], Atifa Ambreen[3]

[1]Government College University, Faisalabad, Pakistan. [2]Fatima Mamorial Hospital, Lahore, Pakistan. [3]Government College University, Faislabad, Pakistan

Abstract

Background: The rise and spread of MDR and XDR Salmonella Typhi is a serious global public health issue. After the first case was discovered in Sindh province, Pakistan in 2016, it has now been reported worldwide mainly in vulnerable patients. Methods: A total of 4,543 blood samples from children's patients with suspected sepsis were collected and processed in the BacT/ALERT system. Confirmed isolates and MIC were determined in the VITEK® 2 system. Molecular identification for blaCTX-M, blaIMP, blaVIM, blaTEM, gyrA, qnrS and gyrB was carried out by PCR. Results: Of 4,543 blood cultures, 458 (10%) were positive for bacterial growth and S. Typhi (415; 90%) remained the principal pathogen. Antibiogram testing indicated that 208 (50.1%) and 137 (33%) were MDR and XDR S. Typhi, respectively. MDR isolates were positive for blaTEM-1 and XDR S. Typhi cases showed positive blaCTX-M, gyrA, gyrB. Conclusion: High prevalence of MDR and XDR S. Typhi in children. Strict hygiene practices must be in place, including hand hygiene, clean drinking water and food and vaccination.

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The Selective Effects of Diclofenac, Metformin, and 17-Beta-Estradiol, on Antimicrobial Resistance in Complex Bacterial Communities

April Hayes ORCID iD[1], Lihong Zhang[1], Jason Snape[2], William Gaze[1], Aimee Murray[1]

[1]University of Exeter, Penryn, United Kingdom. [2]AstraZenaca, Alderley Edge, United Kingdom

Abstract

Non-antibiotic drugs (NADs) have been demonstrated to have antibacterial effects, and several have been shown to increase horizontal gene transfer rates in single species experiments. Our previous work showed that several tested NADs had inhibitory effects on growth of a bacterial community. Three of these NADs were further investigated. Complex communities (wastewater influent) were evolved separately for seven days with concentrations of diclofenac, metformin, and 17-beta-estradiol, ranging from concentrations similar to those found in the human gut, down to environmentally relevant concentrations. Analyses of sequenced metagenomes of the evolved communities shows that diclofenac and metformin did not select for known antibiotic resistance genes (ARGs) at any tested concentration. This was supported by qPCR data that showed that *intI1* (a genetic element associated with acquired AMR) prevalence was not affected by concentration in these evolved communities. However, 17-beta-estradiol was associated with an increase in several metal resistance genes (*arsB*, *arsR*, and *ncrA*) in response to increasing 17-beta-estradiol concentrations in the evolved communities. In agreement with this, qPCR results show that *intI1* was shown to increase in the 17-beta-estradiol communities when compared to the control evolved community. Phenotypic plating showed various changes in resistance to various antibiotics. These results demonstrate that diclofenac and metformin are unlikely to select for known, heritable antibiotic resistance genes in complex communities at tested concentrations. Conversely, 17-beta-estradiol may select for AMR as indicated by increased metal resistance gene relative abundance and *intI1* prevalence, the latter of which is linked with increased AMR evolvability.

P615

Multidrug Resistant Pathogens Isolated from the Plumbing System of Patient Bathrooms in Irish Hospital.

Emma Rossiter[1], Thuy Thi Do[1], Marie Gaffney[2], Cathal Collins[2], Fiona Walsh[1]

[1]Maynooth University, Kildare, Ireland. [2]Cavan and Monaghan Hospital, Cavan, Ireland

Abstract

Background

Within Irish hospitals handwashing sinks, showers and toilets, are shared spaces with connected wastewater systems, creating reservoirs for biofilms and dissemination of Healthcare-Associated Infections (HAIs).

Methods

Employing ESWABs, toilets and drains of sinks and showers were sampled, at three time points for one day a week over three weeks, across three patient rooms. Samples were enriched for extended spectrum beta-lactamases (ESBL) producing and carbapenem-resistant *Enterobacteriales*, imipenem-resistant *Pseudomonas* and *Acinetobacter* spp., Methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *enterococci* (VRE) and isolated on selective agar with antibiotic. Isolates were selected for antibiotic susceptibility testing.

Results

Most frequently isolated was carbapenem-resistant *Acinetobacter* species, followed by ESBL producing *E*. *coli*, MRSA, carbapenem -resistant *Pseudomonas* species, carbapenem -resistant and ESBL *Klebsiella* species and carbapenem-resistant *E. coli*. No VRE were detected. Carbapenem–resistant and ESBL *E*. *coli* and *Klebsiella* species were detected in all rooms and time points. Over 60% of EBSL-positive isolates displayed resistance to imipenem. Additionally, many ESBL *Enterobacteriaceae* were multi-drug resistant with over 80% displaying ciprofloxacin resistance. Metallo-beta-lactamase activity was detected in 93% of carbapenem-resistant *Klebsiella* species.

Conclusion

While no carbapenem-resistant infections were detected in patients in this hospital carbapenem pathogens were present in the hospital sanitary ware. The resistant bacteria identified pose a serious risk to patients and potentiate an environmental resistome within hospital plumbing facilities.

P616

Transforming *Enterococcus faecium* NCTC13165 to confer Chloramphenicol Resistance

Gavin Dillon ORCID iD, Fiona Walsh ORCID iD

Maynooth University, Maynooth, Ireland

Abstract

Background: To study the effect antibiotic resistance genes (ARGs) have on gram-positive pathogens, *Enterococcus faecium* NCTC13165 was transformed with a cfrA containing plasmid via conjugation. Conjugation was used to transform NCTC13165 to circumvent difficulties associated with transforming gram-positive bacteria.

Methods: A cfrA-pUCIDT vector was synthesized and transformed into *Escherichia coli* MG1655, which acts as a conjugation donor. NCTC13165 and MG1655 (cfrA-pUCIDT) cultures were grown overnight at 37°C. These cultures were harvested via centrifugation, and both were resuspended and combined in 1mL LB Broth. The combined bacteria mixture (100uL) was placed onto a sterile nitrocellulose filter that was placed on LB agar and incubated at 30°C overnight. Bacteria were then recovered by vortexing the filters in 5mL 0.9% NaCl for 1-2 minutes. After vortexing, 100uL of this resuspension was plated onto LB plates containing 20ug/mL chloramphenicol and 5ug/mL colistin and were incubated for 72 hours at 37°C.

Results: Colonies that grew on the selective agar plates were screened via PCR with primers designed to amplify a 1.1kB region of cfrA-pUCIDT. PCR positive transformants containing cfrA-pUCIDT were stocked for future research.

Conclusion: Using the above method proved to be an effective means of transforming E. faecium. This method will enable researchers investigating antimicrobial resistance to manipulate these pathogens more easily and reliably.

P617

Investigating the Effects of Aminoglycoside Antibiotic Plant Protection Products on Mixed Microbial Communities

Laura Murray[1], Lihong Zhang[1], Jason Snape[2], William Gaze[1], Aimee Murray[1]

[1]University of Exeter, Penryn, United Kingdom. [2]AstraZeneca, Macclesfield, United Kingdom

Abstract

Antibiotics are applied to the environment during agricultural processes. Some of the most used crop antibiotics are aminoglycosides. Streptomycin and gentamicin are clinical aminoglycosides that are also used as plant protection products (PPPs), kasugamycin is used only as a PPP. PPPs are applied in large quantities, at high concentrations, non-specifically, which then disseminate and degrade, resulting in a range of concentrations being present in the environment.

The effects of aminoglycosides on microbial communities are not fully understood but could include increased antibiotic resistance (ABR) and ABR genes (ARGs) in the environment, which could be transferred between environmentally and clinically relevant bacteria. In this study, we compared antibiotics of the same class for their effects on microbial communities and investigated if crop-specific antibiotic kasugamycin selects for clinically relevant ARGs.

Lowest observed effect concentrations for streptomycin (2mg/L), gentamicin (0.25mg/L) and kasugamycin (50mg/L) were generated by overnight growth experiments. These informed the concentration ranges for seven-day evolution experiments. Following DNA extraction, qPCR for *intI1*/16S rRNA and metagenomic sequencing was performed. Sequencing data was analysed to determine community (metaphlan2) and ARG (ARGs-OAP) changes.

Streptomycin and gentamicin significantly increased *intI1* at concentrations orders of magnitude lower than application concentration. Increased resistance to antibiotic classes (e.g., aminoglycosides, beta-lactams) were also observed. Species richness decreased with increasing concentration. Kasugamycin did not increase *intI1*, nor decrease species richness, nor strongly select for ARGs, however, community changes were observed.

Results provide information on the effects of PPPs on microbial communities, contributing to understanding ABR from a “One Health” perspective.

P618

Novel antimicrobials from *Streptomyces coelicolor* challenged by *Aspergillus* fungi under nutrient-deplete conditions

Ruth Nair, Rajeev Srivastava, Cher-Pheng Ooi

Edge Hill University, Ormskirk, United Kingdom

Abstract

Background: There is an ever-increasing need to identify novel antimicrobials. Public Health England estimates 10 million deaths globally by the year 2050 if the current trend of rampant antimicrobial resistance is left unchecked. Co-culturing *Streptomyces* with competing microorganisms is a viable method to identify novel antimicrobials.

Methods: Wild-type (WT) and mutant (M1146, M1152, M1154) *S. coelicolor* strains were co-cultured with *A. flavus* and *A. parasiticus* under nutrient-deplete conditions to induce secretion of secondary metabolites by *S. coelicolor*.

Results: Anti-fungal activity was only observed under nutrient-deplete conditions. *Aspergillus* radial growth was reduced, with the mean diameter of colonies decreased by ~12 mm when co-cultured with all strains of *S. coelicolor*. Zones of inhibition for *Aspergillus* growth around a seeded *Streptomyces* colony was 31 ± 1.5 mm and 22 ± 6.7 mm when co-cultured with the *S. coelicolor* strains M1152 and M1146 respectively, a significant increase compared to when co-cultured with WT *S. coelicolor* (4 ± 0.6 mm; $P < 0.0001$, $n = 3$). Quantitation by florescence microscopy indicate that *S. coelicolor* suppress *Aspergillus* growth by inhibiting fungal wall formation and hyphae proliferation. Extracts from co-culture conditions which suppress *Aspergillus* growth inhibit ESKAPE pathogens. Analyses of these extracts by LC-MS have identified known compounds with anti-microbial activity, along with novel compounds with predicted anti-microbial activity.

Conclusion: The data argue that *Streptomyces* species competing with *Aspergillus* under nutrient-deplete conditions secrete secondary metabolites with broad range anti-microbial activity. *S. coelicolor* mutant, M1152, exhibits significantly increased antimicrobial activity in co-cultured conditions as compared to WT.

P619

Biodiversity as a barrier to the spread of Antimicrobial Resistance and Escherichia coli

Shauna O'Shea ORCID iD[1], Elisa Catao[2], Xavier Bellanger[2], Christophe Merlin[2], Fiona Walsh[3]

[1]Maynooth Uinversity, Maynooth, Ireland. [2]Université de Lorraine, Nancy, France. [3]Maynooth University, Maynooth, Ireland

Abstract

It's widely hypothesised that high biodiversity acts as a barrier for the spread of antimicrobial resistance (AMR) in various environments.

Glass slides were deployed into one high/low biodiversity river (HBR or LBR) for 6 weeks. Artificial microcosms were set up using fresh, filtered river water and the glass slides from the rivers containing the river biofilms. Microcosms were inoculated with kanamycin resistant (plasmid pG527) Escherichia coli (MG1655). Biofilms were sampled during a 14-day period post inoculation, DNA extracted, and absolute quantification qPCR performed.

Plasmid and E. coli levels varied across time in the biofilm DNA. All copy numbers are per 3uL of DNA: One day post inoculation, plasmids = 10^8 and E. coli were 10^7 in the LBR and plasmids = 10^7 and E. coli 10^4 in the HBR. Day 14 post inoculation, LBR levels of plasmid were 10^5 and E. coli levels were reduced to 10^5. However, in the HBR E. coli levels dipped to 10^3 from 10^4 while plasmid levels were reduced to 10^5 from 10^7.

The presence of the E. coli in the HBR were prevented from increasing in copy number at day 1. Plasmid levels were high in both HBR and LBR experiments at day 1 and day 14 and higher than. Escherichia coli in the HBR, which only persisted in the LBR experiment. Our findings display that both high and low biodiversity do not act as a barrier for the dissemination of mobile AMR but high biodiversity prevented the infiltration of E. coli.

P620

Intracellular survival of the ESKAPE pathogen *Enterobacter bugandensis* in human macrophages

Karolina Wojtania, Georgiana Parau, Miguel Valvano ORCID iD

Queen's University Belfast, Belfast, United Kingdom

Abstract

The genus *Enterobacte*r includes Gram-negative opportunistic pathogens causing nosocomial multidrug antimicrobial resistant (AMR) infections, for which this genus is part of the ESKAPE group of dangerous pathogens for mankind. Despite the wealth of information on *Enterobacter*'s mechanisms of antimicrobial resistance, less is known on pathogenesis, especially interaction of these bacteria with innate immune cells. *E. bugandensis* is an *Enterobacter* species that has gained notoriety for its ability to cause neonatal sepsis and other serious infections. We investigated the ability of a clinical isolate of *E. bugandensis*, strain E104107, to survive in human macrophages employing differentiated THP-1 monocytes and primary human monocyte derived macrophages (hMDM). Macrophages and bacteria (E104107R, expressing a recombinant mCherry) were co-incubated at different MOIs. Extracellular bacteria were removed by washes and kanamycin treatment and infections followed for 3, 5, and 21 hours. Immunofluorescence microscopy revealed that bacteria are engulfed by macrophages and reaches a membrane-bound compartment resembling a late phagolysosome. During the observed times, macrophages appear healthy and they do undergo pyroptotic cell death based on interleukin 1-β (IL1-β) release and Gasdermin-D cleavage. Quantification of the intracellular bacterial burden by colony forming units (CFUs) revealed a small increase in the number of intracellular bacteria (≤ 1 log10 unit), relative to the initial dose. This suggests that intracellular bacteria are unable to replicate, or they replicate at very slow rate. Together, our findings indicate that *E. bugandensis* can survive in macrophages without eliciting a pro-inflammatory response, suggesting macrophages may provide a bacterial niche to escape immune surveillance and antibiotic treatment.

P621

The role of TnaA in the formation of *E. coli* biofilms

Alicia Hoi Ying Liu ORCID iD, Ashraf Zarkan ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

A key aspect of the antimicrobial resistance crisis is the ability of bacteria to form biofilms, which are structural barriers against antibiotics and the host immune system. Biofilms are implicated in recurrent infections, such as urinary tract infections (UTIs) which are predominantly caused by *Escherichia coli* (*E. coli*). Tryptophanase (TnaA) is a promiscuous amino acid degrading enzyme in *E. coli*, and its role in the formation of biofilms has been previously demonstrated. However, the exact amino acids that can be degraded by TnaA remains unclear, and the contribution of each degradation pathway in the formation of *E. coli* biofilms is unknown. Here we describe a set of enzymatic assays to assess the potential interaction of TnaA with all 20 proteinogenic amino acids that can be synthesised by *E. coli*. To investigate the contribution of each degradation pathway in *E. coli* biofilms, we describe a set of biofilm assays in minimal media supplemented with one amino acid at a time. This work investigates the mechanisms underlying the role of TnaA in the formation of *E. coli* biofilms, which may provide a potential route to combat biofilms in UTIs.

P622

MOLECULAR SCREENING OF HEMOPLAMAS IN DONKEYS FROM SOMALIA

Mohamed Abdulkadir Shair ORCID iD

Abrar University, Mogadishu, Somalia. UFPR, Parana, Brazil

Abstract

Hemotropic mycoplasmas (hemoplasmas) are the causative agents of hemolytic infectious anemia in mammals worldwide. Hemoplasmas, closely related to 'Candidatus Mycoplasma haematobovis' (formerly 'Candidatus Mycoplasma haemobos'), were found in horses, with the animals presenting anemia, weight loss, and pallor mucosa. Epidemiological data and molecular characterization of hemoplasma infection in Somalia are lacking since the country has used donkeys for various activities, including carrying goods on their backs or pulling carts loaded with goods like firewood, foods, water, and construction materials. Considering the social-economic importance and the lack of data regarding the epidemiology and molecular characterization of these pathogens in donkeys in Somalia, this study aimed to screen donkeys for hemoplasma species infection and evaluate factors associated with infection in Somalia. A total of 30 donkeys were evaluated. Blood samples were collected, DNA was extracted, and samples were further screened using hemoplasma genus-specific real-time (qPCR) and conventional PCR assays targeting the 16S rRNA gene of this group of bacteria. The animals were not infested by ticks at the time of sampling. The mammal endogenous gene glyceraldehyde-3-phosphate dehydrogenase (gapdh) was consistently amplified from all donkeys' samples. The Gapdh gene was consistently amplified in all donkey samples. All animals tested negative for hemoplasmas. Further studies evaluating a larger number of donkeys are needed to establish if hemoplasmas may be infecting this animal species in Somalia.

Keywords: Hemotropic Mycoplasma spp., domestic ass, hemotropic mycoplasmas, Sub-Saharan Africa.

P623

The anti-plasmid activity of cobalt complexes on antimicrobial drug resistance plasmids in Enterobacteriaceae

Ilyas Alav ORCID iD[1], Pedro Resende[2], Hannah Partington[1], Simon Gibbons[3], Rianne Lord[2], Michelle Buckner ORCID iD[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Liverpool John Moores University, Liverpool, United Kingdom

Abstract

Antimicrobial resistance genes (ARG), such as extended spectrum β-lactamases (ESBL) and carbapenemase genes, are commonly carried on plasmids. Plasmids can transmit between bacteria, disseminate globally, and cause clinically important resistance. Therefore, targeting plasmids could reduce ARG prevalence, and restore the efficacy of existing antibiotics. Here, the effect of cobalt metal complexes on the transmission of ARG (including ESBL/carbapenemase) encoding plasmids in *Escherichia coli* and *Klebsiella pneumoniae* was investigated. Antimicrobial susceptibility testing of ten cobalt complexes revealed no antibacterial activity. The impact of cobalt complexes was tested on the transmission of plasmids with different incompatibility groups in *E. coli* using agar conjugation assays. Four of the most promising cobalt complexes were evaluated for their ability to reduce transmission of ESBL/carbapenemase plasmids pCT and pKpQIL tagged with *gfp*, in *E. coli* and *K. pneumoniae*, respectively using a flow cytometry based assay. Compounds Co4, Co6, and Co8 significantly reduced transmission of R388, pKM101, R6K, and RP4 plasmids in *E. coli*. Compounds Co4, Co5, Co6 and Co8 also significantly reduced pCT*gfp* transmission in *E. coli* and pKpQIL*gfp* transmission in *K. pneumoniae* without affecting bacterial growth. This novel study reports a previously uncharacterised ability of designer cobalt complexes to reduce transmission of clinically relevant plasmids. These cobalt complexes are not cytotoxic towards mammalian cells and are not antibacterial, therefore could be optimised and employed as anti-plasmid compounds to reduce prevalence of AMR genes in bacterial populations.

P624

Advancing phage-based interventions for cattle colonised with Escherichia coli O157:H7

Asim Ullah, Marianne Keith, Alba Park De La Torriente, Sean McAteer, Alison Low, David Gally

Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom

Abstract

E. coli O157:H7 is a zoonotic pathogen that colonises ruminants especially cattle by forming strong intimate attachment with the epithelial cells of the terminal rectum. Once in the food chain, E. coli O157 poses a potential risk to human health causing mild to life-threatening illness and has been associated with sporadic outbreaks globally especially in the UK, USA, and Argentina. Several control interventions have been trialled in cattle including treatment with phage but these have had limited success. We hypothesize that a better understanding of the physiology of E. coli O157 at the site of colonisation will aid the development of a phage intervention. We are studying phage-host interactions under conditions which more closely reflect those in vivo with the bacteria intimately attached to epithelial cells. Under these conditions, phage have been identified which can kill the majority of intimately attached bacteria but there are sub-populations of E. coli O157 that resist phage predation. Our on-going research aims to elucidate the mechanisms responsible for this population heterogeneity and phage resistance in order to identify phage which can lyse these sub-populations. Phage cocktails demonstrated to be effective in cell culture will then be trialled in cattle colonised with E. coli O157.

P625

Deletion of the formicamycin biosynthetic gene cluster (*for* BGC) induces the expression of a previously cryptic NRPS and the production of new antimicrobial molecules

Valerie Chan, Rebecca Devine, Matthew Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

The discovery of new antibiotics is crucial to mitigate the spread of antibiotic resistance, which poses an urgent threat to public health. *Streptomyces formicae* KY5 has several biosynthetic gene clusters (BGCs) that may encode for bioactive metabolites. However, many of these BGCs are silent under laboratory conditions, offering an underexploited source of new antimicrobial compounds. Deletion of the formicamycin BGC (*for* BGC) using CRISPR/Cas9 induces the activation of cryptic BGCs, most significantly, a non-ribosomal peptide synthetase (NRPS) BGC. Bioassays against *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus* (MRSA) show the Δ*for* mutant displays increased inhibitory activity compared to the wild type, suggesting the NRPS encodes for antimicrobial compounds. Analysis of extracts from the Δ*for* mutant by HPLC shows the mutant produces at least 2 new compounds compared to the wild-type. At least one of these compounds is readily purified by preparative HPLC and is bioactive against MRSA and *Bacillus subtilis*. The isolation and characterisation of other compounds from the NRPS BGC is ongoing. In addition, deletions and over-expressions of a *Streptomyces* antibiotic regulatory protein (SARP) located in the NRPS BGC are being conducted, to establish its role in the regulation of the BGC and to aid with compound isolation. As well as characterising new antimicrobial metabolites from *S. formicae*, this work will contribute to our understanding of antibiotic biosynthesis and gene regulation in this talented strain.

P626

Molecular epidemiology of linezolid resistance *Enterococcus faecium* isolated from clinical and Environmental samples.

Vandana Rani[1,2], Mohammad Amin-ul Mannan[3], Rajni Gaind[1]

[1]VMMC & Safdarjung Hospital, New Delhi, India. [2]Lovely professional University, Phawara, Punjab, India. [3]Lovely Professional University, Phagwara, Punjab, India

Abstract

Background: *Enterococcus faecium* (EF) is the 3rd most common cause of nosocomial infections. Vancomycin resistance has emerged globally. WHO has classified linezolid (LZ) as a reserve antibiotic for vancomycin resistant isolates. The present study was conducted to study the etiology of Linezolid resistant E. faecium (LREF) and the role of immediate patient environment as possible source of infection. Characterized for LZ resistance mechanisms, virulence genes and molecular epidemiology.

Methods: LREF isolated from clinical and environmental samples were identified by Vitek. AST was performed by disc diffusion & MIC of linezolid, vancomycin, daptomycin and quinupristin/dalfopristin were determined by E-test. PCR was performed for virulence genes (esp & hyl) and LZ resistance mechanism (mutation in 23S rRNA, *optrA* and *cfr* gene). PFGE was used for molecular typing.

Results: A total of 227 LREF were studied (202 clinical & 25 environmental). The clinical isolates were isolated from urine (50%), blood (41%) and pus (9%). The MIC of LZ ranged 8-256mg/L. Vancomycin resistance (≥80%) and MDR (≥90%) was high. Predominant mechanism of LZ resistance among clinical isolates was *optrA* (48%) and mutation in 23S rRNA among environmental isolates (33%). Virulence gene *esp* was detected in 52% & 71% and *hyl* in 10% &13% clinical and environmental isolates respectively. PFGE analysis demonstrated 30 clones from both clinical and environmental isolates.

Conclusion: Prevalence of *optrA* gene was high in clinical isolates. PFGE suggest spread of LREF isolates via cross transmission. Existence of clinical and environmental LREF in same clones suggests horizontal transmission of resistant genes.

P627

CRISPR-Cas9 as a tool to combat AMR in *E. coli* ST131

Clàudia Morros-Bernaus, David Walker-Sünderhauf, Stineke van Houte

University of Exeter, Penryn, United Kingdom

Abstract

Antimicrobial Resistance (AMR) is a well-known global concern. An important group of AMR genes are those encoding Extended-Spectrum ß-lactamases (ESBL), which include CTX-M enzymes. The extraintestinal pathogenic *Escherichia coli* sequence type 131 (ST131), responsible for urinary tract and bloodstream infections, often harbours *CTX-M* genes, especially *blaCTX-M-15*. Here, we explore the use of CRISPR-Cas9 to combat AMR from *E. coli* ST131.

A CRISPR-Cas9 cassette targeting *blaCTX-M-15* was delivered by either transformation or conjugation to four *E. coli* ST131 isolates, all encoding *blaCTX-M-15*. For transformation, the CRISPR-Cas9 cassette was encoded on a cloning vector (pACYC_csg), and for conjugation on a broad host-range conjugative plasmid (pKJK5::csg), delivered by *E. col*i K12 donors. A control non-targeting CRISPR-Cas9 cassette (targeting a random sequence absent from ST131) was delivered in the same way.

When the CRISPR-Cas9 cassette was targeting *blaCTX-M-15*, a reduction of 2-4 orders of magnitude was observed in the viable population of transformants or transconjugants as compared to the non-targeting control. Interestingly, the isolates responded differently to CRISPR-Cas9 treatment depending on the *blaCTX-M-15* genetic location, with stronger reductions in viable population observed in isolates with a chromosomal *blaCTX-M-15* gene compared to a plasmid-located gene. Further experiments are underway to analyse how the location of *blaCTX-M-15* could influence the escape from CRISPR-Cas9 targeting.

Our study shows that CRISPR-Cas9 can be used against *E. coli* ST131 carrying a clinically relevant AMR gene, by strongly reducing the population sizes of this pathogen. This is a promising approach that could help combat AMR.

P628

Creation of library of carbapenem and extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*

Chloe McKenna ORCID iD, Fiona Walsh ORCID iD

Maynooth University, Maynooth, Ireland

Abstract

Background: The World Health Organisation (WHO) indicated in 2017 that the greatest threat to human health in the next decade will be antimicrobial resistance (AMR). In order to focus research, they released a catalogue of bacteria which they believe pose the greatest threat. The first priority in this list included the carbapenem and extended spectrum beta-lactamase producing Enterobacteriaceae such as *Klebsiella pneumoniae* and *Escherichia coli.*

Results: Five different resistance genes of clinical importance (bla_{KPC}, $bla_{CTX\text{-}M\text{-}15}$, *aac(6')-Ib-cr, armA*, and bla_{NDM}) were individually transformed into *E.coli* MG1655 on Golden Gate puCIDT plasmid backbone using electroporation. These plasmids were then conjugated into *K. pneumoniae* NCTC418 through filter mating. This created a library of strains of *E. coli* and *K. pneumoniae* which all contained the same plasmid backbone, but with a different AMR gene. This library can be used to identify and compare the impact of the specific AMR gene on the host cells.

Conclusion: Generation of highly similar pathogens differing by only the AMR gene is a valuable resource to understand the impact of the AMR gene on different pathogens

P629

Do nanomedicines have the potential to combat antimicrobial resistance (AMR)?

Umaru Bah[1], Nick Wheelhouse[1], Samantha Donnellan[1], Stephanie Dancer[1], Steve Rannard[2], Catherine Unsworth[2]

[1]Edinburgh Napier University, Edinburgh, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Antimicrobial resistance (AMR) causes an estimated 4.95 million deaths annually, with a projected rise to 10 million deaths by 2050. With increasing clinical AMR infections, rapid emergence of multidrug resistance organisms, compounded by a decline in discovery and development of new antibiotics, the need for novel antimicrobial therapies is urgent. The current study will investigate the efficacy of solid drug nanoparticles (SDNs) against clinical antibiotic resistant bacterial strains. Nano-formulations of existing antibiotics are hypothesized to demonstrate increased bioavailability and efficacy, this reducing the effective dosage required, thus mitigating drug resistance. As SDNs are intended for human use, it's vital these are tested in suitable models resembling mammalian systems. In recent years the larvae of the wax moth, *Galleria mellonella*, have been developed as a viable alternative to extensive animal experimentation due to its demonstrated shared commonalities with mammalian models.

As a first stage to validate the infection model, *Galleria mellonella* larvae were infected via the haemocoel with defined doses of E. coli NCTC 12241. The mortality rate and viability of larvae were monitored over a 7-day period using the health index scoring system (HISS). Significant differences ($p = 0.03$) were observed for the doses of $5x10^4$ CFU and $5x10^3$ CFU with mortality rates of 88% and 42% and HISS of 0.76 and 3.31, respectively. Using this model, subtle differences in the effects of different bacterial doses upon larval viability were detectable. Further work will therefore be used for screening SDNs against a range of antibiotic resistant clinical bacterial isolates.

P630

A new twist on drug design: AdhE spirosomes as cross species anti-virulence targets

Ester Serrano ORCID iD, Andrew Roe, Olwyn Byron

University of Glasgow, Glasgow, United Kingdom

Abstract

Enterohemorrhagic Escherichia coli (EHEC) is a major human pathogen that causes bloody diarrhoea, haemorrhagic colitis, and life-threatening haemolytic uremic syndrome. In our quest to develop new compounds to block a range of infections, we have studied the EHEC virulence factor AdhE. Deletion of *adhE* results in a huge attenuation of virulence and a reduction in expression of the major system used by EHEC to attach to host cells. AdhE is a bidirectional enzyme that catalyses the conversion of acetyl-CoA to ethanol. Unusually, AdhE oligomerises *in vivo* and *in vitro* to form long (15-120 nm) filaments heterogenous in length called spirosomes.

Here, we report progress on understanding the solution behaviour of AdhE in partially fractionated, but still functional, form. Analytical ultracentrifugation (AUC) sedimentation velocity and small angle X-ray scattering (SAXS) analysis of fractionated AdhE demonstrates that it is not possible, with conventional size exclusion chromatography, to generate homogenous AdhE samples. Instead, we were able to determine the constituents of fractions and building on this, we observed that the length of the spirosomes has no effect on the enzymatic activity of the protein in the forward reaction. However, in the reverse reaction there is a huge reduction in activity when the spirosome length decreases. Taken together these results suggest that the spirosome formation drives the direction of the enzymatic activity of the protein. These results are key because understanding how AdhE spirosomes work will help us to develop, in the longer-term, specific inhibitors that might function against a range of Gram-negative pathogens.

P631

Rapid Screening of Novel Lantibiotics Using *Streptococcus mutans*

Saswati Biswas

KUMC, Kansas City, USA

Abstract

Widespread antibiotic resistance among pathogenic bacteria is a severe challenge that today's pharma sector is facing. Thus, lantibiotics, which are extensively modified short peptide antimicrobials, are being explored with renewed interest. In this study, we investigated whether a heterologous production system can be established to expedite the discovery of novel lantibiotics by developing a screening process without having access to the original producer. For this screening, we tested a lantibiotic mutacin II producer strain *Streptococcus mutans* T8. This organism is genetically easy to manipulate and is naturally a prolific producer of lantibiotics. Since lantibiotic biosynthetic apparatus use the leader peptide of the lantibiotic as the recognition sequence, we replaced only the core sequence of mutacin II with its homologs in the chromosome by fusion PCR and recombination. For screening novel lantibiotics, we used a target panel of twelve bacterial strains. We used a mutacin II knockout strain as a negative control. To verify the promiscuity of the biosynthetic machinery of mutacin II, we first tested a well-characterized lantibiotic homolog, lacticin 481. Production of the bioactive lacticin 481 ascertained the validity of the system. Using this screening process, we were able to produce a novel bioactive lantibiotic that we named SPP2. SPP2 shares homology with nukacin-ISK1, a potent lantibiotic. Thus, we established a system where simply by electronically accessing the core sequences of novel homologous lantibiotics, one can test the target and potency of novel lantibiotics using various *S. mutans* strains as screening hosts.

P632

Evaluation of diagnostic accuracy of the Rapid antimicrobial susceptibility testing – direct disk diffusion (RAST- dDD) compared with conventional AST in positively flagged blood culture bottles for select gram positive and gram-negative organisms

Rajni Gaind, Anusha Bhatnagar

VMMC and Safdarjung Hospital, New Delhi, India

Abstract

OBJECTIVES: To evaluate the performance of Rapid -direct disc diffusion test (EUCAST-RAST) from positively flagged blood culture (BC) bottles for relevant sepsis pathogens.

MATERIAL AND METHODS- Standardization of RAST was done using BC broths spiked with isolates with defined resistance. In phase2, EUCAST-RAST was validated on 500 positive culture broths - 100 each of E.coli, K.pneumoniae, P. aeruginosa, S.aureus and Enterococcus species. The BC were processed immediately and AST was performed by EUCAST- standard disc diffusion and EUCAST-RAST . Reading was done at 4, 6 and 8 hrs by 3 independent observers. Genetic mechanism of resistance was also studied. Scatter plots were constructed and categorical agreement, very major error, major error, and minor errors were evaluated in accordance with the International Organization for Standardization criteria.

RESULTS: For five pathogens , most inhibition zones could be read after 4 h. Overall the VME and ME decreased from 4-8 hours. For E.coli and K. pneumoniae at 8h , CA> 93% for cefotaxime, ceftazidime, ciprofloxacin, amikacin and CA=100% for piperacillin-tazobactam and meropenem was observed . Excellent results were observed in case of P.aeruginosa for all tested antibiotics at 8 hours. RAST was successfully identified all cephalosporin and carbapenem resistant E.coli, K.pneumoniae, P.aeruginosa. RAST correctly identified all methicillin-resistant S.aureus and vancomycin resistant Enterococcus faecium at 4-6 h. RAST for Gentamicin raised challenges for most pathogens

CONCLUSION: RAST shortens turnaround time, is reliable tool to improve the clinical management of sepsis by providing rapid phenotypic susceptibility data. RAST method can be implemented in routine laboratories with laboratory workflow adjustments

P633

Studying antibiotic resistance evolution against gentamicin using two new in vitro methods; Antibiotic Resistance Growth Plate and Chunking methods

Razan Abbara, Suzy Moody, Simon Gould, Gary Forster-Wilkins, David Mackintosh, Ekaterina Lamber, Mark Fielder

Kingston University London, London, United Kingdom

Abstract

Due to the importance of antibiotic resistance as a global health threat, it is important to understand how de novo resistance occurs, especially in bacteria known to be of medical importance. Various techniques have been implemented to study antibiotic resistance (AR) evolution (Lukačišinová and Bollenbach, 2017). In the current study, two in vitro techniques were used, the Antibiotic Resistance Growth Plate (ARGP) and "Chunking methods", to develop resistance against gentamicin at MIC (0.5-2 mg/l) and 10XMIC (5-20 mg/l) levels in Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. Once a resistant phenotype was observed whole genome sequencing (WGS) and bioinformatic analysis were used to determine the nature of the resistance phenotype. WGS revealed the presence of a mutation in fusA gene encoding elongation factor G (EF-G) in two strains of E. coli mutated at 10XMIC of gentamicin. This was also observed in two strains of S. aureus at both MIC and 10XMIC levels of antibiotic and a strain of K. pneumoniae at both of MIC and 10XMIC antibiotic concentrations. The mutations found were in domains V and IV of EF-G. Additionally, there were mutations in 5S ribosomal RNA gene in two strains of E. coli at MIC, and in 16S ribosomal RNA gene in a strain of E. coli and K. pneumoniae at 10XMIC. The data from this study indicates that in vitro AR evolution against gentamicin is driven by genetic mutations in EF-G and ribosomal genes, and offers a useful insight to the nature of antibiotic resistance.

P634

Studying antibiotic resistance evolution against gentamicin using two new in vitro methods; Antibiotic Resistance Growth Plate and Chunking methods

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Abstract

Due to the importance of antibiotic resistance as a global health threat, it is important to understand how de novo resistance occurs, especially in bacteria known to be of medical importance. Various techniques have been implemented to study antibiotic resistance (AR) evolution (Lukačišinová and Bollenbach, 2017). In the current study, two in vitro techniques were used, the Antibiotic Resistance Growth Plate (ARGP) and "Chunking methods", to develop resistance against gentamicin at MIC (0.5-2 mg/l) and 10XMIC (5-20 mg/l) levels in Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. Once a resistant phenotype was observed whole genome sequencing (WGS) and bioinformatic analysis were used to determine the nature of the resistance phenotype. WGS revealed the presence of a mutation in fusA gene encoding elongation factor G (EF-G) in two strains of E. coli mutated at 10XMIC of gentamicin. This was also observed in two strains of S. aureus at both MIC and 10XMIC levels of antibiotic and a strain of K. pneumoniae at both of MIC and 10XMIC antibiotic concentrations. The mutations found were in domains V and IV of EF-G. Additionally, there were mutations in 5S ribosomal RNA gene in two strains of E. coli at MIC, and in 16S ribosomal RNA gene in a strain of E. coli and K. pneumoniae at 10XMIC. The data from this study indicates that in vitro AR evolution against gentamicin is driven by genetic mutations in EF-G and ribosomal genes, and offers a useful insight to the nature of antibiotic resistance.

P636

Plasmid Mediated Spread of New Delhi Metalloproteinase Genes Within Gram-Negative Organisms

Cosmika Goswami[1], Stephen Fox ORCID iD[2], Alistair Leanord[2], Tom Evans[2]

[1]University of Glasgow, Scotland, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

A major mechanism of carbapenemase resistance in Gram-negative organisms is mediated by genes encoding New Delhi metalloproteinases (NDMs). The genetic basis for their dissemination is not well understood. We set out to assemble complete plasmid and chromosomal sequences from representative carbapenem-resistant organisms.

NDM-containing human isolates of Escherichia coli and Klebsiella pneumoniae were subjected to short and long read sequencing, as well as isolates of multiple organisms containing a blaNDM gene from one patient. Reads were assembled using Unicyler.

We assembled complete genome and plasmid sequences from 9 E. coli and 10 K. pneumoniae isolates. Most blaNDM genes were found in IncF group plasmids. Sequence identity between the different plasmids containing blaNDM genes was in general limited, but some IncX and IncF group plasmids showed much higher similarity. The immediate genetic environment of blaNDM-5 showed a common element found in 6 plasmids of E. coli and K. pneumoniae flanked by an Insertion Sequence Common Region (ISCR) element on one side and IS26 on the other. The immediate environment of the blaNDM-1 genes was less well conserved, although it was flanked by diverse mobile elements. In 1 patient, 3 different organisms containing a blaNDM gene were isolated; detailed genetic analysis showed that these were most likely simultaneously acquired.

Although plasmids carry the genes encoding NDM metalloproteinases, the main route of transfer between microbes is through much smaller mobile genetic units, with conservation of ISCR and IS26 elements flanking blaNDM genes in different bacterial plasmids.

P636

Antibacterial activity of novel dimeric linear polyamines against *Staphylococcus aureus*

Edward Douglas, Abdulaziz Alkhzem, Toska Wonfor, Shuxian Li, Timothy Woodman, Ian Blagbrough, Maisem Laabei

Bath University, Bath, United Kingdom

Abstract

New therapeutic options are urgently required for the treatment of resistant Staphylococcus aureus infections. Accordingly, in this study we sought to exploit an existing chink in the armour of S. aureus, its curious vulnerability towards polyamines. This involved the synthesis of novel linear polyamines, called AHA-1394, -1282, and -1268 based on spermine and norspermine. These molecules carried with them a greater positive charge and resulted in enhanced anti-staphylococcal activity compared to their parental compounds. With AHA-1394 displaying the most impressive bactericidal activity of the three synthetic polyamines, we sought to further investigate its potential as a S. aureus therapeutic option. We found that AHA-1394 displayed minimal toxicity towards human cells and impressive biofilm prevention and dispersal activity. As S. aureus has become renowned for its propensity to gain resistance to antibiotics, we were interested in whether resistance against AHA-1394 could be selected for. We subsequently generated two stable resistant mutants in two different backgrounds which we found linked to a gain of function mutation, S337L, in MprF. However, this mutant MprF allele, although also associated with daptomycin non-susceptibility, appeared to confer resistance in a way unrelated to the lysyl-phosphatidylglycerol synthase or flippase activity of the enzyme. Instead, it appears that this mutation causes activation of the VraRS cell wall stress stimulon which results in a thicker cell wall. Finally, we show that AHA-1394 displays a particular proclivity for antibiotic potentiation. Together this study shows that polyamine derivatives are impressive drug candidates that warrant further investigation.

P637

Effect of genetic background and order of antibiotic selection on fitness and collateral susceptibility in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*

Alasdair Hubbard ORCID iD[1,2], Ellie Allman[2], Adam Roberts[2]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Mutations conferring antimicrobial resistance (AMR) often result in a fitness cost to bacteria in the absence of antibiotics. Understanding these fitness effects following selection in the presence of antibiotics may inform strategies to limit the emergence of AMR.

Ten independent lineages of two clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* were selected in the presence of sub-inhibitory concentrations of amoxicillin-clavulanic acid (AMC) or gentamicin (GEN). Five independent lineages of each isolate-antibiotic combination for one *E. coli* and *K. pneumoniae* isolates were subsequently selected for in the alternative antibiotic, creating 25 AMC-GEN and 25 GEN-AMC independent lineages per isolate. Comparative fitness and disc diffusion assays of all independent lineages were assessed.

We found that, while fitness effects of antibiotic selection in individual lineages varied, overall, the fitness effect of single selection in AMC or GEN is dependent on the genetic background. Additionally, relative to both the original and immediate single-selected ancestors, sequential selection in GEN-AMC resulted in a larger, overall fitness cost compared to AMC-GEN, which is replicated in both *E. coli* and *K. pneumoniae*. Collateral effects on susceptibility to other antibiotics can be either dependent or independent on the order of antibiotic selection.

This study indicates that the order of antibiotic selection can impact the overall fitness costs of AMR and collateral effects on other antibiotics. Therefore, if we can determine the antibiotics, or order of antibiotics, which maximise the potential fitness costs of AMR mutations, we may be able to limit the emergence of AMR.

P638

The N-terminal helix of MarA as a key element in the mechanism of DNA binding

Marina Corbella ORCID iD[1], Cátia Moreira ORCID iD[1], Shina CL Kamerlin ORCID iD[1,2], Jessica MA Blair ORCID iD[3], Enea Sancho-Vaello ORCID iD[3,4]

[1]Uppsala University, Uppsala, Sweden. [2]Georgia Institute of Technology, Atlanta, USA. [3]University of Birmingham, Birmingham, United Kingdom. [4]Universitat Autònoma de Barcelona, Barcelona, Spain

Abstract

Efflux is one of the mechanisms employed by Gram-negative bacteria to become resistant to routinely used antibiotics. The inhibition of efflux by targeting their regulators is a promising strategy to re-sensitise bacterial pathogens to antibiotics. AcrAB-TolC is the main Resistance-Nodulation-Division efflux pump in Enterobacteriaceae. MarA is an AraC/XylS family global regulator that regulates more than 40 genes related to the antimicrobial resistance phenotype, including *acrAB*. The aim of this work was to understand the role of the N-terminal helix of MarA in the mechanism of DNA binding. An N-terminal deletion of MarA showed that the N-terminal helix has a role in the recognition of the functional marboxes. By engineering two double cysteine variants of MarA, and combining *in vitro* electrophoretic mobility assays and *in vivo* measurements of *acrAB* transcription with molecular dynamic simulations, it was shown that the immobilization of the N-terminal helix of MarA prevents binding to DNA. This new mechanism of inhibition seems to be universal for the monomeric members of the AraC/XylS family, as suggested by molecular dynamics simulations done with the two-domain protein Rob. These results point to the N-terminal helix of the AraC/XylS family monomeric regulators as a promising target for the development of inhibitors.

P639

Investigating zinc-mediated antibiotic resistance and associated co-resistance in *E. coli* isolates

Amber Rose[1], Marwa Hassan[2], Mark Chambers[1,2], Roberto La Ragione[1,2]

[1]School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [2]School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Abstract

Background: Antimicrobial resistance (AMR) is global health threat affecting both humans and animals. Pig feed is frequently supplemented with zinc (Zn) as an additive. Zn has important biological and antimicrobial functions; however, supplementation with Zn can result in selection of Zn resistance, which has been demonstrated to be associated with antibiotic co-resistance.

Methods: A pooled pig faeces sample was used to screen for Zn resistant *E. coli* isolates at different Zn concentrations, and in the abscence and presence of cefotaxime, meropenem, colistin, tetracycline, tigecycline, gentamycin and ciprofloxacin. Selected isolates were further assessed for fitness using growth curves.

Results: Large numbers of Zn-resistant and colistin, tetracylcine and gentamycin Zn co-resistant *E. coli* isolates (10^6-10^7 cfu/mL) were demonstrated. The selected *E. coli* isolates with colistin-Zn co-resistance demonstrated a higher fitness cost than the tetracycline-Zn co-resistant strains. Meropenem-Zn co-selection demonstrated only 5 cfu/mL resistant *E. coli* isolates with no co-resistances detected with tigecycline and ciprofloxacin. Cefotaxime-Zn co-resistance was detected in large number of Enterobacteriaceae isolates of non-*E. coli* appreance. Antibiotic suscpetibility testing for the selected *E. coli* isolates demonstrated high resistances to ampicillin, azithromycin, sulfamethoxazole, tetracycline and colistin.

Conclusions: Zn-resistant E. coli isolates were detected in pig faeces, and were demonstrated to be coincident with gentamycin, colistin and tetracycline resistances. Colistin-Zn co-resistant E. coli isolates demonstrated an increased doubling time compared to tetracycline-Zn co-resistant E. coli isolates, which maybe associated with Zn resistance. Further investigations should be carried out to demonstrate if antibiotic-Zn co-resistances are coincidental or through shared molecular mechanisms.

P640

Transient gut colonizing species demonstrate increased resistome diversity in the human gut microbiome

Elle Hill ORCID iD, Jose Fernando Garcia Guevara ORCID iD, Theo Portlock ORCID iD, Sunjae Lee ORCID iD, Saeed Shoaie ORCID iD, David Moyes ORCID iD

King's College London, London, United Kingdom

Abstract

As the incidence of anti-microbial resistant bacteria increases globally, determining how bacteria obtain and maintain antimicrobial resistance within complex communities is imperative. In this study, we investigate the stool resistome (profile of antimicrobial resistance genes (ARGs)) of 100 healthy Swedish individuals over six timepoints. Using a metagenomics approach, we matched ARGs to bacterial species identified in the cohort, and correlated ARGs and bacterial species abundance for each individual across four timepoints. Next, we applied a hidden Markov model to the bacterial species counts over four timepoints in order to identify bacterial species that remain detectable over time (persistent colonizing species, (PCS)) and species that are only detected inconsistently (transient colonizing species, (TCS)). Common PCS and TCS were identified and related to species from our ARG correlation analysis. PCS and TCS displayed different contributions to ARGs over time, with TCS in particular being more strongly correlated with multiple ARGs than PCS across multiple timepoints. We also identified that species that are not a part of the commensal gut microbiome are more likely to have pathogenic characteristics, potentially explaining the increased prevalence of ARGs. By understanding the contribution of PCS and TCS to antimicrobial resistance in healthy individuals, we gained a deeper understanding of “baseline” ARG profiles. Subsequent comparison of this baseline to individuals with various diseases will enable us to observe the impact of specific bacterial species and ARGs in antimicrobial resistance in different disease settings, allowing for improved surveillance of the impact of clinical conditions on the spread of AMR.

P641

The artificial sweetener acesulfame-K inhibits multidrug resistant pathogen growth and potentiates antibiotic activity

Rubén de Dios ORCID iD, Chris R Proctor, Evgenia Maslova, Sindija Dzalbe, Christian J Rudolph, Ronan R McCarthy

Brunel University London, Uxbridge, United Kingdom

Abstract

Antimicrobial resistance is one of the most pressing concerns of our time, as deaths caused by it and its burden to global healthcare systems are increasing at an alarmingly rate, resulting in the antibiotic resistance crisis. To tackle this crisis, novel antimicrobial therapies need to be urgently developed. One possibility to do this is to identify natural compounds with antimicrobial activity or to repurpose compounds whose safety for humans is well established. For example, artificial sweeteners (AS). After screening the antimicrobial activity of a panel of ASs, we selected acesulfame-K (ace-K) for further investigation, as it presented a broad-spectrum activity against both Gram-positive and Gram-negative pathogens. We were able to confirm antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterococcus faecalis* and *Acinetobacter baumannii*. Using the latter as a model, we interrogated the antimicrobial and anti-virulence properties of ace-K using dRNA-seq. The phenotypic validation of our transcriptomic analysis confirmed ace-K can disable virulence factors, such as biofilm formation, twitching motility and natural transformation. Furthermore, sub-lethal concentrations of ace-K can potentiate various antibiotics, including carbapenems, against multidrug-resistant *A. baumannii*. Interestingly, genes encoding membrane-associated proteins were enriched in the dRNA-seq dataset, indicating ace-K can impact the cell envelope. Subsequent live cell imaging experiments confirmed this for *A. baumannii* and an *E. coli* laboratory model, showing that the ace-K treatment leads to bulge-mediated cell lysis, similarly to β-lactam antibiotics. Our findings provide consistent evidence supporting ASs, such as ace-K, as promising antimicrobial candidates or antibiotic adjuvants against multidrug-resistant pathogens.

P642

Evaluation of *Aeromonas* genomes (*in silico*) to assess the potential level of acquired antimicrobial resistance genes uptake from plasmids

Ogueri Nwaiwu[1], Helen Onyeaka[2]

[1]University of Birmingham, Edgbaston, Birmingham, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

The spread of antibiotic resistance genes (ARG) is currently a global concern. In many bacteria like *Aeromonas* species, it was found recently that acquired ARG sequences seemed to be widespread in their plasmids. Whether some of the acquired ARG seen in the plasmid sequences have been fully integrated into the genome assemblies is currently unknown.

In this study, thirty-two out of the 55 (58%) acquired ARG detected in *Aeromonas* plasmids in a previous study were detected in genomes analysed. Overall, sequences of 71 acquired ARG were detected in 85 genomes screened, and the predominant gene was *ampH* (HQ586946.1) which confers resistance to beta-lactams. Up to 25 genomes showed sequences of genes that confer resistance to at least three drug classes and suggest that they may exhibit multiple antibiotic resistance.

The prevalence of the same acquired ARG sequences in both *Aeromonas* plasmids and genomes is a concern and suggests considerable uptake of genes from the environment and possible integration into the chromosome. More studies are required to establish if all the genes detected in both genome and plasmids were because of chance or diversion after a speciation event. Considering that sequence similarity may not necessarily confer homology or imply conservation, more studies, in particular, laboratory verification will be required for phenotype confirmation for all acquired ARG mentioned in this study. The gene sequences detected may be used to gain more understanding of the paralogous and orthologous nature of environmental gene transfer in *Aeromonas* species.

P643

A new way to analyse antibiotic interactions at subinhibitory concentrations.

Dione L. Sánchez-Hevia, Nikolaos Fastis-Kavalopoulos, Dan I. Andersson

Uppsala University, Uppsala, Sweden

Abstract

Antibiotics are frequently prescribed in combinations, but their clinical effectiveness varies since a drug combination may show an increased (synergy), equal (additivity) or reduced (antagonism) effect on efficacy compared to that predicted from the compared to that predicted from the combined effect of the individual antibiotics.

The golden standard methodologies to determine the outcome of antibiotic interactions are checkerboards and time-kill assays. These methods are very time-consuming, hindering both their routine use and screening of large collections of isolates. The novel methodology CombiANT avoids both inconveniences. However, all these methods consider antibiotic interactions at drug concentrations that completely stop growth.

We propose a new method (SIF, sublethal interaction factor) to analyse antibiotic interactions in liquid cultures exposed to sublethal antibiotic concentrations. The cultures are grown in presence of subinhibitory drugs concentrations, either individually or in combination, and the OD600 is measured for 24h. Using the growth rate in medium without drug, the growth rates are transformed into relative growth rates. Subsequently, the theoretical additivity line is calculated multiplying AB1relative growth x AB2relative growth, using the mathematical model proposed by Bliss. In additive antibiotic interactions, the actual and theoretical relative growth lines overlap, whereas for synergistic or antagonistic interactions a difference between the two lines is detected. These differences can be analysed measuring the area under the curve of each relative growth line. Using well-known antibiotic interactions, we verified that statistical differences between the actual and theoretical growth lines are detected in synergistic and antagonistic cases, but not in additive situations.

P644

Understanding the Therapeutic Potential of Hydrogen Sulfide for Treating Microbial Skin Infections

Ka Ho Ho ORCID iD[1], Sara Nasereddin[2], Osamah Malallah[2], Stuart Jones[2], Albert Bolhuis[1]

[1]University of Bath, Bath, United Kingdom. [2]Institute of Pharmaceutical Science, London, United Kingdom

Abstract

Skin and nail infections with fungi and bacteria are very common, affecting 20-25% of the world population. The administration of antimicrobials is challenging due to the properties of skin and nails as protective tissues of the body. Topical treatments can be lengthy and often fail due to poor penetration of antimicrobials. However, we hypothesise that hydrogen sulfide (H_2S), which is small and has antimicrobial activity, provides an alternative treatment strategy as this may penetrate much better into skin or nails as compared to conventional antimicrobial agents. This study is to understand the effectiveness and mechanism of actions of H_2S to pathogens that cause skin and nail infections, including fungal dermatophytes and *Staphylococcus aureus*.

The H_2S was generated by the H_2S donor NaHS. To study H_2S' activities on different species, fungicidal, serial dilution spot, DCHF-DA fluorescence and cytochrome *c* oxidase (COX) assays were used.

H_2S was shown to be fungicidal against *Trichophyton rubrum* and *interdigitale*. H_2S was fungistatic against other fungi that cause nail infections, including *Aspergillus niger*, *Neoscytalidium dimidiatum*, *Fusarium oxysporum*, and *Candida albicans*. Early confocal and flow cytometry studies showed that H_2S kills dermatophytes in a time- and fungal life cycle-dependent manner. It also has a strong bactericidal activity against methicillin-resistant *S. aureus* (MRSA), even at high bacterial density levels. The mechanism of action is not clear yet, but early results suggest that H_2S leads to the production of ROS and inhibition of the COX complex.

The study demonstrated the antimicrobial activity of H_2S on several dermatophytes and MRSA.

P645

Teamwork makes the dream work: investigating potential Phage-Antibiotic Synergy (PAS) between pleurotin and phage K in S. aureus

Michaël Tadesse ORCID iD

University of Warwick, Coventry, United Kingdom

Abstract

Background

Multidrug-resistant organisms pose a major healthcare challenge. Current limitations on antibiotic discovery, have necessitated the search for novel discovery methods and sources as well as non-antibiotic alternatives. A very promising strategy is phage-antibiotic combination (PAC) therapy, where cocktails of phage and antibiotics are employed against problematic bacterial strains. In this project, the combination of the basidiomycete-derived, secondary metabolite pleurotin, which was shown to have anti-gram-positive activity, and bacteriophages targeting Staphylococcus aureus was examined.

Methods

Pleurotin was isolated from the basidiomycete Hohenbuehelia atrocaerulea grown in YM broth. Purification and structure characterisation was performed using flash chromatography, liquid chromatography mass spectrometry (LCMS), high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Purified pleurotin was compared to vancomycin, in combination with Phage K against S. aureus (NCTC 9318) in single agent and phage-antibiotic Broth Microdilution assays. Pleurotin and vancomycin twofold dilutions were prepared around reported MICs. Phage K inoculum was kept at 107 PFU/mL. Bacterial cell density was adjusted to 0.5 McFarland standard in accordance with CLSI guidelines.

Results

Pleurotin and vancomycin both showed expected inhibitory activity against NCTC 9318. Reduced growth was seen in most concentrations. Phage K in combination with both pleurotin and vancomycin proved to be synergistically effective compared to treatment with phage or antibiotic alone.

Conclusion

Results suggest that phage K can lower the working MIC for pleurotin and vancomycin. Subsequent work revealing efficiency against clinically relevant strains and biofilms will be necessary. Also, cytotoxicity of pleurotin in human cells will need to be evaluated.

P646

Multi-drug resistant *E. coli* displace commensal *E. coli* from the intestinal tract, a trait associated with elevated levels of genetic diversity in carbohydrate metabolism genes

Christopher Connor ORCID iD[1], Amanda Zucoloto[2], Ian-Ling Yu[3], Jukka Corrander[4], Braedon McDonald[2], Alan McNally[5]

[1]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia. [2]International Microbiome Centre, University of Calgary, Calgary, Canada. [3]Department of Critical Care Medicine, Cumming School of Medicine, University of Calgary, Calgary, Canada. [4]Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway. [5]Institute of Microbiology and Infection, College of Medical and Dental Science, University of Birmingham, Birmingham, United Kingdom

Abstract

Extra-intestinal pathogenic *E. coli* (ExPEC) can cause a variety of infections outside of the intestine and are a major causative agent of urinary tract infections. Treatment of these infections is increasingly frustrated by antimicrobial resistance (AMR) diminishing the number of effective therapies available to clinicians. Incidence of multi-drug resistance (MDR) is not uniform across the phylogenetic spectrum of *E. coli*. Instead AMR is concentrated in select lineages, such as ST131, which are MDR pandemic clones that have spread AMR globally. Using a gnotobiotic mouse model we demonstrate that an MDR *E. coli* ST131 is capable out-competing and displacing non-MDR *E. coli* from the gut *in vivo*. This is achieved in the absence of antibiotic treatment mediating a selective advantage. In mice colonised with non-MDR *E. coli* strains, challenge with MDR *E. coli* either by oral gavage or co-housing with MDR *E. coli* colonized mice results in displacement and dominant intestinal colonization by MDR *E. coli* ST131. To investigate the genetic basis of this superior gut colonization ability by MDR *E. coli*, we used a functional pangenomic analysis of 19,571 *E. coli* genomes revealing that carriage of AMR genes is associated with increased diversity in carbohydrate metabolism genes. The data presented here demonstrate that independent of antibiotic selective pressures, MDR *E. coli* display a competitive advantage to colonise the mammalian gut and points to a vital role of metabolism in the evolution and success of MDR lineages of *E. coli* via carriage and spread.

P647

Through the Looking-Glass: A curious outbreak of extended-spectrum beta-lactamase-producing Escherichia coli in New Zealand

Rhys White ORCID iD[1,2,3], Melinda Ashcroft ORCID iD[2,3], Kok-Gan Chan ORCID iD[4,5], Teik-Min Chong[4], Brian Forde ORCID iD[6], Kate Peters[2], Minh-Duy Phan[2], Xiaoyun Ren ORCID iD[1], Mark Schembri ORCID iD[7], Wai-Fong Yin[4], Deborah Williamson ORCID iD[8], Kristin Dyet ORCID iD[1], Scott Beatson ORCID iD[7,3], Joep de Ligt ORCID iD[1]

[1]Institute of Environmental Science and Research, Porirua, New Zealand. [2]The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Infectious Disease Research Centre, Brisbane, Australia. [3]The University of Queensland, Australian Centre for Ecogenomics, Brisbane, Australia. [4]University of Malaya, Institute of Biological Sciences, Division of Genetics and Molecular Biology, Kuala Lumpur, Malaysia. [5]Jiangsu University, International Genome Centre, Zhenjiang, China. [6]The University of Queensland, UQ Centre for Clinical Research (UQCCR) and Australian Infectious Disease Research Centre, Royal Brisbane & Women's Hospital Campus, Brisbane, Australia. [7]The University of Queensland, School of Chemistry and Molecular Biosciences, Australian Infectious Disease Research Centre,, Brisbane, Australia. [8]The University of Melbourne at The Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, Microbiological Diagnostic Unit Public Health Laboratory and Doherty Applied Microbial Genomics, Melbourne, Australia

Abstract

Before the 2000s, New Zealand's surveillance of antibiotic resistance in human pathogens detected less than nine annual cases of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (ESBL-E). In 2001, however, the Hawke's Bay region experienced an increase in ESBL-E associated with clinical infections. Throughout 2001 and 2002, 95 isolates were collected from the Hawke's Bay region during continuous annual surveillance. Another two isolates were collected during a 2007 survey. All isolates were characterised using phenotypic testing for ESBL production and underwent short-read whole-genome sequencing (WGS). Multilocus sequence typing, antibiotic resistance and virulence-associated genotyping were performed *in silico*. The genome for isolate ARL02201 was completed using additional long-read sequencing. This reference genome was then used to call single-nucleotide variants (SNVs) and reconstruct a core-genome phylogeny. Phenotypic and genotypic testing showed all isolates were ESBL producers. Most ESBL-E genomes from the Hawke's Bay region (*n*=84/97) were sequence type (ST)410. A cluster of 83 ST410 genomes had a median pairwise distance of eight SNVs. Notably, 72 ST410 genomes were collected from the same testing facility in 2001-2002. The ARL02201 genome (ST410) revealed the ESBL-encoding $bla_{\text{CTX-M-15}}$ gene, and narrow spectrum $bla_{\text{OXA-1}}$ and $bla_{\text{TEM-1B}}$, are carried within a 25 Kb island, alongside other resistance genes, on an F-type plasmid. All ST410 genomes from the cluster were confirmed to carry this 25 Kb resistance island. WGS of ESBL-E offers increased detail over conventional typing methods, particularly for investigations into outbreaks and understanding if they are related to introduction or endemic events.

P648

Tetraspanin CD9 peptides inhibit the adhesion of *P. aeruginosa* to HaCaT cells

Khairiyah Murad ORCID iD[1], AP Dr Sharaniza Ab Rahim ORCID iD[2], Dr Hassanain Al-Talib ORCID iD[3]

[1]Institute For Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Malaysia. [2]Department of Biochemistry, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Malaysia. [3]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Malaysia

Abstract

Multidrug-resistant (MDR) *P. aeruginosa* strains are evolving at an alarming rate because there are few effective treatments. Moreover, anti-adhesion therapies are relatively new in the treatment of bacterial infections. Therefore, this work aims to investigate the potential anti-adhesion properties of CD9 peptides on the adherence of *P. aeruginosa* to human keratinocytes modelled by the HaCaT cell line.

This study included a standard strain of *P. aeruginosa* (ATCC 27853) and an MDR- *P. aeruginosa* isolate. We determined the cytotoxicity effects of CD9 peptides on cell viability through an MTT assay. The rate of bacterial adhesion was determined quantitatively by counting viable bacterial cells expressed in the colony-forming unit while qualitatively by Giemsa staining and transmission electron microscopy (TEM) to examine the morphology of cells treated and non-treated.

The results showed that CD9 peptides were well tolerated by HaCaT cells with viability greater than 80%. A statistically significant difference at $p < 0.01$ in colonies formed by both *P. aeruginosa* isolates treated with CD9 peptides compared to the untreated samples. Photographs from Giemsa staining & TEM revealed that the treated samples had lower bacterial density and were located farther from the cells than the untreated cells. Besides, CD9 peptides had no negative effect on the morphologies of both HaCaT and *P. aeruginosa*.

This study successfully showed that the CD9 peptides as a promising therapeutic alternative against *P. aeruginosa* because it inhibits bacterial adhesion to host cells. Furthermore, it is suggested that CD9 peptides work by indirect bacterial killing that does not negatively affect the nature of host cells.

P649

Emergence of carbapenemase-producing *Pseudomonas aeruginosa* related to clinical infections at shrimp farming

Wandee Sirichokchatchawan ORCID iD[1,2], Nuvee Prapasarakul[3,2]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand. [2]Diagnosis and Monitoring of Animal Pathogens Research Unit, Chulalongkorn University, Bangkok, Thailand. [3]Department of Veterinary Microbiology, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok, Thailand

Abstract

Emergence and spreading of carbapenemase-mediated carbapenem-resistant bacteria are the major concern globally. Numerous antibiotics have been used to prevent diseases and enhance farm management in aquaculture production. Therefore, the potential for emergence of carbapenemase-producing bacteria in shrimp farming is incredibly high. The present study aimed to determine the occurrence of carbapenemase-producing Gram-negative bacteria from a shrimp farming. Shrimps and water samples were collected from 16 shrimp ponds in Central Thailand. Samples were cultured on MacConkey-meropenem agar, and incubated overnight. Different colony morphologies were selected for Carba NP test and MALDI-TOF Mass Spectrometry. A broth-dilution method was performed to determine the antimicrobial resistance profiles of isolated bacteria. PCR and whole genome sequencing were carried out for carbapenemase genes confirmation. Only two carbapenemase-producing isolates were discovered from shrimp intestines, and identified as *P. aeruginosa*. One of the isolates was found to be a high-risk sequence type (ST) 132 that associated with patients with septicemia in Czech, and urinary and respiratory tract infection in Croatia, and never been reported in Thailand. The genomic analysis of carbapenemase-producing *P. aeruginosa* ST132 confirmed that it also possessed the genes *aph(3')-IIb, fosA, catB7*, and bla_{OXA-50} and bla_{PAO} encoded for aminoglycoside, fosfomycin, chloramphenicol, and Beta-lactamase. Our findings highlighted the importance of the emergence of carbapenemase-producing bacteria in aquaculture production with high potential impacts on human from the spreading of these bacteria through food products and spill-over.

P650

Isolation, identification, and exploration of the deep-sea sponge derived *Streptomyces microflavus* and its potential for novel antimicrobial compound production

Jazmin Conway[1], Grant January[1], Rosemary Dorrington[2], Kerry Howell[1], Mathew Upton[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]Rhodes University, Makhanda, South Africa

Abstract

In an era where current antibiotics are becoming less effective towards resistant strains of bacteria, there is a need for new antibiotics. Antimicrobial Resistance is one of the top 10 global public health threats and is described as 'the highest health endangerment in the 21st century'. Here we isolated a deep-sea *Streptomyces microflavus* with promising antimicrobial activity.

Application of carefully prepared sponge material to various types of media, selective and non-selective, led to the cultivation of more than 200 isolates. Various antimicrobial assays were carried out against ESKAPE pathogens. Whole genome sequencing (WGS; Illumina and ONT) and hybrid assembly allowed exploration of putative biosynthetic gene clusters (BGCs) for novelty of one strain of interest. Liquid-liquid extraction followed by Reversed Phase--HPLC and Mass Spectrometry (LC-MS/MS) enabled characterisation of bioactive compounds.

This study reports, for the first time, the isolation of *Streptomyces microflavus* from the deep-sea sponge *Pheronema carpenteri*. WGS (8.4MBp) analysis allowed identification of the isolate as *Streptomyces microflavus.* AntiSMASH revealed 38 predicted BGCs, 7 of which are potentially novel and 22 being unique to this isolate when compared with the most closely related *Streptomyces microflavus.* The isolate showed Gram positive antimicrobial potential through various bioassay tests and through downstream compound purification this isolate was shown to secrete multiple bioactive compounds. Further compound characterisation, including LC-MS/MS and NMR spectroscopy, are being used to dereplicate known and unknown bioactive compounds. Overall, this strain of *Streptomyces microflavus* is a promising candidate for the discovery of novel antimicrobial compounds.

P651

A semi-synthetic natural product cocktail based on Bald's eyesalve: antibacterial activity, and interactions with other antimicrobials

University Warwick, Jessica Furner-Pardoe, Freya Harrison

University of Warwick, Coventry, United Kingdom

Abstract

Antimicrobial resistance is a major global challenge due to the increase in disease mortality, increased hospital stay, and cost of treatment. Natural products from plants amongst others are some of the alternatives that have been suggested to treat drug-resistant infections. Bald's eyesalve is a medieval remedy for treating eye infections that have shown broad-spectrum antibacterial activity. Biofilm eradication by a reconstruction of Bald's eyesalve was previously shown to require the presence of all four ingredients in the remedy. We have found that this biofilm eradication activity can be recapitulated by a semi-synthetic cocktail comprising a compound purified from one ingredient (compound A) and a second ingredient from the remedy (ingredient B). We report that the semi-synthetic cocktail has good antibacterial activity against both planktonic and biofilm-associated populations of Staphylococcus aureus, Acinetobacter baumanii, and Pseudomonas aeruginosa in standard lab media and in host-mimicking models. The cocktail showed >4-log killing against S. aureus Newman and A. baumanii ATCC 19606 in an in vitro wound biofilm model, and against P. aeruginosa LESB58 in an ex vivo model of cystic fibrosis lung biofilm respectively. We confirmed that the cocktail has better activity than its individual components, especially in biofilm models. We also explored interactions with other antimicrobials using a planktonic checkerboard assay. Our study showed that we can make a synthetic cocktail from our current formulation that could be used as treatment for chronic wound and cystic fibrosis lung infections.

P652

Genomics of MDR *Escherichia coli* and *Klebsiella pneumoniae* reveals antibiotic resistant genes and virulence factors from clinical isolates of sepsis patients

Nishitha R Kumar ORCID iD[1], Akila Prashant ORCID iD[1], Kerry Cooper ORCID iD[2], Tejashree A ORCID iD[3]

[1]JSSAHER, Mysuru, India. [2]University of Arizona, Tucson, USA. [3]JSSAHER, Mysore, India

Abstract

Background: Bacterial infection is always a starting point for sepsis. Newly evolved bacterial genes provide an insights into the genetic network essential for the species' replication, adaptation, and survival. We compared the phenotypic and genotypic factors underpinning the multidrug resistance (MDR) in *Escherichia coli (EC)* and *Klebsiella pneumoniae(KP)* and the patterns of their virulence factors to understand their mode of action in ICU patients.

Materials & Methods: A hospital-based research was conducted on clinical isolates of MDR *EC* and *KP*. Thirty-six bacterial DNA was isolated which was subjected to bacterial genome sequencing on Illumina platform. The fastqc was checked using Unicycler. The assembled fasta files examined for functional annotation using prokka for % coverage and % identity to reference genome. Bacterial genomes were deposited in the NCBI under BioProjects PRJNA821629 and PRJNA790696 for *EC* and *KP,* respectively. The downstream NGS analysis was performed with ResFinder, Virulence factor database, and Plasmid Finder using ABRicate tool to find respective genes.

Results: The novel AMR core genes such as blaEC and fosA5 (100%), blaSHV-11 (55%), PmrB (44.4%), blaCTX-M-15 (22%), blaSHV-14 and sitABCD (11.1%), blaSHV-28 and blaSHV-1 (16%), blaSHV-27 and blaOKP-B and blaTEM-1B (5.5%), sitABCD (11.1%) and virulence genes such as ybtP, ybtQ, fdeC, (100%); MLST of *EC* showed ST69, ST405, ST131, ST361, ST2851, ST167, ST1287, ST8346, ST450, ST73, and *KP* showed ST2278, ST16, ST280, ST273, ST2096, ST23, ST4, ST147, ST437, ST48.

Conclusion: Our study successfully concluded that bacterial MDR and virulence genes significantly regulate the survival and transmission of bacterial activity in patients diagnosed with sepsis.

P653

Combining Hi-C and long-read metagenomic sequencing to link antimicrobial resistance genes to their bacterial hosts in the human gut microbiome

Gregory McCallum[1], Amanda Rossiter[1], Sarah Kuehne[2], Joshua Quick[1], Willem van Schaik[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom. [2]The School of Dentistry, University of Birmingham, Birmingham, United Kingdom

Abstract

The complex microbial ecosystem inhabiting the human gut, termed the gut microbiome, can act as a reservoir for antimicrobial resistance genes (ARGs), collectively known as the gut resistome. A metagenomic technique called Hi-C can link bacterial genes to phylogenetic markers, allowing ARGs to be linked to their bacterial host.

Here, we implemented Hi-C to investigate the bacterial hosts of ARGs in 4 human faecal samples. We developed a bioinformatic workflow utilising Hi-C sequencing data and metagenomic binning techniques that was able link 87 ARGs to their hosts across the four samples, including ARGs carried on plasmids in an Acinetobacter pittii strain that was used as a spike-in. We found that the main limiting factor in identifying the bacterial hosts of the ARGs was the success of the binning process and the ability to taxonomically classify the bins. To improve this, long-read metagenomic nanopore sequencing was implemented on the faecal samples, and ongoing analyses aim to greatly improve the construction of metagenome assembled genomes. For this, high-molecular weight DNA extraction methods were optimised to achieve high-quality, long-read sequence data.

Following Hi-C analysis, the hosts of several ARGs were successfully cultured and whole-genome sequenced. These sequencing data provided genomic context for the ARGs, and offered insights into the limitations of using Hi-C to link ARGs to their host in complex metagenomic samples. Overall, our data highlight the complementarity of Hi-C and culture-based approaches to fully characterise the gut resistome.

P654

The Efficacy Novel Lytic Phages Against Clinical Bacterial Isolates from Urinary Tract Infections in Biofilms and In vivo

Libby Duignan[1], Craig Winstanley[2], Chloe James[3], Rachel Floyd[1], Jo Fothergill[4]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Univeristy of Liverpool, Liverpool, United Kingdom. [3]University of Salford, Salford, United Kingdom. [4]University ofLiverpool, Liverpool, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a major global health issue. *Escherichia coli* is a common cause of urinary tract infections (UTIs). These infections can be problematic to treat and can develop into kidney infections and urosepsis. One potential alternative with growing traction is the use of lytic phages.

E. coli isolated from patients with UTIs and bacteraemia (151 isolates), were used to test the efficacy 56 novel lytic phages isolated from sewage. The bacterial panel were genome sequenced and extensively tested for antimicrobial resistance. This revealed a range of ST types including a high prevalence of major types ST131, ST73, ST95 and ST69. Antibiotic resistance testing showed many of the isolates were multidrug resistant, with high resistance to penicillins, cephalosporins and fluoroquinolones. Widespread carriage of AMR genes were identified using whole genome sequencing.

A panel of 10 phages were defined through determining host range, phage resistance, phage receptors and phage genome analysis. Phage effectiveness was further analysed against *E.coli* biofilms using an in vitro biofilm model utilising the Ebba biolight dyes to study phage killing-curves against biofilms. An invertebrate in vivo infection model, *Galleria mellonella*, to test phage efficacy in vivo. Variation in activity between strain and between phages was identified.

This study highlights the potential use and challenges of phage therapy against clinical MDR *E. coli* isolates. There is a need for combined approaches that combined well-characterised phages with robust preclinical testing in relevant, clinical isolates.

P655

Vancomycin treatment increases biofilm biomass in *Clostridioides difficile* RT012 strains: Implications for UK first line treatment of CDI

Megan G. Taggart ORCID iD, James S. G. Dooley ORCID iD, Nigel G. Ternan ORCID iD

Ulster University, Coleraine, United Kingdom

Abstract

C. difficile infection (CDI) is the most common nosocomial diarrhoeal infection and is often onset by broad spectrum antibiotic treatment that disrupts the gut microbiome. Current treatments are further antimicrobials, including metronidazole, fidaxomicin and the UK first line option, vancomycin as recommended by UKHSA. Biofilm formation, and spore presence within *C. difficile* biofilms has been linked to CDI recurrence and indeed, biofilms are induced by, and exhibit increased resilience to antibiotic treatment.

In the current investigation, we explored the efficacy of antibiotics (MIC and MBC) against *C. difficile* in broth microdilution assays with either vancomycin or fidaxomicin. Using representative strains from prevalent N.I. *C. difficile* ribotypes, we initially determined conclusively that fidaxomicin is 10 x more effective, mg for mg, than the currently recommended first line treatment, Vancomycin.

Additionally, in biofilm disruption models, our investigations suggest that vancomycin significantly increases biofilm biomass in strains of RT012 lineage when early-stage (24 h old) biofilms are treated for an additional 24 h with both MIC and 2X MIC Vancomycin. Critically, this effect is not observed with fidaxomicin. Taken together, our data suggest that the reported decrease in CDI recurrence in patients treated with fidaxomicin could be due to this antibiotic not only being more effective against *C. difficile* cells, but also by not inducing further biofilm growth, as does vancomycin. Further investigation will determine if fidaxomicin can disrupt established biofilms or indeed prevent their development but, overall, our observation has quite profound implications for prescription practices in regard to CDI.

P656

Hospital wastewater pipes as reservoir of multi-drug resistant bacteria and antimicrobial resistance determinants

Jiaqi Wu, Stephen Kelly, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

Hospital acquired infection caused by multi-drug resistant microorganisms is an increasingly common problem in medical and healthcare settings. Antimicrobial resistance (AMR) genes can be transmitted by direct human contact or via environmental transfer. Clinical environments have been shown to harbour such microbes and resulting in risk of infection outbreaks. Hospital wastewater systems have been identified as ideal ecological niches for infection-causing bacteria, given their favourable conditions for growth and proximity to human activity.

In this study, 34 pipe sections were collected from a large teaching hospital following outbreaks of antibiotic-resistant infection. These wastewater pipes were analyzed using both culture-based and metagenomic approaches. 11 different extended-spectrum β-lactamase producing species were isolated, identified and their antimicrobial susceptibility profiles were determined. Metagenomic datasets obtained from 20 different pipes have been analyzed for both taxonomic and AMR profiles. Nine bacterial isolates from infected patients were sequenced and analyzed, and the subsequent comparative study was performed to determine the correlation between the bacteria causing infection in the patients and the clinical wastewater system. Results showed that the hospital wastewater system is an environment with a high diversity of microbial species and AMR genes.

These results can improve understanding of environment-mediated nosocomial infection and help inform infection control policy. Influencing and controlling the microbiome of the wastewater environment may reduce the proliferation of multi-drug resistant microorganisms and their AMR genes, thereby reducing the spread of infection and the subsequent demand for antibiotic treatment.

P657

The probability of resistance cell establishment is influenced by antibiotic dose and interaction with the sensitive population.

Grace Taylor-Joyce, Helen Alexander

Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom

Abstract

Designing effective treatment strategies is crucial in reducing antimicrobial resistance (AMR) burden. A robust understanding of how antibiotic exposure impacts the evolution of AMR is needed. An essential step in this process is successful proliferation of a resistant cell and establishment of a resistant population. Previous work has shown that antibiotic concentrations well below the minimum inhibitory concentration (MIC) are sufficient to reduce establishment probability. However, under specific antibiotic concentrations a sensitive population provides a protective effect to the resistant cell, significantly increasing establishment probability. The work presented here has built upon these findings. Our aim is to understand whether this phenomenon is seen across multiple antibiotics and resistance mechanisms or is specific to the strains and antibiotics studied. While the previous study focused on strains expressing resistance genes from a plasmid, this work has also used strains that have evolved resistance by de novo genetic changes under antibiotic pressure. Our results confirm that antibiotic concentrations below MIC are sufficient to reduce establishment probability, but the strength of this effect varies between different antibiotics. Ongoing work is looking at whether protection of a resistant cell by the sensitive population is seen under treatment with other antibiotics with differing mechanisms of action. The effect of nutrient availability on the interaction between a resistant cell and the sensitive population under antibiotic treatment is also being studied. This work furthers our understanding of the establishment of a resistant population and helps inform the choice of antibiotic concentrations best able to restrict AMR evolution.

584

Know your river – rivers as a resource for all

Kimberley Summers, Emma Travis, Elizabeth Wellington, Lijiang Song, Chiara Borsetto ORCID iD

University of Warwick, Coventry, United Kingdom

Abstract

Rivers are a valuable resource for leisure, agriculture, and economic activities and can support extensive, dynamic ecosystems. Industrial or agricultural run-offs and discharges from wastewater treatment plants, particularly during heavy rainfall, can cause pollution, damage ecosystems, and raise the risk of antibiotic bacteria surviving in waterways. “Know your river” is a citizen science project aimed at understanding recreational river use across England and river microbial communities. We invited members of the public to collect samples from their local waterways, returning these to us for analysis and providing information on the recreational activity performed in these locations. We performed chemical analysis, using Triple Quad LC-MS, to detect antibiotics and microbiology to enumerate *E. coli* and other coliforms and determine their resistance profile. The majority of samples contained coliform bacteria and 33% showed *E. coli p*resence, some with extensive multi-drug resistance. We found the most resistant *E. coli* came from a small number of the waterways, which also often had high levels of *E. coli* and coliforms. Two antibiotics, trimethoprim, and sulfamethoxazole were found in most rivers. Integration of the reported river usage data and the chemical and microbiological results allowed to report back to the public risk maps on local rivers’ health status and raise awareness on links between environmental pollution and human health with a particular focus on antimicrobial resistance.

P658

NITROSATIVE STRESS RESPONSE MACHINERY AS A PLAUSIBLE ANTI-PATHOGENIC TARGET IN *Pseudomonas aeruginosa*

Vijay Kothari ORCID iD

Nirma University, Ahmedabad, India

Abstract

Background: Antibiotic-resistant phenotypes of Pseudomonas aeruginosa have been listed as priority pathogens by various agencies like WHO and CDC. There is an urgent need for identifying novel antibacterial targets, leads, and mechanisms against this notorious pathogen.

Methods: Effect of certain polyherbal formulations (Panchvalkal and Enteropan), and colloidal silver (Silversol) on virulence of a multi-drug resistant P. aeruginosa strain was investigated, employing the worm Caenorhabditis elegans as a model host. Molecular mechanisms associated with the anti-pathogenic effect of these formulations were elucidated through whole transcriptome analysis (WTA).

Results: Test formulations exerted their anti-pathogenic effect by displaying multiplicity of targets. Among the multiple targets identified through WTA, components of the bacterial nitrosative stress-response machinery appeared to be among common targets of all the three test formulations. Nitric Oxide Reductase (NOR) being an important enzyme employed by P. aeruginosa in combating nitrosative stress, its possible inhibitors were predicted first through in silico approach, and then approximately 80 compounds from among the top in silico leads were assayed in physical form for their ability to protect the model host C. elegans when challenged with the pathogen P. aeruginosa. This resulted in identification of 8 compounds with potent anti- Pseudomonas activity, and also serendipitous identification of 4 anti-nematode compounds.

Conclusion: Generation of nitrosative stress in bacterial pathogens, particularly P. aeruginosa, should further be investigated as a potential anti-pathogenic strategy. Assessing activity of the identified anti-Pseudomonas compounds against other gram-negative bacterial pathogens is also warranted.

P659

Lysis-independent killing by vancomycin in *B. subtilis* and *S. aureus*

Laura Dobby[1], Sandra Laborda-Anadon[1], Gary Sharples[2], Kevin Waldron[3], Rebecca Corrigan[4], Henrik Strahl[1]

[1]Newcastle University, Newcastle Upon-Tyne, United Kingdom. [2]Durham University, Durham, United Kingdom. [3]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland. [4]The University of Sheffield, Sheffield, United Kingdom

Abstract

Vancomycin is a last-resort antibiotic used against Gram-positive multidrug resistant pathogens such as MRSA. While vancomycin's inhibitory mechanism through Lipid II-binding is well characterised, how the resulting inhibition of cell wall synthesis leads to bacterial cell death is surprisingly poorly understood. Preliminary data from our lab has shown that, despite full inhibition of cell wall synthesis, *Bacillus subtilis* cells continue to produce biomass in a cell envelope unable to expand. Using microscopic, single cell analyses of *Staphylococcus aureus* and *B. subtilis*, we could show that this initial stage is followed by membrane depolarisation that is, surprisingly, not linked to the cell lysis process. Interestingly, the stringent response alarmones (p)ppGpp, which act by down-regulating metabolic processes including transcription and translation, have been implicated in tolerance towards vancomycin. Thus, we hypothesised that vancomycin-induced biomass accumulation and (p)ppGpp-linked vancomycin-tolerance may be linked. Here, we show that artificial induction of (p)ppGpp synthesis indeed protects both *S. aureus* and *B. subtilis* against vancomycin-induced killing. Strains lacking (p)ppGpp synthesis capability, in turn, are hypersensitive to vancomycin. Our data suggest that, by inhibiting cell wall synthesis, vancomycin induces severe and pleiotropic cellular disturbances that are linked to biomass production-imbalance and membrane depolarisation. By modulating biomass production, (p)ppGpp can, thus, act as a natural antagonist for the lysis-independent bactericidal activity of vancomycin.

P660

The conserved response regulator, MtrA, regulates antibiotic production in *Streptomyces* on a transcriptional and post-translational level

Rebecca Devine ORCID iD, Barrie Wilkinson, Matt Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

Antimicrobial resistance presents a significant threat to global health and new antibiotics are urgently needed. The specialised metabolites of *Streptomyces* species are a promising source of new antimicrobials, but the genes responsible for these molecules are often silent under standard laboratory conditions. Understanding what regulates the expression of these genes will allow us to design tools to switch on biosynthetic pathways and discover new molecules. The two-component system (TCS), MtrAB, is highly conserved in *Streptomyces* species, and has been shown to regulate genes involved in antibiotic biosynthesis as well as general growth and development in model organisms. Previous work has shown that in *S. venezuelae*, the response regulator, MtrA, regulates over 1000 target genes, but little is currently understood about the molecular mechanisms of this. Here, we characterise the DNA- and protein-binding abilities of this transcriptional regulator using surface plasmon resonance (SPR) to understand how MtrA can regulate such a diverse range of targets. Preliminary data suggests MtrA can act as both a transcriptional and post-translational regulator, and is part of a complex regulatory cascade with other global regulators. This work also aims to understand whether MtrA regulates antibiotic production in non-model organisms, to determine how manipulation of the MtrAB TCS might be used as a tool for antibiotic discovery in the libraries of *Streptomyces* strains isolated from the environment by the wider research community.

P661

The costs and benefits of multidrug resistance in clinical *Escherichia coli.*

Rosie Clover ORCID iD, Chris Knight, Danna Gifford

University of Manchester, Manchester, United Kingdom

Abstract

In clinical environments, antibiotic resistance has become an extensive threat to public health, specifically the increasing rise in multidrug resistance. Current antimicrobial stewardship programs are based upon the understanding that resistance comes at a cost to the organism, typically a fitness cost in the absence of antibiotics; a concept known as the 'cost of resistance'. However, how these cost can change as a organism becomes multidrug resistant is unclear. Moreover, the cost is typically observed in laboratory strains. It remains to be seen whether the cost of resistance is a common feature in clinical isolates. To assess this, we exposed 91 clinical isolates of *Escherichia coli* to several clinically relevant antibiotics to determine their ability to grow in an antibiotic and antibiotic-free environment. Whole-genome sequencing provided a antibiotic resistance gene profile for the isolates. Further measures were implemented to consider genomic variability by factoring in phylogenetic structure. A comparison of the number of antibiotics the isolates were resistant to and their growth in an antibiotic-free environment demonstrated no clear association between multidrug resistance in antibiotic-free environment. Furthermore, among diverse resistance genes, there isn't a clear 'fittest resistance gene', either in the presence or absence of drug (for trimethoprim, beta-lactams and gentamicin). However when we looked at SNP-mediated resistance to ciprofloxacin, triple mutants (*gyrA* D87N+S83L with *parC* S80I) have higher fitness in the presence of drug, with no obvious fitness cost in the absence of drug.

P662

Investigating the effect of pharmaceutical manufacturing waste on the environmental and human resistome in India

Chiara Borsetto ORCID iD[1], Sebastien Raguideau[2], Arpita Bhatt[3], Chandradeo Narayan[3], Vinay Modgil[3], Christopher Quince[2], Lijiang Song[1], Neelam Taneja[3], Elizabeth Wellington[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Earlham Institute, Norwich, United Kingdom. [3]PGIMER, Chandigarh, India

Abstract

Pharmaceutical manufacturing waste is often neglected as a source of environmental antimicrobial pollution contributing to antimicrobial resistance (AMR). However, it could adversely affect the microbiome and resistome of the surrounding contaminated natural environment, representing a risk to human health promoting AMR in the human gut microbiome of the population working/living in these areas. We studied the effects of pharmaceutical manufacturing waste on the environmental and the human faecal resistome in a pharmaceutical manufacturing hub in India, Baddi, and a control area, Kangra. River water, sediment, soil and drinking water were collected across the areas in 2019, 2021 and 2022. Chemical analysis of water samples showed higher concentrations of antimicrobials (e.g. sulfamethoxazole, trimethoprim, fluoroquinolones) in 2019 and lower/undetectable concentrations during 2021-2022. Seasonal variation and COVID19 lockdown restrictions might have influenced the release of pharmaceutical waste in the environment due to closure of manufacturing plants. Environmental resistome profiling by HT-qPCR showed a significant impact potentially linked to exposure to manufacturing waste, with Baddi presenting higher prevalence of the selected 52 ARG targets compared to Kangra. Water was the major reservoir of ARGs in these environments. Metagenome analysis showed presence of ARGs on chromosomal and mobile elements and correlation analysis between HT-qPCR and metagenomes were performed.

The integration of the environmental data with faecal resistome data linked to social science information collected through interviews with selected stakeholders will allow the development of risk models to evaluate the impact of manufacturing waste on human health and promote changes in policy.

P663

Water Treatment Plants: A Reservoir of Antimicrobial Resistance?

Erin Corbett[1], Kiri Rodgers[1], Ronnie Mooney[1], Andrew Hursthouse[2], Elisa Giammarini[1], Soumyo Mukherji[3], Suparna Mukherji[3], Fiona Henriquez[1]

[1]University of the West of Scotland, Hamilton, United Kingdom. [2]University of the West of Scotland, Paisley, United Kingdom. [3]Indian Institute of Technology Bombay, Mumbai, India

Abstract

Antimicrobial resistance (AMR) is amongst the most significant growing threats to human health, with bacteria, viruses, fungi, and parasites developing resistance to commonly used medicines. Environmental factors, including anthropogenic pollutants such as potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs), are known to contribute to AMR – for example via the co-selection of resistance genes for antibiotics and metals. However, a greater understanding of the causes of environmental AMR is required in order to develop preventatives strategies and policies.

Wastewater treatment plants (WWTPs) can act as a hub for anthropogenic pollutants and therefore represent an ideal environment to explore a vast array of parameters. Our research focuses on WWTPs in India and in Scotland, treating domestic, industrial, and pharmaceutical waste streams. By analysing water and sediment from WWTPs and the surrounding environment, we aim to identify correlations between AMR prevalence and chemical parameters including PTEs, antibiotics, and PAHs. This data can then be used to develop surveillance strategies and implement effective methods of intervention and prevention.

We are currently exploring the relationship between AMR and the common protozoan *Acanthamoeba*. *Acanthamoeba* harbours bacteria able to resist amoebic digestion. Upon encountering stressful conditions such as anthropogenic contamination, *Acanthamoeba* encysts – increasing its resistance to antibiotics. The cyst may also act as a hub for bacterial interaction, including the spread of AMR. Our results so far indicate that intracellular bacteria exhibit greater rates of AMR compared to extracellular bacteria, suggesting that *Acanthamoeba* and other eukaryotes could act as vectors for environmental AMR spread.

P664

Antimicrobial resistance (AMR) and Free-Living Amoebae – the overlooked vector?

Elisa Giammarini, Kiri Rodgers, Ronnie Mooney, Fiona Henriquez

University of the West of Scotland, Paisley, United Kingdom

Abstract

Free-living amoebae (FLAs) are ubiquitous, unicellular protists that can harbour bacteria that survive intracellular digestion, known as Amoeba-resisting bacteria (ARB). FLAs can then act as reservoirs, vectors and training grounds for potentially pathogenic microorganisms. Furthermore, the accumulation of antimicrobial substances in the environment has a key role in the selection of the resistant microorganisms. This study investigated the role of FLAs on levels of antimicrobial resistance (AMR) in heavily polluted environments. Sediment samples were collected up and down stream of a chemical wastewater treatment plant (WWTP), which predominantly processes pharmaceutical waste. The FLAs were isolated and characterised, then, were brought to the state of monocultures and mechanically opened to retrieve the intracellular bacteria. The isolated bacteria were screened for their resistance against seven classes of antibiotics and compared to the antimicrobial resistance pattern of the environmental bacteria selected from the corresponding sample. Results showed that among the FLAs, the Acanthamoeba species were predominant, with recurrent amoebic intracellular bacterial species: Pseudomonas spp. Furthermore, preliminary results suggest higher resistance within the intracellular bacteria in contrast to their environmental counterpart. These outcomes highlight the potential role amoebae have in AMR prevalence and will increase our understanding of protists' role in the emergence of AMR.

P665

Conjugatively delivered CRISPR-Cas systems reduce retention of IncI1 resistance plasmids

Joss Lazenby[1], Caroline Rymer[1], Arnoud Van Vliet[2], Holger Kneuper[3], Roberto La Ragione[2], Martin Woodward[3], Simon Andrews[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Surrey, Guildford, United Kingdom. [3]Folium Science, Bristol, United Kingdom

Abstract

Conjugatively delivered CRISPR-Cas systems reduce retention of IncI1 resistance plasmids

Antimicrobial resistance (AMR) is one of the greatest public health threats of the 21st century, associated with nearly 5 million deaths per annum. Despite the fundamental role of antibiotics in the clinic, around 75% of manufactured antibiotics are designated for use in animals. Thus, antimicrobial resistance is harboured in the microflora of food-producing animals, frequently on mobile elements that could transfer resistance into pathogens. We targeted the resistance gene blaCTX-M-1 encoded by animal-associated IncI1 resistance plasmids with the type II-A and type I-E CRISPR systems from Streptococcus pyogenes and Escherichia coli, respectively. Ten analogous guide sequences were designed against the blaCTX-M-1 gene from each system and cloned into CRISPR-Cas plasmid vectors mobilizable by the RP4 conjugation system chromosomally integrated into E. coli S17-1. These were then conjugated into recipient E. coli strains containing the IncI1 resistance plasmids pESBL-138 and pPE13096 and retention of these plasmids assessed by plating onto media containing cefotaxime. Transconjugants of both systems had significantly reduced carriage of pESBL-138 and pPE13096 with no significant difference between the two plasmids. All spacers of the type II-A system decreased the retention of blaCTX-M-1 by >100 fold, but only 6 type I-E spacers were able to achieve the same effect. Suggesting that although both systems are highly effective against IncI1 plasmids there are yet unknown limitations to type I-E spacer design. This work provides the impetus for use of CRISPR-Cas AMR remediation in agricultural contexts through conjugative delivery.

P666

Aerobic acclimation and acquisition of fluoroquinolone resistance have a similar impact on the phenotype of *Campylobacter* isolates.

Gillian Carney[1], Marguerite Clyne[2], Tadhg Ó Cróinín[1]

[1]University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland. [2]University College Dublin, School of Medicine, Dublin, Ireland

Abstract

The survival of *Campylobacter*, a microaerophilic human pathogen, within the aerobic supermarket environment is poorly understood. We have recently observed growth under aerobic stress on solid media and found that it was associated with increased levels of fluoroquinolone resistance (FQ^r). This study further investigates aerobic growth and its association with FQ^r, by generating aerobic acclimated and fluoroquinolone resistant isolates and determining the impact on the phenotypes of *Campylobacter* isolates.

An aerobic acclimated panel (n=4) was generated through the subculturing of visible growth under aerobic stress on solid media for a minimum of 5 passages. Aerobic acclimation led to an increase in autoagglutination and protein secretion. Fluoroquinolone resistant isolates of the *C jejuni* strain 11168 were generated through subculture onto increasing concentrations of the fluoroquinolones, ciprofloxacin and nalidixic acid. Acquisition of FQ^r led to an increase in autoagglutination and protein secretion. Fluoroquinolone resistant isolates then underwent aerobic acclimation and it was found that this process led to a decrease in protein secretion and altered autoagglutination in an isolate dependant manner.

These results demonstrate how passaging under aerobic stress or in the presence of fluoroquinolones can lead to similar changes in the phenotype of *Campylobacter*. This observation supports previous work highlighting an association between FQ^r and aerobic tolerance and the prevalence of FQ^r within supermarket chicken *Campylobacter* isolates. Together this suggests that fluoroquinolone resistant isolates may be better suited to survival under aerobic stress and growth under aerobic stress may lead to the spontaneous generation of FQ^r in the absence of antibiotics.

P667

Phage Therapy Re-sensitises Carbapenem-Resistant *P. aeruginosa* Infection to Antibiotics *In Vivo*

Eleri Ashworth[1], Dr Rebecca Shears[2,3], Professor Aras Kadioglu[2], Dr Joanne Fothergill[1]

[1]University of Liverpool, liverpool, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Pseudomonas aeruginosa is a major nosocomial, opportunistic pathogen that displays intrinsic resistance to antibiotics and can cause severe disease including sepsis. Carbapenem-resistant *P. aeruginosa* has been recognised by the World Health Organisation (WHO) as a priority 1 pathogen with the urgent need for new therapeutics. Since the advent of increasing antibiotic resistance, there has been renewed interest in using bacteriophages as a therapeutic. Phage therapy has many advantages compared to antibiotics, including increased specificity, replication at the site of infection, low manufacturing cost and little to no reported toxicity. However, the dynamics of treating disseminated, pan-resistant *P. aeruginosa* with phage *in vivo* are poorly understood. Using a novel, clinically relevant, *in vivo* model for pan-resistant *P. aeruginosa* systemic infection, phage cocktail displayed strong therapeutic potential *in vivo*, clearing infection from the blood, kidneys, and spleen. The remaining bacteria in the lungs and liver displayed phage resistance due to reduced adsorption. Yet, resistance to phage resulted in re-sensitisation to a range of clinically relevant antibiotics. Phage steering, was utilised *in vivo* and pre-exposure with phage cocktail re-sensitised bacteria to tobramycin and meropenem, resulting in bacterial clearance of pan-resistant *P. aeruginosa* infection. The lungs were a challenging site to treat with bacterial adaptation to the lungs, even in the absence of phage treatment, resulting in resistance to phage. Overall, phage-bacteria dynamics can be exploited however, site of infection and interaction with the host environment must be considered. Phage steering could be an exciting potential therapeutic alternative for treating pan-resistant *P. aeruginosa* infections

P668

Development of efflux resistant antibiotic using a novel efflux resistant breaker technology

Charlotte Hind ORCID iD[1], Mark Laws[2], Kazi Nahar[2], Melanie Clifford[3], Caleb Marsh[1], Matthew Wand[1], Mark Sutton[1], Khondaker Miraz Rahman[2]

[1]UKHSA, Salisbury, United Kingdom. [2]KCL, London, United Kingdom. [3]DSTL, Salisbury, United Kingdom

Abstract

Background: We have developed an 'efflux resistance breaker' (ERB) technology which can be applied to multiple antimicrobial classes. Here, we validate the technology on a class of antibiotics where efflux is a common resistance mechanism, the fluoroquinolones.

Methods: ERB pharmacophores were identified through advanced structural modelling and were synthetically incorporated into existing fluoroquinolone antibiotics. Antimicrobial activity was assessed through minimum inhibitory concentration (MIC) assays and target inhibition was assessed through biochemical gyrase inhibition assays with both wild-type and mutant gyrase enzymes. Compounds were assessed *in vivo* in a murine *Staphyloccocus aureus* thigh infection model. Off-target toxicity was tested using SafetyScreen44.

Results: ERB-modified fluoroquinolones demonstrated up to a 512-fold reduction in MIC in multidrug resistant Gram-positive and Gram-negative bacteria compared to the parent compound (MIC90 0.03 to 2 µg/mL). The ERB compounds maintained target activity against DNA gyrase, both wild-type and S84L mutants (IC50 ~3.8 µg/mL). Two lead compounds demonstrated *in vivo* efficacy, with a 5-log reduction of bacterial load at 20 and 50 mg/kg in a thigh infection model and oral and IV PK/PD profiles comparable to levofloxacin. ERBs did not show any toxicity in mice at 1200 mg/kg/day, nor in off-target toxicity screens such as the hERG channel and cytochrome p450 enzymes.

Conclusion: This technology, which reduces the susceptibility of compounds to efflux, has the potential to revive the use of several classes of current antibiotics, as well as improve novel scaffolds.

P669

Marine bugs for novel drugs - investigating the metabolomes of marine microorganisms for antimicrobial compounds

Grant January ORCID iD[1], Jazmin Conway[1], Jarmo-Charles Kalinski[2], Melody Clark[3], Rosemary Dorrington[2], Mathew Upton[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]Rhodes University, Makhanda, South Africa. [3]British Antarctic Survey, Cambridge, United Kingdom

Abstract

Antimicrobial resistance is a global public health emergency and new anti-infective agents are needed to overcome this "silent" pandemic. Natural products are a well-known source of antimicrobial agents and offer more promising sources for drug discovery compared to synthetic compounds. In this study, Antarctic marine invertebrates and their associated microbial symbionts were investigated for antimicrobial activity.

A bacterial collection produced from representatives of seven Antarctic invertebrates (octocorals; sea lemons; bristle and polychaete worms; tunicates; nudibranchs and sponges) was screened for antibacterial activity on agar-based assays against ESKAPE pathogens. Isolates with promising bioactivities were triaged for further characterisation including "One Strain Many Compounds (OSMAC)" fermentations, in vitro bioassays (cell viability, anticancer, and cytotoxicity), metabolomics, whole genome sequencing, and in silico mining for biosynthetic gene clusters (BGCs).

One promising isolate, Streptomyces finlayi, displayed preliminary antibacterial activity against Staphylococcus aureus. Following OSMAC experiments, the crude extracts were fractionated using C18 flash chromatography. Two UV-vis peaks were isolated with concentrated activity against S. aureus and Escherichia coli, respectively, and have been submitted for LC-MS2 and molecular networking chemical dereplication. In vitro cell liquid assays showed no significant (<50% cell viability) cytotoxicity towards mammalian cells at 50 ug/mL. This genome was sequenced and antiSMASH prediction revealed 24 BGCs, 9 of which could putatively produce antimicrobial compounds with broad and narrow spectrum antibacterial activity. One of these BGCs, which is potentially novel and could produce a chemical derivative of the antibiotic salinamide, is being investigated in more detail.

P670

Assay Development for Antibiotic Discovery Against Salmonella Typhimurium Persister Cells

Edna Mokeira Ondari ORCID iD[1], Jay Hinton ORCID iD[2], Ian Gilbert ORCID iD[1], Megan Bergkessel ORCID iD[1]

[1]University of Dundee, Dundee, United Kingdom. [2]Universisty of Liverpool, Liverpool, United Kingdom

Abstract

Bacterial persisters are non-replicating cell sub-populations that can survive clinical antibiotic concentrations, posing a significant challenge for infection control and can potentiate the development of antibacterial resistance. However, conventional methods for screening and identifying antibacterial drugs aim to inhibit bacterial replication and growth and have limited utility for identifying compounds that target bacteria in non-replicative states.

We have developed a screening assay for identifying candidate compounds capable of killing non-replicating S. Typhimurium of the ST313 lineage. We use D23580, a representative strain of invasive, multi-drug resistant isolates that causes severe bloodstream infection in vulnerable African populations. We have validated the assay on a small compound library.

We describe experiments for characterizing compounds active against persister cells in both axenic and infection contexts. These used bio-orthogonal non-canonical amino acid tagging (BONCAT), to investigate biosynthetic activity in environmental conditions that reduced or arrested bacterial growth. Whilst many classical antibiotics have the most potent bactericidal activity against actively growing cells, we show found that carbonyl cyanide m-chlorophenyl hydrazine (CCCP) has higher bactericidal activity under growth-arrested conditions than during active growth.

Our findings contribute new insights into the modes of action of compounds with activity against bacteria during dormancy, and could prove relevant to a range of persistent infectious diseases.

P671

Taxonomic and resistance profiling of the ureteric stent microbiome following renal transplant surgery

Katie Brown[1], Laura McClenaghan[1], Thomas Thompson[1], Hannah Magowan[2], Tim Brown[2], Brendan F. Gilmore[1], Stephen A. Kelly[1]

[1]Queens University, Belfast, United Kingdom. [2]Belfast City Hospital, Belfast, United Kingdom

Abstract

Kidney transplantation is often the preferred treatment for end-stage renal failure as it improves the quality of life for recipients and allows them to live without the need for dialysis. Ureteric stents are regularly inserted during kidney transplants to prevent post-operative blockages of the ureter. Kidney transplant rejection happens in 10-15% of patients, and one cause of rejection is post-operative infection. The ureteric stent is one potential location for infection, as bacteria can form biofilms within the channel of the stent. The aim of this study was to characterise the microbiome and the resistome (the genes associated with antibiotic resistance) of ureteric stents removed from post-kidney transplant patients.

Total bacterial DNA was extracted from ureteric stents using the Qiagen DNeasy PowerSoil Kit, and DNA was subsequently sequenced. Following total DNA extraction and sequencing, metagenomic DNA was studied using a number of computational tools, including MegaHIT, Kraken2, and the Resistance Gene Identifier (RGI). Genome binning was carried out to produce metagenomically assembled genomes (MAGs). Taxonomic annotation was carried out using DNA contigs produced by MegaHIT, and with the generated MAGs.

The overall microbiome and resistome of each stent varied, but common patterns were observed. This study has the capacity to aid in the treatment of infections in kidney transplant patients and may inform the choice of antibiotic used in treatment. More understanding into the role of bacterial infections associated with the ureteric stent in successful kidney transplants versus transplant rejection will help in tailoring potential treatment plans for patients.

P672

Artificial sweeteners inhibit multidrug-resistant pathogen growth and potentiate antibiotic activity

Rubén de Dios ORCID iD, Chris R Proctor, Evgenia Maslova, Sindija Dzalbe, Christian J Rudolph, Ronan McCarthy ORCID iD

Brunel University London, London, United Kingdom

Abstract

The antibiotic resistance crisis poses a major threat to our global health care infrastructure. It is now widely recognised that there is an urgent need to discover new compounds with antimicrobial activity to tackle this crisis. In this work, we explore the human diet as a reservoir for compounds with potential antimicrobial activity. We identify three artificial sweeteners, saccharin, cyclamate and acesulfame-K (ace-K), that have a major growth inhibitory effect on priority pathogens including multidrug resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. We further characterise the impact of ace-K on *A. baumannii*, demonstrating that it can disable a suite of virulence behaviours required for infection and environmental persistence such as biofilm formation, motility and the ability to acquire exogenous antibiotic-resistant genes. Live cell imaging revealed that the mechanism of growth inhibition is through bulge-mediated cell lysis and that cells can be rescued by cation supplementation. We have also demonstrated that ace-K, when used at sub-lethal concentrations, can lead to a dramatic increase in the sensitivity of *A. baumannii* to last resort antibiotics, including carbapenems and polymyxins. Using a novel *ex vivo* porcine skin wound model, we show that the antimicrobial and antibiotic potentiating activities of ace-K are maintained in the wound microenvironment and that it is more effective than a leading commercial wound sterilization solution. Our findings demonstrate the influence of artificial sweeteners on pathogen behaviour and uncovers their previously unrecognised therapeutic potential.

P673

Investigating bacterial colonisation of the lower respiratory tract in alpha-1 antitrypsin deficiency (AATD)-related chronic obstructive pulmonary disease (COPD)

Daniella Spittle, Jan Stanka, Anita Pye, Alice Turner

University of Birmingham, Birmingham, United Kingdom

Abstract

Half of acute exacerbations of COPD (AECOPD) are caused by bacterial infection, yet most episodes are treated with antibiotics. Infection may be due to the introduction of a new species or the expansion of a colonising bacteria in the lower respiratory tract (LRT). Alpha-1 antitrypsin deficiency (AATD) is a genetic risk factor for early-onset COPD, independent of smoking history. Longitudinal sputum samples from AATD-COPD patients, in stable and AECOPD states, were processed for quantitative culture. Contemporaneous clinical data was collected which included age, sex and smoking status. Post-hoc analyses sought to calculate the odds of an exacerbation in the presence of colonisation by recognised, potentially-pathogenic bacteria of the LRT. A total of n=1337 sputum samples from n=262 patients were included for analysis. *Haemophilus influenzae* was the most frequently isolated organism and was detected in 22% samples. Isolation of *Moraxella catarrhalis*, *Staphylococcus aureus* and *Pseudomonas* species during stable disease were associated with a 2.69- ($p=0.001$), 1.96- ($p=0.011$) and 2.5-fold ($p<0.001$) increase in the odds of AECOPD, respectively. AECOPD were associated with increased numbers of *Haemophilus influenzae* ($p=0.002$), *Moraxella catarrhalis* ($p=0.048$) and total bacterial load of pathogens ($p=0.002$). AECOPD are a leading cause for the antibiotic prescription in the UK. It is therefore vital that the underlying microbiology in COPD is understood. Our findings show that expansion of colonising bacteria increase the odds of exacerbation in AATD-COPD, which informs the targeted prevention of these organisms. Future work will use 16S rRNA sequencing to investigate the LRT microbiome in AATD-COPD in greater depth.

P674

Testing the antimicrobial properties of water extract of Aerial roots of *Ficus benghalensis* against *Staphylococcus Aureus* and *Escherichia Coli*

Sudarshan Vishvani[1], Dhruvinka Seneviratne[2], Ayesha Khalid[3], Imashi Weerakkody[4], Dinelka Ranasingha[5], Sharon Nicholas[3], Nishani Jayathilake[3]

[1]International Institute of Health Sciences, wattala, Sri Lanka. [2]International Institute of Health Sciences, Negombo, Sri Lanka. [3]International Institute of Health Sciences, Colombo, Sri Lanka. [4]International Institute of Health Sciences, Ja - Ela, Sri Lanka. [5]International Institute of Health Sciences, Kadana, Sri Lanka

Abstract

Introduction: Ficus benghalensis is a plant native to the Indian subcontinent that is widely used around the world specially in Ayurvedic medicine. The problem of the research was, there had been few studies on the bioactive properties of aerial roots of Ficus benghalensis. The aim of this study was to test the anti-microbial properties of the extract.

Methods: Water extraction method was performed to obtain extract of aerial roots of Ficus benghalensis. A concentration series were prepared according to a simple dilution method (10, 1, 0.1, 0.01, and 0.001 mg/ml). The anti-microbial tests were conducted in a triplicate according to the disk diffusion method on ATCC 25922 Escherichia coli and ATCC 25923 Staphylococcus aureus, and inhibition zones were recorded after 24 hours.

Results and Discussion: The positive controls were standards antibiotics, of Ampicillin and Penicillin. At a concentration of 10, 1, and 0.1 mg/ml of plant extract, the average inhibition against E. coli was 1.8, 1.5, and 0.7 cm, respectively. No inhibitory effect was shown for 0.01 and 0.001mg/ml. Against Staphylococcus aureus, an inhibition zone of 1.5 and 0.8 cm was shown at 10 and 1mg/ml respectively. 0.1, 0.01, and 0.001 mg/ml did not shown inhibition. As observed, when the concentration was high, the inhibition zone was greater.

Conclusion: Despite the fact that Ficus benghalensis is used in traditional medicine, scientific evidence supporting bioactivities and toxicity studies is scarce. This study demonstrates antibacterial activities based on experimental results. Further studies are needed to identify other bioactive properties.

P675

Investigating the mechanism of novel antimicrobial peptoids against *P. aeruginosa*

Melanie Burger[1], Jennifer S Lin[2], Annelise E Barron[2], Madeleine G. Moule[1]

[1]Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, United Kingdom. [2]Department of Bioengineering, School of Medicine & School of Engineering, Stanford University, Stanford, USA

Abstract

Pseudomonas aeruginosa is a human pathogen for which there is an urgent need for novel treatment options. The list of effective antibiotics is becoming shorter and shorter as this pathogen has diversified its strategies of evading their activity. *P. aeruginosa's* ability to form persister (dormant) cells and biofilms in order to escape antibiotics is especially harmful to cystic fibrosis patients. This evasion can lead to chronic and reoccurring infections. We aim to overcome these evasion strategies by using novel antimicrobials "peptoids" in combination with current antibiotics to resensitise the bacteria. Checkerboard assays were used to determine which antibiotic-peptoid combinations are synergistic. These also provide initial guidance on the optimal concentrations required to treat biofilms and persister cells formed by *P. aeruginosa.* Antibiotic-peptoid combinations that were deemed synergistic, included antibiotics that *P. aeruginosa* displays intrinsic resistance to and are, therefore, not usually used to treat such infections. Synergistic combinations also included those antibiotics that, due to their toxicity, are used sparingly. When combining these antibiotics with our peptoids their therapeutic window could be lowered to nontoxic concentrations. Furthermore, understanding how these combinations exert their superiority over conventional treatment will lead to more treatment options against *P. aeruginosa* infections. This will help to individualise drug regimes as a precision medicine approach by identifying the best therapeutic combinations for various antibiotic resistant strains.

P676

In-vitro susceptibility of Omadacycline vs Tigecycline among multidrug resistant *Acinetobacter baumannii clinical isolates*

HITENDER GAUTAM, Priyanka Sharma, Farhana Jahan, Aranya K. Das, Sangeeta Pandey, Sarita Mohapatra, Seema Sood, Benu Dhawan, Arti Kapil, Bimal K. Das

AIIMS, New Delhi, India

Abstract

Background –

Antimicrobial resistance in *Acinetobacter baumannii* is a matter of concern and there are limited treatment options available for multidrug-resistant (MDR) strains. Omadacycline is a derivative of tetracycline (3rd generation tetracycline) and reported to have good activity against MDR pathogens as it counters the effect of efflux pumps.

Methods –

Minimum inhibitory concentration of omadacycline and tigecycline was tested in 120 strains of *A. baumannii* by microbroth dilution to compare the in vitro activity. Polymerase chain reaction (PCR) was performed to check the presence of AdeABC efflux pump genes and compared with the in vitro microbiological activity.

Results –

Out of the total 120 strains included in the study 91 (75.8%) were susceptible to omadacycline and 87 (72.5%) were susceptible for tigecycline. MIC for omadacycline and tigecycline ranged from 0.5 to 64 µg/ml and 0.5 to 8 µg/ml, respectively. MIC_{50} and MIC_{90} was 4µg/ml and 32 µg/ml for omadacycline, and 2 µg/ml and 16 µg/ml for tigecycline, respectively. The presence of AdeB, AdeC, AdeR and AdeS efflux pump genes was observed in 38 (31.6%), 78 (65%), 1 (0.8%) and, 44 (36.6%) strains respectively.

Conclusion –

Omadacycline may serve as an alternative treatment option in MDR Acinetobacter baumannii strains and was found to be less affected by the AdeABC efflux mechanism in the strains used in our study. There is a need to carry out more genomic studies to find out the exact resistance mechanisms and clinical studies to evaluate the therapeutic outcomes.

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Convergence of antibiotic resistance and virulence traits in *Klebsiella pneumoniae* through plasmid hybridisation in One Health settings

Keira Cozens[1], Natacha Couto[2], Marjorie Gibbon[1], Matthew Avison[3], Edward Feil[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]University of Bristol, Bristol, United Kingdom

Abstract

Background

Plasmids are major drivers of antimicrobial resistance, and recent advances in long-read sequencing make it possible to generate complete assemblies of plasmid genomes for large population samples. Here we apply this technology to the critical priority pathogen *Klebsiella pneumoniae* from humans, animals, and the environment, to determine how frequently hybrid plasmids emerge that possess both antimicrobial resistance and virulence traits.

Methods

We utilised *K. pneumoniae* genome sequence data from two large One Health studies from Italy (1705 strains) and Thailand (607 strains) generated by the SpARK and OH-DART consortia, respectively. We focused on strains carrying the plasmid-mediated virulence gene *iuc*3, which is associated with pigs, and used long-read sequencing to generate hybrid assemblies of 80 isolates. We used Kleborate to identify plasmids with acquired resistance genes, and those with iuc3 and a resistance score of 1 or more were defined as hybrid plasmids.

Results

In total we identified 7 hybrid plasmids, all from Thailand; six were isolated from neighbouring fresh markets (2 isolated from pork, 4 from duck meat), and one isolated from an inpatient from a nearby hospital. Each plasmid was identified from distinct sequence types (ST408, ST2791, ST8, ST3415, ST43, ST607, ST193) and conferred resistance to at least 6 classes of antibiotics. Whilst we found 44 plasmids harbouring *iuc*3 in the strains from Italy, none of these were hybrid plasmids.

Conclusion

Here we report the first instance of hybrid plasmids arising outside of the hospital environment, emphasising the importance of monitoring virulence alongside antimicrobial resistance surveillance.

P677

The diversity of antimicrobial resistance genes in commensal and pathogenic *Escherichia coli* strains from Scottish cattle

Andrew Bease[1], Sabine Delannoy[2], Patrick Fach[2], Kareen Macleod[1], Anne Holmes[3], Bryan Wee[1], Lesley Allison[3], Sue Tongue[4], Deborah Hoyle[1]

[1]The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom. [2]Laboratoire de Sécurité des Aliments, Agence Nationale De Sécurité Sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Paris, France. [3]Scottish E.coli O157/STEC Reference Laboratory (SERL), NHS Lothian, Little France, Edinburgh, United Kingdom. [4]Centre for Epidemiology and Planetary Health, North Faculty, Scotland's Rural College (SRUC), Inverness, United Kingdom

Abstract

Antimicrobial resistance genes (ARGs) in *Escherichia coli* are typically monitored in pathogenic strains during clinical diagnostics, but are less often documented in commensal bacteria. In this study we investigated the diversity of ARGs within commensal and pathogenic *E. coli* strains isolated from cattle, using two different methodologies.

Commensal *E. coli* strains (n=251) and a collection of enteropathogenic (EPEC) and Shiga toxin-producing strains (n=134) were isolated from bovine faecal pat samples obtained from 65 Scottish cattle herds between 2014–2015. Both strain collections were generated from the same set of herds, across differing faecal pat samples, isolated using agar that was nonselective for antimicrobial resistance. The presence of ARGs was determined in the commensal collection by Fluidigm PCR microarray and in the pathogenic collection by whole genome sequencing.

The majority of commensal strains (51.8%) belonged to the B1 phylogroup and 7.6% possessed *eae* or *sTa* genes, indicative of EPEC or enterotoxigenic *E. coli*, respectively. Overall, ARG carriage was relatively low: 8% of commensal and 13% of pathogenic strains carried at least one ARG. We detected the genes *aph(3')*, *aph(6')*, *tet*, *sul*, *blaTEM*, *dfrA* and *floR* in 0.5-7% of strains across both collections. The majority of resistant strains carried multiple ARGs. The only ARG detected by genome sequencing that was not present in the PCR microarray was *fosA7*, found in three EPEC strains. The plasmid IncQ1 was significantly associated with AMR positivity in commensal strains. These data support the robustness of different assay methods for monitoring ARGs across bacterial populations.

P678

Targeting small RNA-mediated regulation of virulence and antibiotic resistance to develop non-traditional therapeutic options against *Pseudomonas aeruginosa*

Tarcisio Brignoli ORCID iD, Giovanni Bertoni, Silvia Ferrara

Università degli Studi di Milano, Milan, Italy

Abstract

Pseudomonas aeruginosa is an opportunistic human pathogen known for its antibiotic tolerance and ability to readily develop new resistance. The development of compounds with anti-virulence effects or capable of re-sensitizing antibiotic-resistant strains is an innovative approach that may be capable of producing drugs with high specificity and narrow spectra to target *P. aeruginosa* in diseases such as cystic fibrosis. In this perspective, regulatory small RNAs (sRNAs) represent an unexploited category of therapeutical targets. We focused on the *P. aeruginosa* sRNA ErsA which is involved in the regulation of several functions linked to lung pathogenesis and antibiotic resistance. An ErsA knock-out mutant indeed fails to form a mature biofilm and is significantly less virulent following the infection of both bronchial epithelial cells and a murine model. Moreover, ErsA deletion induces sensitization to ceftazidime, cefepime, and meropenem in a multidrug-resistant clinical strain. The main aim of this work was to develop and test anti-ErsA Peptide Nucleic Acids (PNAs) potentially able to bind to ErsA, block its regulatory function, and induce the phenotypes that we observed in the ErsA knock-out mutants. We showed that the anti-ErsA PNAs can interfere with ErsA regulatory function, although the inhibitory effects depend on the target tested. Furthermore, we provided evidence of the ability of anti-ErsA PNAs to re-sensitize a resistant clinical strain to meropenem. Overall, our results strongly suggest the potential of targeting ErsA as a novel strategy to contrast *P. aeruginosa* and the use of anti-ErsA PNAs to contrast *P. aeruginosa* resistance by re-sensitization to antibiotics.

P679

Susceptibility of bacteriophage-producing Pseudomonas aeruginosa isolates from cystic fibrosis sputum to ceragenins

Ewelina Piktel[1], Urszula Wnorowska[2], Magdalena Zakrzewska[2], Krzysztof Fiedoruk[2], Robert Bucki[2]

[1]Medical University of Bialystok, Bialystok, Poland. [2]Medical University of Bialystok, Białystok, Poland

Abstract

Production of filamentous bacteriophages such as Pf4 by Pseudomonas aeruginosa has been shown to be associated with increased bacterial resistance to phagocytosis and decreased inflammatory response of the host, which accounts for chronic lung infections in CF subjects. Moreover, the inactivation of endogenous antimicrobial molecules and positively-charged antibiotics as a result of their electrostatic interaction with bacteriophage was described. Additional studies are necessary to understand the role of Pf-like bacteriophages in infections caused by P. aeruginosa from the perspective of their pathophysiology and treatment.

This study aimed to evaluate the antibacterial activity of ceragenins against clinical isolates of P. aeruginosa using strains that differ based on their ability to express Pf-like bacteriophages. Minimal inhibitory concentrations of ceragenins CSA-13, CSA-44, and CSA-131 were estimated using the broth dilution method, while the P. aeruginosa biofilm mass developed in the presence of ceragenins [administrated alone or combined with DNase I or poly-aspartic acid (pASP)], was assessed using crystal violet staining.

The results obtained indicate that ceragenins retain strong antimicrobial activity, both against strains with bacteriophage expression and isolates that do not produce bacteriophages. Moreover, ceragenins are able to significantly prevent the formation of biofilm by P. aeruginosa. Ceragenin CSA-13 displays an increasing effect in combined therapy with pASP and DNase, although higher sensitivity characterized strains lacking production of bacteriophages.

Our data strongly suggest the potential of ceragenins to develop a new treatment against P. aeruginosa-caused chronic lung infections in CF subjects.

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P680

Influence of Lipid Remodelling on Phage Therapy in Pseudomonas aeruginosa

Rhiannon Lyon[1], Yin Chen[1], Dave Scanlan[1], Andy Millard[2]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Leicester, Leicester, United Kingdom

Abstract

Pseudomonas aeruginosa is an important Gram-negative pathogen with intrinsic resistance to certain antibiotics. It is of particular significance in individuals with cystic fibrosis or the immunocompromised. Antimicrobial resistance (AMR) is a massive threat to global health, predicted to kill 10 million people by 2050 if nothing is done to tackle it. A promising therapy for combatting AMR infections is phage therapy. However, more research needs to be done into the mechanisms which affect whether phage therapy is successful or not, as demonstrated by mixed results from clinical trials. One aspect that needs to be investigated is the role of the bacteria cell surface in phage therapy. Bacteria can undergo changes in the lipid makeup of their cell membranes in response to environmental factors. This has the potential to influence membrane proteins and lipopolysaccharides of Gram-negative bacteria, which are known phage receptors. The environments inside a human body are very different to in vitro environments often used to study phage therapy in the lab, therefore it is important to understand the effect this change in environment has on phage therapy. Our results indicate that the lipids that make up the outer membrane of P. aeruginosa can influence the efficacy of phage infection. This could be an important factor to consider in future development of phage therapy.

P681

The activity of ceragenin CSA-13 against multispecies biofilms associated with chronic infections in cystic fibrosis subjects.

Urszula Wnorowska[1], Dawid Łysik[2], Ewelina Piktel[1], Krzysztof Fiedoruk[1], Magdalena Zakrzewska[1], Tamara Daniluk[1], Robert Bucki[1]

[1]Medical University of Bialystok, Bialystok, Poland. [2]Bialystok University of Technology, Bialystok, Poland

Abstract

Opportunistic pathogens such as *P. aeruginosa, S. aureus*, and *C. albicans* are usually growing in biofilm form in which increased antibiotic resistance was noted. The development of such biofilms triggers life-threatening infections in cystic fibrosis patients, which are associated with impaired response to antibiotic therapy, rapid decline in lung function, and poor clinical outcomes. An additional key factor in the development of drug-resistant phenotypes and the persistence of chronic lung infections in cystic fibrosis patients is the high viscosity of biofilms developed by mucoid (alginate-producing) strains of *P. aeruginosa*, which considerably impairs the diffusion of antimicrobials within biofilm matrix and thus, hampers the eradication of biofilm-embedded bacteria.

This study was designed to evaluate the ability of ceragenin CSA-13, being a lipid analog of endogenous antimicrobial peptides to eradicate multispecies biofilms associated with lung infection development in cystic fibrosis subjects. The mass of multispecies biofilms developed in the presence of CSA-13 was measured using crystal violet staining. Rheological properties of bacterial biofilms exposed to the different concentrations of ceragenin were investigated using a rheometer in a plate-plate arrangement.

We observed considerable reductions in single- and multi-species biofilm masses upon treatment with CSA-13. Alterations in the viscoelastic properties of biofilms developed in the presence of CSA-13 were also noted.

The observed effects of CSA-13 strongly suggest the possibility to develop novel lung infection therapies for cystic fibrosis subjects based on lipid antimicrobials from the ceragenin family.

This work was financially supported by grant from the National Science Centre, Poland: UMO-2018/30/M/NZ6/00502 (RB).

P682

In vitro evolution of *Klebsiella grimontii* to TZP resistance reveals identical and unique genomic changes between lineages compared to in-patient based evolution

Ellie Allman[1], Natasha Niethamer[1], Aakash Khanijau[2,3], Rachel Mcgalliard[2,3], Richard Goodman[1], Christopher Parry[1,3], Enitan Carrol[2,3], Adam Roberts[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom

Abstract

Experimental evolution of pathogenic bacteria to antimicrobial resistance (AMR) has the potential to rapidly inform antibiotic therapy. However, there is limited understanding of how *in vitro* AMR evolution can replicate evolution of AMR within the human host. A trio of *Klebsiella grimontii* isolates cultured from a hospital in-patient with a recurrent bloodstream infection showed development of piperacillin-tazobactam (TZP) resistance over a four-month period due to a single SNP in the promoter of a chromosomal $bla_{OXY\text{-}6\text{-}4}$. To test if the same evolutionary pathways are followed in laboratory evolution compared to in-patient, the susceptible ancestor was exposed to sub-inhibitory concentrations of TZP in LB broth, followed by growth on TZP-supplemented LB agar. Resistant colonies were selected, and fitness, TZP susceptibility and genomes were compared to the ancestor and the *in vivo* evolved resistant isolate. In one *in vitro* evolved lineage, we observed the same $bla_{OXY\text{-}6\text{-}4}$ promoter SNP as seen in the *in vivo* evolved resistant strain which conferred high-level TZP resistance, however all other adaptive mutations were unique to either the *in vivo* or *in vitro* evolved lineages. The acquisition of TZP resistance did not confer any negative fitness consequences in the laboratory-evolved strains, in contrast to reduced fitness of the in-patient evolved strain. This study highlights different evolutionary trajectories in laboratory-based AMR evolution when compared to in-patient evolution and emphasises the need for comprehensive experimental design and cautious translation of findings to the clinic, particularly when interpreting fitness data from laboratory experiments.

P683

Pseudomonas aeruginosa antimicrobial resistance in sputum mimicking media

Hollie Leighton[1], Dilip Nazareth[2], Aras Kadioglu[1], Daniel Niell[1], Jo Fothergill[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Liverpool Heart and Chest Hospital, Liverpool, United Kingdom

Abstract

In the lungs of a person with cystic fibrosis (CF), microbes can colonize and form biofilms containing multiple species of bacteria and fungi. Interactions between these organisms are overlooked when determining the effectiveness of antimicrobials. The lung environment is also altered in CF and CF sputum contains high levels of mucin, extracellular DNA and amino acids. The environment can also affect the resistance of bacteria in this niche.

In order to develop a more representative therapeutic screening platform, the first aim was to compare growth and resistance in available sputum mimics including synthetic CF media (SCFM2), artificial sputum media (ASM) and CF lung media (CFLM) using two widely studied *P. aeruginosa* strains.

There was no significant difference in growth of bacteria in the three media. Bacterial load increased over the first 24 h to and then remained constant. Antibiotics were applied at 24 h and studied at 48 h. Using reduction in bacterial load and metabolic activity, very high levels of resistance to meropenem and tobramycin were observed for both strains. Colistin resistance in human sputum was most similar to SCFM2 and CFLM.

Ongoing work involves studying the impact of complex polymicrobial biofilms on resistance to these antibiotics and novel therapeutics including phage. Transcriptomes will be utilised in order to develop models that closely reflect the gene expression of *P. aeruginosa* during chronic respiratory infection.

This project contributes to a wider project (PIPE-CF): to develop a robust preclinical framework for the development of novel CF antimicrobials.

P684

Large deletion of chromosomal regions harbouring nitroreductase gene *nfsB* is associated with nitrofurantoin heteroresistance in *Escherichia coli*

Yu Wan ORCID iD[1,2], Akshay Sabnis ORCID iD[3], Zaynab Mumin[4], Isabelle Potterill[4], Elita Jauneikaite ORCID iD[1,5,6], Colin Brown[1,2], Matthew Ellington[1,2], Andrew Edwards ORCID iD[3], Shiranee Sriskandan ORCID iD[1,3]

[1]NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Department of Infectious Disease, Imperial College London, London, United Kingdom. [2]HCAI, Fungal, AMR, AMU and Sepsis Division, London, United Kingdom. [3]MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, London, United Kingdom. [4]Reference Services Division, National Infection Service, UK Health Security Agency, London, United Kingdom. [5]Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, London, United Kingdom. [6]MRC Centre for Global Infectious Disease Analysis, School of Public Health, London, United Kingdom

Abstract

Background Nitrofurantoin is a broad-spectrum first-line antimicrobial used for managing uncomplicated urinary tract infections. Loss-of-function mutations in chromosomal genes *nfsA*, *nfsB*, and *ribE* of *Escherichia coli* are known to reduce nitrofurantoin susceptibility. Here, we report monoclonal nitrofurantoin heteroresistance in *E. coli* and a novel genetic mechanism associated with this phenomenon.

Methods Subpopulations with reduced nitrofurantoin susceptibility in cultures of two *E. coli* blood isolates 26B.P and 880B.P were identified using population analysis profiling that followed a two-fold gradient of nitrofurantoin concentrations. Four colonies of each isolate growing at its ½ nitrofurantoin MIC were sub-cultured with the same nitrofurantoin concentration (n=2) or without nitrofurantoin (n=2). Genomic DNA was extracted from parental and derivative isolates and sequenced with Illumina and Nanopore MinION systems. Genetic variation among isolates was determined using assembly- and mapping-based bioinformatics methods.

Results Nitrofurantoin MICs of both isolates were 64 mg/L. The proportion of cells grown at ½ MIC was 2×10-6 and 9×10-5, respectively, which is distinct to that of a homogeneously susceptible or resistant isolate. All derivative isolates showed deletions in chromosomal regions harbouring *nfsB* when compared to parental isolates. The length of deleted regions ranged between 11–20 kbp (26B.P) and 24–66 kbp (880B.P), and interestingly, breakpoints of all deletions were associated with IS*1*-family insertion sequences. Nevertheless, mechanisms causing such deletions remain unknown.

Conclusion Isolates 26B.P and 880B.P were heteroresistant to nitrofurantoin, with subpopulations expected to have stably reduced nitrofurantoin susceptibility and therefore may compromise nitrofurantoin chemotherapy. Nitrofurantoin heteroresistance needs to be considered by future methods of susceptibility testing.

P685

Genomic characterisation of piperacillin/tazobactam resistant, cephalosporin susceptible *Klebsiella spp.* isolates causing bloodstream infections, from patients within a UK hospital

Alice Fraser[1], Ralfh Pulmones[1], Eva Heinz[1], Thomas Edwards[1], Christopher Parry[1,2], Laura Brettell[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom

Abstract

Piperacillin/tazobactam (TZP) is an important β-lactam/β-lactamase inhibitor combination antibiotic, used in hospitals for the treatment of serious infections caused by members of the *Klebsiella* genus. Like other members of the Enterobacteriaceae family, resistance to TZP in *Klebsiella spp*. predominantly occurs with resistance to third-generation cephalosporins. Previously we have investigated the genetic diversity and resistance mechanisms of this unusual piperacillin/tazobactam-resistant, third-generation cephalosporin-susceptible (TZP-R/3GC-S) phenotype in *Escherichia coli.*

In this study, we sought to investigate the same phenotype in invasive *Klebsiella spp.* (n=25). Isolates were collected at the same tertiary hospital in Liverpool, UK, during the same period (2014-2017), as our previous study in E. coli.

The TZP-R/3GC-S phenotype was identified in *K. pneumoniae* (n=20), *K. variicola* (n=3), *K. oxytoca* (n=1) and *K. michiganensis* (n=1). A broad range of sequence types were associated with the phenotype and the isolates contained a diverse range of plasmids, indicating multiple acquisition events of TZP resistance mechanisms, rather than clonal expansion of a specific plasmid or sequence type.

The dominating resistance mechanism associated was carriage of an OXA-48 β-lactamase (which was also associated with carbapenem resistance), followed by OXA-1 and TEM-1. Six (21%) of the isolates only contained β-lactamase genes which are usually chromosomally encoded (SHV, LEN and OXY), indicating that TZP-R/3GC-S in Klebsiella spp. may be the result of mutated or hyperproduced, chromosomally-encoded β-lactamases. Our findings highlight both the complexity of this phenotype within *Klebsiella spp*. and the distinct difference in mechanisms between E. coli and *Klebsiella spp.*

P686

Host-associated biomarkers of pulmonary exacerbation resolution in Cystic Fibrosis following antimicrobial intervention

Sian Pottenger[1], Freddy Frost[1,2], Martin Bachman[3], Bruno Bellina[3], Maike Langini[3], Martin Walshaw[2], Dilip Nazareth[2], Daniel R Neill[1], Joanne L Fothergill[1]

[1]Clinical Infection, Microbiology & Immunology, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]Adult CF centre, Liverpool Heart and Chest Hospital, Liverpool, United Kingdom. [3]Discovery Science & Technology, Medicines Discovery Catapult, Alderley Park, United Kingdom

Abstract

Background: Development of new therapeutics targeting lung infections in cystic fibrosis (CF) is challenging, given that commonly used clinical trial endpoints, such as lung function and Quality of Life improvements, cannot be assessed in preclinical models. Identification of biomarkers that correlate with positive treatment outcomes but which can also be measured in preclinical models, could lead to better assessment of novel therapeutics.

Methods: AZTEC-CF (NCT02894684) was a randomised clinical trial comparing inhaled antibiotics (AZLI) to standard intravenous antibiotics (IV) for treatment of acute pulmonary exacerbations of CF. This post-hoc exploratory analysis utilised Mesoscale Discovery (Mesoscale, UK) assays and LC- MS analysis (Medicines Discovery Catapult, UK) to quantify host and pathogen biomarkers in sputum samples. We assessed how changes in these biomarkers correlated with the clinical outcomes of lung function measured as forced expiratory volume in 1 second (FEV1) and the respiratory domain of the CF Quality of Life Score (CFQ-R).

Results: We identified five candidate biomarkers (Matrix Metalloproteinase-9, Myeloperoxidase, Vascular Endothelial Growth Factor-A, YKL-40 and IL-1β) that decreased in abundance over the course of treatment and that were all significantly associated with improved FEV1 and CFQ-R scores. Interestingly, 4/5 of these biomarkers were markedly reduced when pwCF received inhaled AZLI compared to IV treatment.

Conclusion: This study identified 5 biomarkers associated with important clinical outcomes in exacerbations of CF. All 5 biomarkers can potentially be measured in pre-clinical models and prioritising the improvement of models to assess biomarker responsiveness to new therapeutics is key

P687

Identifying Antimicrobial resistance reservoirs in neonatal calves and their associated environments.

Katie Lawther[1], Aaron Brown[2], Fernanda Godoy Santos[1], Linda Oyama[1], Francesco Rubino[1], Lucy Dillon[1], Gillian Scoley[2], Steven Morrison[2], Sharon Huws[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]AFBI, Hillsborough, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a serious threat to animal and human health. Livestock and their housing may act as a reservoir for AMR bacteria. In this study, we aimed to contribute to AMR surveillance within dairy farms by characterising the resistomes associated with neonatal calves and their associated environments. Ten dairy farms were surveyed for >90 parameters including hygiene practices and antibiotic usage. Both culture-based and culture independent techniques were used to characterise the resistomes present in calf faeces and calf house environment including feed equipment, calf feed and milk fed to calves. Samples underwent shotgun metagenomic sequencing and were also tested *in vitro* for phenotypic AMR against seven antibiotic classes: penicillins, macrolides, phenicols, aminoglycosides, tetracyclines, synthetic and polymyxins. Resistant bacteria were isolated and underwent 16S rRNA gene and genome sequencing, and multidrug resistance (MDR) testing. High AMR diversity and abundance were reported in calf houses, with AMR levels varying between farms. Metagenomics revealed high resistance gene abundances within faecal samples, with transcripts per million values reaching 13,085. Phenotypic resistance to 6 antibiotic classes was detected on all farms, with resistance against neomycin and trimethoprim being highest. Of the 84 anaerobic isolates sequenced, 78.6% were MDR, while 36.0% of aerobic isolates were MDR, also isolated was *Escherichia coli* that was phenotypically resistant to 7 antibiotics classes. This study reveals the AMR reservoirs within dairy calf houses, and AMR risks associated with farming practices, including sanitation and milk replacer utilisation, that may contribute to high AMR levels in calf gastrointestinal tracts.

P688

Targeting iron homeostasis as a means to potentiate colistin treatment

Kavita Gadar[1], Rubén de Dios[1], Nikol Kadeřábková[2], Thomas Prescott[3], Despoina Mavridou[2,4], Ronan McCarthy[1]

[1]Brunel University, London, United Kingdom. [2]The University of Texas at Austin, Austin, USA. [3]Royal Botanic Gardens, London, United Kingdom. [4]John Ring LaMontagne Centre for Infectious Diseases, Austin, USA

Abstract

Acinetobacter baumannii is a Gram-negative multi-drug resistant pathogen that commonly causes bloodstream, wound, and urinary tract infections. Carbapenem-resistant *A. baumannii* is currently at the top of the World Health Organisation's list of priority pathogens in urgent need of novel therapeutic interventions. The broadening resistance profile of *A. baumannii* clinical isolates has led to a large increase in clinical reliance on our last resort antibiotic, colistin. As a result, the emergence of colistin-resistant *A. baumannii* strains has rendered our last-line drug ineffective. As such, the identification of compounds which increase the efficacy of colistin or, overcome colistin resistance, are currently of great interest.

In this study, we used high throughput screening methods to identify the plant derived phytochemical kaempferol as a potentiator of colistin activity. We found that the presence of kaempferol causes a dysregulation of iron homeostasis in the bacteria, which leads to bacterial killing when in combination with sub-inhibitory concentrations of colistin. We demonstrate that this occurs due to the disruption of Fenton's reaction causing a lethal build-up of toxic reactive oxygen species within the cell, resulting in death. Furthermore, we demonstrate that kaempferol displays the remarkable ability to overcome both intrinsic and acquired colistin resistance mechanisms in clinical strains of critical pathogens. We also demonstrate that the combination treatment is effective *in vivo* in *Galleria mellonella* model of infection. Overall, our findings provide a promising treatment strategy that can prolong the lifespan of colistin and overcome colistin-resistant infections.

P689

Cecacin: an AI-derived antifungal peptide as a potential combinatorial therapeutic for mutidrug resistant *C. auris* infections

Emma Hilditch, Linda Oyama ORCID iD

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistance (AMR) is ranked as one of the top 10 global public health threats facing humanity. Among these resistant organisms of rising concern is multidrug-resistant *Candida auris*, a pathogen on the WHO critical list, which causes invasive candidiasis (IC) in immunocompromised individuals. IC is associated with high fatality rates of up to 60% and with ~90% of *C. auris* strains being intrinsically resistant to fluconazole and pan-resistant to other first-line anticandidal drugs within the echinocandin and polyene classes, urgent and novel antifungal solutions which are more effective and less cytotoxic than current treatments are required to combat antifungal resistance. For this reason, we investigated the potential of an artificial intelligence derived peptide, **cecacin** with antifungal activity as a primary or combinatorial therapeutic for treatment of *C. auris* infections. Cecacin had potent fungicidal activity against all four strains of *C. auris* tested including *C. auris* DSM 105986, DSM 105987, DSM 105988, and DSM 105990 with minimum inhibitory concentrations between 32 µg/mL and 128 µg/mL. We also observed an additive effect of cecacin when used in combination with amphothericin B with a fractional inhibitory concentration (FIC) index of 0.625. Although investigations into the mode of action of this combined additive effect is currently being explored, these findings show great promise of cecacin and its derivatives as templates for future anti *C. auris* therapeutic development.

P690

Tracking of the regional expansion and characterization of New Delhi Metallo-β-Lactamase (*bla*$_{NDM}$) producing Klebsiella pneumoniae using whole genome sequencing

Noor Ul Ain Noor Ul Ain[1,2], Muhammad Umer Asghar[3,4], Saba Riaz[1]

[1]Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. [2]Center for Clinical Microbiology, University College London, London, United Kingdom. [3]National Institute for Biotechnology and Genetic Engineering College, Pakistan institute of Engineering & Applied Sciences (NIBGE), National Probiotic Lab-HBD, Faisalabad, Pakistan. [4]Pakistan Institute of Engineering & Applied Sciences (PIEAS), Nilore, Islamabad, Pakistan

Abstract

Background

The study aims to characterize the blaNDM producing K. pneumoniae strains from Pakistan, in terms of genetic context and investigate the regional expansion and genetic relatedness of NDM producing K. pneumoniae strains genetic relatedness within South Asian countries.

Methods

Eight NDM -positive K. pneumoniae clinical isolates were collected from 4 clinical settings of the region. Antimicrobial resistance was assessed and whole genome sequencing (WGS) was used for the genome and Phylogenetic analysis to investigate the relatedness of new and existing STs of NDM producing K. pneumoniae in the region.

Results

The study determined ST11, ST29, ST231, ST340, ST2096 among the blaNDM possessing K. pneumoniae included in the study. NDM-1, NDM-5 and NDM-20 were the three different variants of blaNDM identified. The co-acquisition of different blaESBL genes (*bla*$_{TEM}$, *bla*$_{CMY}$, *bla*$_{CTXM-15}$, *bla*$_{SHV}$) along with the carbapenemase (*bla*$_{NDM-1}$, *bla*$_{OXA-232}$) and efflux pumps genes (oqxAB, KpnE, KpnG, marA and rsmA) conferring multi drug resistance was observed in all isolates. The comparison of STs within the South Asian countries presented similar STs existing in Pakistan, China, India,and Nepal with overall 12 distinct STs prevailing in Pakistan. To our knowledge, the ST2096 has not been found before in South Asia, associated with blaNDM.

Conclusion

The diverse STs and genetic variability of blaNDM possessing K. pneumoniae among multiple clonal variants prevailing in the region presents wide ranging dissemination of the NDM gene. The study emphasizes regular surveillance, antibiotic stewardship programs and effective interventions in infection control to develop counter action plans for minimizing the problem.

P691

Where Phages 'Phit' into the Pangenome of *Streptococcus suis*: Informing Decisions for Phage-therapy

Emmet Campbell, Chris Creevey, Timofey Skvortsov, Nicolas Dimonaco

Queen's University Belfast, Belfast, United Kingdom

Abstract

As antibiotic efficacy decreases with continued misuse, novel therapeutics for tackling multi-drug resistant pathogens are overdue. *Streptococcus suis*, a pig pathobiont and model organism for developing novel bacteriophage therapies, severely impacts the swine industry. Understanding it's broader genomic picture may allow for forming connections between shared phage-associated elements.

From BV-BRC, 2121 *S. suis* genomes (classified as 'good' quality) were assessed for similarity using average nucleotide identity. Genomes were annotated *de novo* with Prokka, Stop-Codon-Delimited ORFs identified with StORF-Reporter, and the pangenome analysis performed with Roary. Pangenomes of all isolates, those with <=3 contigs (complete), and genetically-similar isolates were compared. Further analysis will capture the phage susceptibility profiles across these isolates.

The 132 complete (not necessarily genetically-similar) isolates produced a smaller core than ~500 genetically-similar isolates. Increasing sample size of a pangenome analysis will often result in a reduced core-gene size, which was not observed. The composition of the pangenome was found to be significantly dependent on the diversity between genomes selected for analysis. High diversity is likely out of scope for prophage/phage-resistance profiling, due to reduced possibility of geographically varied or non-contemporaneous isolates sharing these factors. Functional analysis of the core-genome could reveal targets for non-whole-phage therapies.

Preliminary results provide a library of genetically-similar *S. suis* genomes to analyse. Characterising the presence/absence of prophages and phage-defence systems will guide the selection of phages for phage cocktails. Incorporating this into the pangenome will allow us to contextualise susceptibility and resistance factors across the core and accessory genome.

P692

Distribution of virulence genes and antimicrobial susceptibility among Group B Streptococcus (GBS) isolated from diverse clinical syndromes: A report from India

Ruma Das, Rajni Gaind

Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India

Abstract

Background:

GBS is associated with maternal colonization, neonatal sepsis, and adult infections. It has a variety of virulence factors. Penicillin is the drug of choice. Reports of a rise in minimum inhibitory concentration (MIC) of penicillin have emerged.

Objective: To study the distribution of virulence genes and antibiotic susceptibility pattern (AST) of GBS isolated from various clinical syndromes.

Material and methods:

GBS isolates (2020-2022) from various clinical samples were included. Identification was confirmed by PCR (*cfb* and *dlt*S). Seven virulence genes were screened by using multiplex PCR. AST was performed as per CLSI guidelines using disk diffusion method. MIC of Ampicillin was determined by gradient strips.

Results:

Fifty-two isolates of GBS from maternal colonization (9), neonatal sepsis (20), and adult disease (23) were studied. Virulence genes *cfb* and *dlt*S were present in all isolates and other commonly observed virulence genes were *bca* (63%) and *psp* (61.5%). All isolates were susceptible to beta-lactams, and vancomycin but resistance to tetracycline was high (90%). All urinary isolates (15) were susceptible to nitrofurantoin. Ampicillin MIC range was 0.125-0.25 with MIC50 of 0.19 µg/ml. Resistance to erythromycin, clindamycin, and levofloxacin was significantly higher among isolates from adult disease. Erythromycin resistance was mediated by cMLSB. MDR was observed in > 65% of isolates.

Conclusion:

Potential vaccine candidates were *cfb*,*bca,* and *psp*. Penicillin remains the drug of choice for both prophylaxis and treatment. Use of Erythromycin or Clindamycin should be guided by AST. High resistance among isolates from adults may be associated with prior antibiotic exposure.

P693

Biofilm eradicating properties of a polyphenolic flavonoid against multidrug resistant Klebsiella pneumoniae

Blessing Oyedemi, Samantha McLean

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Naturally derived antimicrobial has a longstanding reputation due to wide array of pharmacological potential currently investigated as a front-line treatments. In this study, we evaluated biofilm eradicating properties of a flavonoid polyphenol (FP) as a standalone or when in combination with clinically relevant antibiotics. Results showed that combinations of curcumin (MIC=512µg/ml) with meropenem (MIC=16 µg/ml, gentamycin (MIC=32 µg/ml or ciprofloxacin (MIC=8 µg/ml potentiated synergistic inhibitory activities against Pseudomonas aeruginosa FIC <0.5 (128 µg/ml, 8 µg/ml and 2.1 µg/ml) respectively. FP completely eradicated in-vitro peg biofilms formed by P. aeruginosa, Staphylococcus aureus and Klebsiella pneumonia at MBEC values of higher original MIC at 2048 µg/ml but 2 folds lower when in combination with meropenem against K. pneumonia. The effect of this drug agent on their biofilm forming genes and metabolites produced by the breakdown of the extracellular polysaccharides is currently investigated. These results are promising because the global burden of antibiotic resistance and how biofilms increase ;their high tolerance to antibiotics is worrisome; and this could be addressed by way of discovering new molecules that could treat antibiotic-resistant infections.

P694

WILBR: Contribution of wild birds to antimicrobial resistance in the environment and on farm

Olivia Turner[1,2], Nathaniel Storey[3], Francesca Martelli[1], Shaun Cawthraw[1], William Gaze[2], Manal AbuOun[1], Muna Anjum[1]

[1]Animal and Plant Health Agency, Addlestone, United Kingdom. [2]University of Exeter, Exeter, United Kingdom. [3]Great Ormond Street Childrens Hospital, London, United Kingdom

Abstract

Aim: Despite wild birds not being intentionally exposed to antimicrobials, antimicrobial resistance (AMR) is widespread in bacteria in some wild bird populations. In the WILBR project the aims included exploring the likelihood of wild birds as a vector of transmission of AMR to farms.

Methods: Faecal samples were collected from gulls and pigs over 3 time-points at 12 month intervals over 2017-2019 on a low antimicrobial usage UK pig farm. Escherichia coli were isolated on antibiotic-free and antibiotic-supplemented agar plates and underwent whole genome sequencing. Sequence analysis was carried out to assess the diversity of E. coli strains and characterise the AMR genes, to help assess the potential for transmission between gulls and pigs.

Results: In total, 632 E. coli were isolated from pig and gull faeces (n=342 and n=290 respectively). E. coli ST 744 (31.5%), 10 (14.2%), 88 (10.7%), and 44 (8.4%) were most prevalent, and were also the only ST types present in both gull and pig faeces. Over 44% of pig isolates from non-selective agar harboured 1-12 AMR genes, and 36% of gull isolates harboured up to 15 AMR genes.The majority of ST744s were isolated from ciprofloxacin supplemented plates and harboured multiple AMR genes.

Conclusions: The presence of E. coli strains of the same ST type in both gull and pig faeces across multiple time points indicates the persistence of antimicrobial resistance in the farm environment and the possible transmission or exchange of multi-drug resistant E. coli between these compartments

P695

Microbial Genome-wide association studies (mGWAS) reveal novel loci associated with distinct clinical phenotypes and antibiotic resistance in Staphylococcus aureus.

Cosmika Goswami, Stephen Fox, Alistair Leanord, Thomas Evans

University of Glasgow, Glasgow, United Kingdom

Abstract

Background: mGWAS have great power in detecting genetic loci associated with phenotypic traits. We conducted such an analysis on 1496 bloodstream isolates of Staphylococcus aureus with rich associated clinical and microbiological data.

Methods: Using the mGWAS tool Pyseer, we interrogated the genomic data for single nucleotide polymorphisms (SNPs) and altered kmers. These changes were related to specifc phenotypic characteristics of antimicrobial resistance or association with clinical features such as portal of entry.

Results: SNPs and k-mers associated with antibiotic resistant markers revealed a number of significantly associated mutations in unsuspected targets. Mutations in the gene for thymidylate synthase (thyA) were associated with resistance to trimethoprim and mupiricin; knockout of thymidylate synthase is known to confer a small colony phenotype which may underlie this association with multiple antibiotic resistance. Fusidic acid resistance was associated with mutations in the gene for clumping factor (clfA), which faciltitates colonization of biomaterials . A number of mutations were associated with infection resulting from indwelling central vascular catheters. These included mutations in genes encoding a staphylococcal superantigen-like protein, ssl13 and an adhesin, sasA. Staphylococcal discitis was associated with mutations in the hemA gene, another regulator of the small colony phenotype, and in the upstream region of the nmrA gene, a regulator of biofilm formation.

Conclusion: This study has revealed a number of novel loci associated with antibiotic resistance and clinical features associated with infection. Further work will determine the role of these changes in the phenotypic characteristics concerned.

P696

Preparation of liposome-encapsulated bacteriophages by microfluidics for enhancing intracellular phage delivery

Xinyao He, Wafa Al-Jamal, Timofey Skvortsov

Queen's University Belfast, Belfast, United Kingdom

Abstract

Phage therapy as one of the main alternatives to the use of antibiotics has been actively investigated and successfully applied in the treatment of various bacterial infections, but is still challenging to apply clinically. The encapsulation of phages in liposomes can improve their storage stability and help to address some of the difficulties associated with their clinical application such as the rapid clearance of phage particles by the host immune system. Moreover, as the phospholipid bilayer of liposomes readily fuses with cell membranes, liposomes can help the phage to access the pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella spp.* inside the infected human and animal cells. In this project, we focus on the development of liposome formulations loaded with corresponding therapeutic phages against the foodborne pathogen *Listeria monocytogenes*. *Listeria* phage P100 was selected for its broad host range and desirable physical and chemical properties, showing no loss of activity within the pH range 4-6 and in certain organic solvents (25% ethanol, 0.6% Triton X-100, 0.002% Tween-20). Liposomes were prepared using the microfluidic mixing technology. Several formulations have been assessed, with cationic liposomes (C12/DSPC/Chol 50:38.5:11.5) and neutral liposomes (DOPC/Chol 4:1) achieving encapsulation rates of around 60% and 40% respectively. Preliminary results are encouraging and cell culture assays are currently under way. We anticipate that the encapsulated phages will improve the efficacy of elimination of intracellular bacterial pathogens using relevant *in vitro* and *in vivo* infection models.

P697

Genotypic and Phenotypic Assessment of Antimicrobial Resistance of Helicobacter pylori by Whole Genome Sequencing: a multicentric study in Oman

Zaaima ALJabri[1], Amal AL Hinai[1], Meher Rizvi[1], Said AL Busafi[2], Zakariya AL Muharrmi[2]

[1]Sultan Qaboos University, Muscat, Oman. [2]Sultan Qaboos University Hospital, Muscat, Oman

Abstract

Introduction: Statistics on antibiotic resistance and genetic mechanisms of resistance of H pylori are lacking in Oman, which is important for choosing the right medications to eradicate H. pylori. This study aims to investigate antimicrobial resistance of clarithromycin, amoxicillin, levofloxacin, metronidazole, and tetracycline both phenotypically and genotypically in H. pylori.

Methods: H. pylori gastric samples with positive rapid urease test; were phenotypically tested for antibiotic susceptibility using broth microdilution and Epsilometer test (E-test) for six antibiotics. Data obtained from WGS was analyzed for the genotype of antimicrobial resistance, virulence factors, Multi-Locus Sequence Typing (MLST), and phylogenetic relatedness of the strains.

Results:The highest levels of resistance was in MTZ (93.3%-100%) by the two methods. CLA resistance was (53.3%-33.3%) by the two methods with a strong positive correlation coefficient ($r= 0.665$, $p< 0.05$). AMX showed disagreement between the methods in one isolate only, with a very strong correlation ($r = 0.894$, $p = 0$).

Among the 20 strains, 55%, 66.6%, and 65.5% strains showed resistance to Clarithromycin, amoxicillin and rifampicin, respectively with point mutations at various loci. MLST analysis showed that twelve strains belonged to ST 3120, and seven isolates were novel. The phylogenetic analysis suggests that the strains circulating in the Omani population are very similar.

Conclusion: In summary, our findings indicate that H. pylori demonstrated significant resistance to clarithromycin, metronidazole, and amoxicillin with contributing point mutations. Furthermore, antibiotic susceptibility and molecular testing by WGS are essential to guide appropriate and population-specific antibiotic therapy.

Session Topic: Negative strand and double strand RNA viruses

P698

Elucidating the molecular mechanisms of vRNP bundling that drive influenza virus pandemics

Samantha Hover ORCID iD[1], Jean-Baptiste Brault[2], Kuang-Yu Chen[2], Nadia Naffakh[2], Juan Fontana[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Institut Pasteur, Paris, France

Abstract

Sporadically, novel and potentially devastating pandemic influenza A viruses (IAVs) arise during co-infection of differing IAV strains. Such pandemic viruses occur due to the segmented nature of the viral genome into 8 distinct ribonucleoproteins (vRNPs), which must all come together to produce progeny virions. This enables the emergence of novel viruses containing vRNPs from two or more strains. However, the mechanisms by which vRNPs are transported from the nucleus to the cell membrane and assemble into virions, during which reassortment occurs, are largely uncharacterised.

Previous work demonstrated that IAV remodels the endoplasmic reticulum (ER) of infected cells, which becomes tubulated and extends throughout the cell. Both vRNPs and the host Rab11 protein, required for vRNP trafficking, are present upon this modified ER and cover the surface of unique vesicle-like structures; termed irregularly coated vesicles (ICVs). These ICVs are thought to be the transport vesicles for vRNPs to the budding sites at the plasma membrane, forming a platform for vRNP bundling of the 8 segments in transit. To investigate the molecular mechanisms of ER re-shaping and of ICV generation, we have developed an extraction protocol for enriching ICVs from infected cells, to assess their composition using a range of approaches. We have successfully extracted ICVs coated in vRNPs and imaged them using electron microscopy, and we are continuing to study ICVs using cryo-electron tomography, confocal microscopy and proteomic analysis.

P699

Detrimental impact of Newcastle Disease Virus on the exocrine and endocrine functions of the pancreas in chickens

Zahid Manzoor[1], Zaib Ur Rehman[2], Chan Ding[3], Chunchun Meng[3], Syeda Maryam Hussain[4], Zishan Ahmad[5], Zia Ur Rehman[6], Ali Ahmad[7]

[1]Pir Mehr Ali Shah Arid Agriculture University, Dept. of Parasitology and Microbiology, Rawalpindi, Pakistan. [2]Pir Mehr Ali Shah Arid Agriculture University, Dept. of Poultry Sciences, Rawalpindi, Pakistan. [3]Shanghai Veterinary Research Institute, Shanghai, China. [4]Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. [5]Pir Mehr Ali Shah Arid Agriculture University, Dept. of Pathology, Rawalpindi, Pakistan. [6]The Islamia University of Bahawalpur, Dept. of Physiology, Bahawalpur, Pakistan. [7]University of Veterinary and Animal Sciences, Lahore, Narowal Campus, Narowal, Pakistan

Abstract

Newcastle disease virus (NDV) causes a highly contagious and devastating disease in poultry, Newcastle disease (ND). ND causes heavy economic losses to the global poultry industry by decreasing the growth rate, decrease egg productions, mortality, and morbidity. Although, significant advances have been made in the vaccine development, but outbreaks are reported in vaccinated birds leading to overall decreased growth rate. In this study, we report the damage caused by the NDV infection in the pancreatic tissues of vaccinated as well as specific pathogen free chickens. The histopathological examination of the pancreas showed sever damage in the form of partial depletion of zymogen granules, acinar cell vacuolization, necrosis, and apoptosis, congestion in the large and small vessels, sloughing of epithelial cells of pancreatic duct, and mild perivascular edema. Increased plasma levels of corticosterone, somatostatin, were observed in NDV infected chicks at 3 and 5-day post infection (DPI). Slight decrease in the plasma concentrations of the insulin were noticed at 5 DPI. Significant changes were not observed in the plasma levels of glucagon. Furthermore, NDV infection has decreases the activity and mRNA expression of amylase, lipase, and trypsin from the pancreas. Taken together, our findings highlight that NDV induces extensive tissue damage in pancreas, decrease the activity and expression of pancreatic enzymes and increase plasma corticosterone and somatostatin.

P700

Multimodal bioimaging of bovine respiratory syncytial virus inclusion bodies

Emily Lacey ORCID iD

University of Oxford, Oxford, United Kingdom. Diamond Light Source, Didcot, United Kingdom. The Pirbright Institute, Surrey, United Kingdom

Abstract

Bovine respiratory syncytial virus (BRSV) is an agricultural pathogen that significantly burdens animal health, food security, and international trade. Membraneless inclusion bodies are reported to form within BRSV-infected cells and are considered a cornerstone for successful viral replication activities, while simultaneously contributing to the evasion of host cell innate immune responses. To date, characterisation of these inclusion bodies has only captured the morphology of these liquid-liquid phase-separated organelles in microscopic detail; however, the structure of inclusion bodies at Angstrom resolution remains to be solved. We investigated BRSV inclusion bodies, in vivo, under near-native frozen-hydrated conditions using a multi-modal and multi-scale cryo-EM approach. Cryogenic focussed-ion-beam (cryo-FIB) milling guided by precision targeting of correlative light electron microscopy (cryo-CLEM) was used to thin fluorescently tagged BRSV-infected cells. Thinned lamellae, <200nm, were captured at high resolution using a dose-symmetric tilt-series scheme for cryo-electron tomography (cryo-ET). Novel 3-Dimentional reconstructions of BRSV inclusion bodies were generated to elucidate inclusion body functions and contributions to viral infection. Our results aim to characterise functional compartments within inclusion bodies named inclusion body-associated granules (IBAGs). While at the whole cell level, serial block-face scanning electron microscopy was used to capture several inclusion bodies in the context of the wider cellular landscape. Overall, this research overcomes the limited resolution structural understanding of inclusion bodies using a cryo-EM approach. By modelling these organelles down to Angstrom resolution, we have observed the whole cell context of inclusion bodies through to the detailed internal organisation within these organelles.

P701

Tools to investigate the dynamic composition of viral replication factories.

Xinyu Wang, Julia Acker, Alexander Borodavka

University of Cambridge, Cambridge, United Kingdom

Abstract

Rotaviruses (RVs) cause life-threatening gastroenteritis in children under 5 years old, particularly in developing countries. To date, there are no efficient anti-RV therapeutics. Replication of the RV genome is believed to occur within cytoplasmic replication factories, termed viroplasms. Recently, we have shown that RV viroplasms represent protein-RNA condensates formed via phase separation (PS) of viral proteins. We have examined several small molecules known to disrupt LLPS and identified propylene glycol (PG) as a compound with low toxicity suitable for dissolving viroplasms. We have shown that PG treatments of RV-infected cells significantly reduced the viral titres approximately 150-fold compared to the untreated samples. Dissolution of viroplasms also resulted in dephosphorylation of the viroplasm-forming protein NSP5 that could be reversed by inhibiting cytoplasmic phosphatases. Interestingly, during late infection stages (> 6 hours post-infection), viroplasms became refractory to the PG treatments, altering their liquid-like behaviour consistent with a liquid-to-gel/solid phase transition. Remarkably, PG treatments of cells during late infection stages also did not result in a significant drop in viral titres. To further investigate the dynamic composition of viroplasms, we have used reverse genetics approaches to rescue RVs harbouring a highly efficient peroxidase (APEX2), fused to the viroplasmic scaffold protein NSP5 (NSP5-APEX2). Biotinylated proteins were detected in recombinant RV-infected cells by Western Blot and visualised in situ by immunofluorescence. We will use mass spectrometry-based proteomics followed by genetic validation to identify host cell factors involved in RV replication and viroplasm formation.

P702

The anti-viral role and therapeutic potential of novel seaweed-derived compounds

Daniele Mega[1], Chloe Bramwell[1], Anja Kipar[1,2], James Stewart[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Zurich, Zurich, Switzerland

Abstract

Influenza viruses are segmented single stranded negative sense RNA (-ssRNA) viruses in the *Orthomyxoviridae* family and cause respiratory infections. Influenza A viruses (IAV) are responsible for the majority of human disease and 5 pandemics since 1889, the most recent of which was 2009 and the most lethal in 1918 with over 50 million recorded deaths worldwide. Vaccines and antiviral drugs are available, however, these are often ineffective due to rapid virus evolution. This study focuses on seaweed extracts (SE) isolated from *Ascophyllum nodosum,* which show potent broad anti-viral activity and low toxicity *in vitro*. SE reduced virus induced cytopathic effects (CPE) and growth against H1N1 and H3N2 subtypes. The data indicates SE primarily interacts directly with virions, preventing virus cell binding and entry. Intranasal administration of SE in mice prior to sub-lethal infection with IAV or at the time of infection reduced virus mediated weight loss and viral load in lung tissue. SE may be a promising antiviral agent in treatment or prophylactic treatment of Influenza infections.

P703

The impact of rotavirus VP3 and NSP1 on IFN-mediated host-restriction of viral replication

Lee Sherry ORCID iD, Xinyu Wang, Daniel Desiro, Alex Borodavka

University of Cambridge, Cambridge, United Kingdom

Abstract

Rotaviruses, a member of the Sedoreoviridiae family, are the leading cause of gastroenteritis in young children globally. These are non-enveloped, triple-layered viruses which encapsidate 11 segments of double-stranded (ds)RNA. During viral replication, the virus limits the production of dsRNA and viral core assembly to membrane-less viral replication organelles, known as viroplasms. Through limiting dsRNA production to nascent viral cores within viroplasms, these viruses are able to avoid detection by the host's cytosolic dsRNA-sensors, such as RIG-I, MDA-5 and PKR, which is highly important in the viruses ability to avoid triggering innate antiviral responses, such as the activation of the interferon (IFN) signalling cascades. Unfortunately, this strategy of shielding dsRNA from the host is not 100% efficient and can lead to the production of IFN and with that, the induction of hundred's of IFN-stimulated genes (ISGs) which act in an antiviral manner to make an unfavourable replication environment for the virus.

To combat this, rotaviruses are able to subvert this activation through two separate mechanisms; either through the E3-ligase related activity of NSP1 that degrades proteins necessary for IFN signaling, or via the phosphodiesterase (PDE) activity of VP3 that hydrolyzes the RNase L-activator 2',5'-oligoadenylate. Here, we investigate the impact of these two viral proteins on host-restriction, both individually and in concert using reverse genetics, through the production of reassortant viruses between the Simian rotavirus strain SA11, and the Bovine rotavirus strain RF, in the context of IFN-treated cells.

P704

Genome-Scale CRISPR-Cas9 Knockout Screening in Avian Cells to Identify Host Factors Essential for Influenza Virus Infection

Rose Blake[1], Abraham Lee[1], Nick Parkinson[1], Spring Tan[1], Vayalena Drampa[1], Kenneth Bailley[1], Paul Digard[1], Rachel Hawken[2], Finn Grey[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]Cobb-Vantress, Arkansas, USA

Abstract

IAV is a critically important pathogen in terms of human health, food security and animal welfare. Despite coordinated international surveillance and control strategies, IAV regularly causes globally significant outbreaks of disease. Genome editing has the potential to generate livestock that are resistant to IAV. However, high confidence host targets that may confer resistance must be identified. Here, we performed a genome-scale CRISPR/Cas9 knockout screen in chicken lung epithelial cells (CLEC 213) with the avian H3N2 strain with a 3:5 re-assortment virus. Both sorted screens based on the level of influenza infection in a cell and survival screens which generated cells resistant to influenza infection were conducted to identify host specific restriction factors against IAV. Several genes involved in sialic acid biosynthesis and N-linked glycosylation were enriched post infection in resistant cells from the survival screen and the low infection population in the sorted screens. These include SLC35A1 and host specific factors which have previously been identified in the avian host in the low sorted population, including ANP32A. This indicated that our CRISPR sort and survival screens were capable of identifying host specific dependency factors, and the validation and characterisation of several targets novel for avian influenza infection is ongoing.

P705

Influenza A viruses and direct cell-to-cell spread of infection.

Daniel Weir ORCID iD, Edward Hutchinson ORCID iD

MRC University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Influenza A viruses (IAVs) produce pleiomorphic virions. Strains that cause natural infections often form filamentous virions. The selection of this morphology implies a fitness advantage, but the reason why is unclear. Recently it was discovered that IAVs are not solely transmitted by virions but can also spread directly from cell-to-cell by trafficking viral material through tunnelling nanotubules (TNTs). TNTs are dynamic membrane structures that connect cells and have striking morphological and compositional similarities to filamentous IAV virions. IAV infection increases TNTs, suggesting that these structures could facilitate the spread of infection within a host while evading host immune responses. However, suboptimal fixation methods for imaging likely underestimates the influence of TNTs in infection spread and the significance of these structures continues to be revealed. Here we tested the hypothesis that filamentous IAVs, whose dimensions and composition resemble TNTs, could increase the efficiency of direct cell-to-cell spread. Using antiviral drugs to inhibit virus release, we analysed the frequency of direct cell-to-cell spread by microplaque assay. Furthermore, we performed super resolution confocal imaging of TNT-like structures following infection with these strains to assess whether the difference in morphology of the virions correlate with an increase in the observation of TNTs. IAV strains demonstrated to be filamentous or predominately spherical were directly compared in these assays. These methods are allowing us to determine how virion morphology relates to how influenza viruses spread and demonstrates the potential of direct cell-to-cell spread in efficiently propagating infection.

P706

The proviral effect of autophagy during arenavirus infection

Robert Stott-Marshall ORCID iD, Toshana Foster ORCID iD

University of Nottingham, Loughborough, United Kingdom

Abstract

Autophagy is an evolutionarily conserved process that is most notably a mechanism of degradation of long-lived proteins or damaged organelles in response to cellular stress. Autophagy may also play a key role in the degradation of intracellular pathogens and many viral pathogens including influenza A (IAV), hepatitis C virus (HCV) and human immunodeficiency virus-1 (HIV-1), have evolved the ability to inhibit, avoid or even utilize autophagy to enhance their own replication.

The Arenaviridae are the largest family of haemorrhagic fever-causing viruses, and many have fatality rates of 15-35% for which there are currently no vaccines or effective treatments. Hence, given the immense impact arenavirus infections have on human health and on the socio-economic status of the developing world, it is imperative to understand the molecular basis of viral pathogenesis. The role of autophagy has been explored during infection of a range of RNA viruses and has been implicated to play a role in the pathogenesis of arenavirus infections.

In this work, arenavirus infection induced autophagy to enhance virus infection. Ectopic expression of arenavirus proteins showed that autophagy induction was mediated by both NP and Z proteins, but not triggered by arenavirus entry alone. Mass spectrometry and co-immunoprecipitation analyses showed interactions between arenavirus NPs and tripartite motif (TRIM) protein 23 which plays a role in inducing autophagy in response to virus infection. Using CRISPR/Cas9 knockouts to create autophagy-deficient cell lines, we also showed that inhibiting autophagy reduced virus replication and production, implicating a proviral effect of autophagy during arenavirus infection.

P707

Deciphering aberrant RNA synthesis during influenza A virus infection

Michael Oade[1], Emmanuelle Pitré[1,2], Elizaveta Elshina[1,2], Benjamin Nilsson-Payant[3], Aartjan te Velthuis[1]

[1]Princeton University, Princeton, USA. [2]University of Cambridge, Cambridge, United Kingdom. [3]Institute of Experimental Virology, TWINCORE Centre for Experimental and Clinical Infection Research, Hannover, Germany

Abstract

Severe disease caused by highly pathogenic influenza virus infections is associated with a dysregulated innate immune response. During influenza A virus replication, the viral polymerase produces both full-length RNAs and shorter, aberrant RNAs including mini viral RNAs (mvRNAs) and defective viral genomes (DVGs). Both mvRNAs and DVGs have been associated with innate immune activation, but only mvRNA synthesis has been shown to be increased in infections with emerging or pandemic influenza A viruses. Presently we do not understand how mvRNAs are formed during infections with these viruses. To further our understanding of mvRNA diversity and their generation, we utilized high-throughput sequencing data of RNA extracted from influenza-infected ferret lung samples and A549 cells. We developed templates representing both negative and positive-sense genome segments whose replication products cannot be further utilised in replication. We find that mvRNAs vary in abundance with unique mvRNAs predominately deriving from segments 1, 2 and 5. The majority of mvRNA generating template-switches occur in U-rich regions of the genome. Collectively, our findings indicate that some mvRNAs are more readily generated compared to others and/or that mvRNAs themselves can be replicated. Indeed, we also find in differences in mvRNA replication level in minigenome assays and that mvRNAs that are not efficiently replicated play a key role in innate immune induction.

P708

Rabies virus can enter mGluR2, ITGB1 and nAChR knockout A549 human cells.

Manar E. Khalifa ORCID iD[1], Muhammad Munir[2]

[1]Lancster University, LANCASTER,Lancashire,UK, United Kingdom. [2]Lancaster University, Lancaster, United Kingdom

Abstract

Rabies is a lethal zoonotic viral disease which causes neurological disorders in a wide range of hosts with a high fatality rate of up to 100%. Multiple cellular receptors have been known to be involved in RV glycoprotein (RV-G) entry into host cells. However, there is a gap in identification the receptor mediated preference of RV-G. To address this problem, we developed a recombinant vesicular stomatitis virus (rVSV-dG-RV-G-GFP), encoding RV G protein and expressing green fluorescent protein (GFP) through a reverse genetics system. Also, we generated three different A549 knockout human cell lines through CRISPR/cas9 system each is deficient in one of RV cellular receptors; A549 ITGB1 KO, A549 mGluR2 KO and A549 nAChR KO cell lines. We compared the obtained results of infecting each of the KO cell lines and A549 wild type (WT) cell lines with rVSV-dG-RV-G-GFP. Our results clearly showed significantly reduced replication of rVSV-dG-RV-G-GFP in A549 ITGB KO, A549 mGluR2 KO and A549 nAChR KO cell lines compared to A549 WT cells. Over expressing of ITGB1 and mGluR2 in A549 ITGB1 KO and A549 mGluR2 KO cell lines; respectively resulted in significant increase in viral titres. While slight increase of RV titres was observed upon over expressing of nAChR in A549 nAChR KO cells. Our results support the capabilities of RV-G to bind to multiple cellular receptors, facilitating its entry into multiple hosts. Thus, genetic ablation of one of those receptors could reduce but not prevent RV replication.

Keywords: Rabies Virus; CRISPR-Cas9; Knockout; Receptor preference.

P709

A BioID2 proximity-based approach reveals TRIM25 and ZAP as arenavirus host restriction factors

Finley Old[1], Robert Stott-Marshall[1], Ivan Campeotto[2], Janet Daly[1], Toshana Foster ORCID iD[1]

[1]Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Science, Wolfson Centre for Global Virus Research, University of Nottingham, Loughborough, United Kingdom. [2]Faculty of Science, 2School of Biosciences, University of Nottingham, Loughborough, United Kingdom

Abstract

Haemorrhagic fever-causing arenaviruses pose serious risks to public health in West Africa and other endemic regions. Annual outbreaks of the most prevalent of these RNA viruses, Lassa virus (LASV), are associated with significant morbidity and high case-fatality rates, thus highlighting the need to develop effective treatments and vaccines. To achieve this, a better understanding of the arenavirus-host interactome and mechanisms of pathogenesis is crucial.

The arenavirus nucleoprotein (NP) is a multifunctional orchestrator of arenavirus replication and immunosuppression. To map the viral NP- host molecular interactome, we used proximity-dependent biotinylation followed by proteomic analysis of the captured biotinylated proteins. In this way, we identified multiple interactions of BioID2-tagged Mopeia virus (MOPV) NP, closely related to LASV, with host cell proteins.

We demonstrated that NP associates with several proteins involved in host translation and identified interactions with E3 ubiquitin ligase and ISG15, and TRIM25 and its cofactor the zinc-finger antiviral protein, ZAP, both of which are involved in regulation of the innate immune response against viruses. Ectopic expression of TRIM25 inhibited MOPV replication whilst knockdown of TRIM25 expression relieved attenuation of MOPV replication. ZAP is expressed as long (L) and short (S) isoforms, and we found that both had potent antiviral activity against MOPV replication and like TRIM25, ZAP knockdown significantly increased MOPV replication. Furthermore, we demonstrated a significant reduction in the attenuation of MOPV replication in both TRIM25 and ZAP knockdown cells pre-treated with type-I interferon. TRIM25 and ZAP therefore play a major role in the IFN-induced antiviral restriction of arenaviruses.

P711

The interactome of the influenza A virus polymerase during genome replication

Amalie B. Rasmussen, Olivia C. Swann, Carol M. Sheppard, Wendy S. Barclay

Imperial College London, London, United Kingdom

Abstract

Influenza A virus (IAV), which is associated with a significant public health burden through seasonal epidemics and occasional pandemics, is dependent on host cell proteins for viral RNA synthesis and other steps of its lifecycle. Host factors, rather than viral proteins, are attractive therapeutic targets that may reduce the ease with which influenza viruses develop antiviral resistance. Therefore, understanding their distinct roles in viral RNA synthesis is an important area of study. The influenza polymerase catalyzes both transcription and replication of the IAV genome by assuming different complexes that almost certainly include various host proteins. Previous genetic screens have identified many host factors supportive of influenza virus polymerase activity, however it remains unclear which host factors are specifically required for genome replication. Using a co-immunoprecipitation mass spectrometry approach, we aimed to identify host factors that uniquely associated with the replication conformation of the IAV polymerase under conditions that uncouple viral transcription and replication. We generated a recombinant H1N1 influenza virus that expresses an affinity-purification tag on the polymerase, which allows study of virus-host interactions in the context of live virus. We compared the interactome of the IAV polymerase in wild-type cells, that allow both viral transcription and replication, and in cells that lack expression of ANP32 proteins so support viral transcription but not replication. We identified a set of host factors that predominantly interact with the IAV polymerase in the wild-type but not the knockout cells, whose proviral function is likely supporting and regulating the activity of the replicase machinery.

P711

Investigating the antiviral role of IFIT proteins in human and bovine RSV

Michal Varga[1,2], Ian Brierley[1], Trevor Sweeney[2], Dalan Bailey[2]

[1]University of Cambridge, Cambridge, United Kingdom. [2]The Pirbright Institute, Guilford, United Kingdom

Abstract

Interferon-induced protein with tetratricopeptide repeats 2 (IFIT2) is an innate antiviral factor, often exhibiting its antiviral function via binding AU-rich viral RNAs or by interacting with parts of the innate immune response. Human and bovine respiratory syncytial virus (RSV) are respiratory pathogens causing significant economic strain for the global healthcare and livestock industries alike. During infection, RSV forms inclusion bodies, membrane-less biomolecular condensates, which are the sites of viral replication. Previous studies confirmed IFIT2 to be antiviral with respect to RSV infection, however, its mode of action remains to be elucidated.

Using confocal microscopy we assessed the localisation of nascent and exogenously expressed human and bovine IFIT2 in the context of RSV infection, and RSV pseudo inclusion bodies (pIB), generated by exogenous expression of nucleoprotein and phosphoprotein of RSV. We also assessed the involvement of the RNA binding capacity of IFIT2 using exogenously expressed, previously reported human IFIT2 RNA-binding mutant and novel bovine IFIT2 RNA-binding mutant.

We observed IFIT2 colocalises to the periphery of the IB in human and bovine cell lines. Additionally, nascent human, bovine and monkey IFIT2 colocalise with the exogenously formed pIBs. After expressing either human or bovine IFIT2 RNA-binding mutants, the interaction with pIBs and IBs remained unchanged, suggesting RNA is not involved in this interaction.

While the precise role and nature of the interaction are to be elucidated these results hint at the significance of IFIT2 protein during RSV infections effects on the inclusion bodies, which is conserved between species.

P712

Generation of a Mucosal Virus Vectored Vaccine for Bovine Parainfluenza Virus 3

Courtney Hawthorn[1], Olivier Touzelet[1], Yemmy Paredes-Pizarro[1], Sara Louise Cosby[2], Ultan Power[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom. [2]Virology Department, Veterinary Science Division, Agri-Food and Biosciences Institute, Belfast, United Kingdom

Abstract

Bovine PIV3 (bPIV3) is a major aetiological component of the bovine respiratory disease complex (BRDC), which is a leading cause of calf morbidity and mortality worldwide. BRDC also causes major economic losses to the agricultural industry. While there are some commercial vaccines available for bPIV3, there is a clear need for improvement.

This project aims to develop recombinant vaccine candidates using a Sendai virus (SeV) vector to express bPIV3 structural proteins. There is no evidence that SeV naturally infects cattle, so the host will have no prior immunity. SeV also has a tissue tropism for the respiratory tract epithelium.

Reverse genetics protocols are in place to enable the generation of these vaccines, including strategies to generate replication competent (rc) and replication incompetent (ri) SeV vectors expressing the bPIV3 genes of interest. The (ri) SeV vaccine strategy eliminates minimal vaccine biosafety issues that may be associated with (rc) SeV vaccines.

bPIV3 structural proteins (codon optimised for expression in cattle) were inserted into a SeV infectious clone plasmid. Insertions were confirmed by restriction enzyme analysis and PCR.

Encouragingly, both replication incompetent and competent rSeV/bPIV3 recombinant viruses were successfully rescued from the respective SeV infectious clones. This was confirmed by RT-PCR, sequencing, immunofluorescence, and western blot. Further in vitro characterisation of the recombinant viruses is currently underway. Ultimately, our aim is to rapidly take these vaccine candidates into in vivo trials in mice and/or cattle.

P713

Development of a split-luciferase complementation assay to measure interactions of influenza virus proteins

Fangzheng Wang ORCID iD, Ervin Fodor ORCID iD

Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Abstract

The replication of influenza virus RNA genome relies on fine-tuned interactions of the viral RNA-dependent RNA polymerase (RdRp), comprised of PB2, PB1 and PA subunits, with other viral proteins such as nucleoprotein (NP) and nuclear export protein (NEP). However, the molecular mechanisms of genome replication and the interplay of the viral RdRp with NP and NEP are not fully understood. Here, we develop a split-luciferase complementation assay that can rapidly and sensitively measure NEP-RdRp and NP-RdRp interactions by fusing the N-terminal and C-terminal halves of *Gaussia princeps* luciferase to the targeted viral proteins. We show that all three polymerase subunits are required for a robust NEP-RdRp or NP-RdRp interaction. Using a series of truncated PB2, we demonstrate that only the N-terminal 110 amino acids of PB2 are required to maintain a robust NEP-RdRp interaction. On the other hand, the NP-RdRp interaction requires a larger part of PB2, including the central cap-binding domain. Intriguingly, deletion of the PB2 627 and nuclear localization signal (NLS) domains does not affect the NP-RdRp interaction, indicating PB2 627 and NLS domains are not essential for the NP-RdRp interaction. Furthermore, our results suggest that the C-terminal tail (residues 491-498) of influenza A virus NP is critical for cRNA accumulation in infected cells potentially by contributing to NP-RdRp interactions. Overall, we have developed a sensitive method that can rapidly monitor the interactions of the influenza virus RdRp with other viral proteins.

P714

Investigating the contribution of internal gene segments in the recent emergence of panzootic H5N1 avian influenza virus

Jessica Quantrill ORCID iD[1], Joe James[2], Ashley Banyard[2], Ian Brown[2], Thomas Peacock[1], Wendy Barclay[1]

[1]Imperial College London, London, United Kingdom. [2]APHA, Weybridge, United Kingdom

Abstract

In recent years, the UK and Europe have experienced record numbers of avian influenza virus (AIV) infections in both wild birds and poultry, most recently dominated by highly pathogenic H5N1 AIV subtypes. The emergence of these dominant H5N1s has co-incided with a selective sweep of internal gene segments giving rise to a few distinct genotypes, particularly differing in their polymerase genes. Here we investigate the effects of these differences in internal genes on the activity and host range of the influenza polymerase.

Using chicken cells, or mammalian cells complemented with chicken ANP32A host factor, we show that contemporary H5N1 polymerases appear to have higher polymerase activity than those replaced. However, they do not show significantly higher activity than other highly poultry-adapted strains. We further investigate the molecular basis for this higher activity in contemporary strains by switching segments between high and low activity strains. Furthermore, we show that introducing the mammalian-adapting PB2 E627K substitution into these H5N1 polymerases increases their ability to utilise human host factor ANP32, in a similar manner to AIV strains which are known to have previously infected humans. Finally, we investigate the compatibility of these contemporary H5N1 genotypes with ANP32A proteins from different poultry, wild fowl and seabird species. Overall, this work suggests that internal gene segments may be playing a role in the emergence and predominance of the current panzootic H5N1 virus.

P715

Dabie bandavirus NSs as a molecular determinant of infection in tick cells

Mazigh Fares ORCID iD[1], Kelsey Davies[1], Lesley Bell-Sakyi ORCID iD[2], Benjamin Brennan ORCID iD[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

Abstract

Dabie bandavirus (SFTSV) is a tick-borne pathogen that emerged in 2009 in China. The genome of SFTSV comprises two negative sense segments (M and L) and an ambisense segment (S) that encodes for the nucleocapsid protein (N) in a negative sense orientation and a non-structural protein (NSs) in a positive sense orientation. Here, we characterise the replication of SFTSV (strain HB29) using cell lines derived from various tick species. We show that SFTSV replicates selectively in tick cell lines derived from a natural vector, *Rhipicephalus microplus* (BME/CTVM6 and BME/CTVM23), and does not replicate in cell lines derived from the species *Ixodes ricinus* (IRE/CTVM19 and IRE/CTVM20) or *Ixodes scapularis* (ISE6), not known to naturally transmit SFTSV. Interestingly, we did not detect the expression of the NSs protein at any point following infection of BME/CTVM6 cells, and very low levels of NSs mRNA were produced as evidenced by Northern blotting. Small RNA sequencing of infected cells revealed that not only NSs mRNA but the whole viral genomic and antigenomic RNAs were targeted by the siRNA pathway (indicated by short 22nt RNAs). Using a previously engineered NSs deletant mutant (rHB29delNSsGFP), we showed that the lack of NSs impairs the ability of SFTSV to infect BME/CTVM6 cells. We are currently investigating the mechanisms by which NSs enhances SFTSV-infection of ticks. This work shows that, although its expression is repressed, NSs is required for SFTSV replication in tick cells and suggests a specific biology of SFTSV and a key role of NSs in arthropod infection.

P716

The human immune response to Crimean-Congo haemorrhagic fever virus: A longitudinal study.

Emma Kennedy[1], Stuart Dowall[1], Nazif Elaldi[2], Roger Hewson[1]

[1]UKHSA, Salisbury, United Kingdom. [2]Cumhuriyet Universityy, Sivas, Turkey

Abstract

Crimean-Congo haemorrhagic fever virus (CCHFV) causes severe haemorrhagic disease in humans that can be fatal. A detailed understanding of the immune responses to CCHFV infection and the pathogenesis of disease remains limited.

In this unique study spanning a two-year period, samples from 68 Turkish CCHF patients were collected to study immune responses to infection. Based on a well characterised severity grading score of 12 parameters measured in the first 5 days of hospitalisation, patients were categorised into mild, moderate or severe risk groups of mortality. Patients' symptoms included fever, myalgia, nausea and headache, with increased age correlating with disease severity. Serum analysis of survivors against fatal cases showed that a strong B-cell response is correlated to survival. Patients in the most severe group (all non-survivors) had significantly lower IgM and IgG levels compared to moderate and mild groups. All survivors developed a strong IgG response that was still elevated 2 years post-infection. Bio-marker assays show 16 cytokines (Eotaxin, G-CSF, GM-CSF, IFN-α, IFN-γ, IL-5, IL-6, IL-8, IL-10, IL-17a, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α and VEG-F) were elevated in severe patients suggesting a 'cytokine storm' phenomenon is linked to severe disease. Patients with moderate and mild symptoms had elevated levels in fewer cytokines, compared to the naïve cohort.

This study is part of a wider programme aimed at developing a detailed understanding of the range of immune responses to CCHFV infection. It will contribute to informed vaccine design and support other interventions to this emerging neglected disease.

P717

Convalescent human plasma protection against CCHF virus challenge in an A129 mouse model

Sarah Kempster[1], Mark Hassall[1], Stuart Dowall[2], Emma Kennedy[2], Steve Findlay-Wilson[2], Neil Almond[1]

[1]MHRA, South Mimms, United Kingdom. [2]UKHSA, Salisbury, United Kingdom

Abstract

Crimean-Congo Haemorrhagic Fever (CCHF) virus is spread by infected ticks or direct contact with blood or tissues and fluids from infected patients or livestock. Infection with CCHF virus causes severe haemorrhagic fever in humans which is fatal in 10-40% of cases.

CCHF virus is listed as a priority pathogen by the World Health Organization (WHO) and there are currently no widely-approved vaccines available.

Definition of the antibody level and characteristics that correlate with protection against CCHF virus infection would support the development of vaccines by providing a 'target threshold' for immunogenicity to achieve, in order to confer protection. We therefore sought to demonstrate titratable protection with antibodies against CCHF from pooled human convalescent plasma in a mouse model.

Convalescent plasma collected from seven individuals with a known previous CCHF virus infection were characterised by ELISA using both commercially available and in-house ELISAs. All plasma recognised nucleoprotein and the Gc glycoprotein, but some had a lower Gn glycoprotein response. Pooled convalescent plasma and two individual donations representing 'high' and 'low' Gn reactivity were administered intraperitoneally to A129 mice 24 hours prior to intradermal challenge with CCHF virus (strain IbAr10200).

A protective effect was seen with all three convalescent plasmas with a longer survival and a reduced clinical score. The response was titratable with a loss of protection when less plasma was administered. Further characterisation of the antibody response within these samples will support vaccine and assay developments for CCHF virus.

P718

Chaperone Proteins Efficiently Drive the Assembly of Newcastle Disease Virus-Like Particles in *Nicotiana benthamiana* Transient Expression System

Ika Nurzijah ORCID iD[1,2,3], Kostya Kanyuka ORCID iD[4], Janet Daly ORCID iD[1], Stephen Dunham ORCID iD[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham, United Kingdom. [2]Rothamsted Research, Harpenden, United Kingdom. [3]Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Purwokerto, Indonesia. [4]National Institute of Agricultural Botany (NIAB), Cambridge, United Kingdom

Abstract

Newcastle Disease (ND) is economically important viral disease in poultry. Despite the availability of commercial vaccines, ND remains a challenge for poultry industry, particularly in poor-and middle-income countries where the strict biosecurity measures are unachievable. Therefore, there is an urgent need of cost-effective technology for developing ND vaccines. This study aims to combine Agrobacterium transient expression system in *Nicotiana benthamiana* with bioinformatics studies to generate novel ND vaccines. We successfully produced optimum yield of NDV envelope proteins (Fusion/F, Haemagglutinin Neuraminidase/HN, and Matrix/M) in *N. benthamiana* at 9 days post-infection (dpi). The co-expression of heterologous chaperone proteins (calnexin and/or calreticulin) in plant expression system and the pH of extraction buffer noticeably influenced the yield of NDV envelope proteins. NDV virus-like particles (VLPs) consisting of the envelope proteins (NDV F, HN, and M) were assembled in *N. benthamiana*. The assembly of NDV VLPs in *N. benthamiana* was efficiently driven by the co-expression of heterologous chaperone proteins. Mice immunisation is underway to evaluate the efficacy of purified NDV VLPs and sub-unit proteins. An evidence-based bioinformatics approach on NDV glycoproteins F and HN was able to identify molecular targets with a potential benefit for ND seed vaccine development against global variant of NDV. Overall studies show future prospect for more efficient ND control in poor-and middle-income countries.

P719

Antiviral activity of IFNε against respiratory syncytial virus and other negative strand viruses in airway epithelial cells

Mary McCabe ORCID iD, Helen Groves, Guillermo Lopez Campos, Erin Getty, Ultan Power

Wellcome-Wolfson Institute for Experimental Medicine, Belfast, United Kingdom

Abstract

Respiratory syncytial virus (RSV), a single-stranded negative-sense RNA virus, is the commonest cause of acute lower respiratory tract infection in infants. Using RNA-seq analysis of previously established well-differentiated primary nasal epithelial cell cultures (WD-PNECs) derived from infants sampled at birth and one-year-old, interferon epsilon (IFNε) was significantly increased following RSV infection versus uninfected controls. Furthermore, IFNε expression was higher in 1 year- versus newborn-derived WD-PNECs both at baseline and following RSV infection, suggesting a more robust role in antiviral activities at 1 year compared to newborns.

To further interrogate the potential antiviral role of IFNε we characterised IFNε expression in airway epithelial cell lines (HEp-2, BEAS-2B, and A549) infected with RSV (RSV-A2/mKate2) or mock-infected. Constitutive IFNε expression was detected in all cell lines, irrespective of infection status. To determine whether IFNε had antiviral properties compared to IFNβ and IFNλ1, cell lines pre-treated with recombinant IFNε, IFNβ or IFNλ1 were infected with RSV (MOI=0.3). IFNε pre-treatment resulted in a dose-dependent reduction in RSV-A2/mKate2 fluorescence in all cell lines, although the reduction was less than that for IFNβ or IFNλ1. Similar dose-dependent antiviral activity of IFNε was evident against Sendai virus/eGFP and influenza PR8/mCherry infection, confirming IFNε has antiviral activity against a range of negative-strand RNA respiratory viruses. Future work will explore the antiviral activity of IFNε against SARS-CoV-2 and the consequences of IFNε gene knockdown and IFNε pre-treatment of the more physiologically relevant WD-PNECs on viral replication and induction of antiviral responses.

P720

Visualisation of bluetongue virus infection in the salivary apparatus of *Culicoides* biting midges highlights the accessory glands as a primary arboviral infection site

Marc Guimerà Busquets[1], Faye Brown[1], Simon Carpenter[1,2], Karin Darpel[1,3], Christopher Sanders[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]Institute of Virology and Immunology, Bern, Switzerland

Abstract

Culicoides biting midges are biological vectors of economically important viruses of livestock, including bluetongue virus (BTV). Arthropod-borne viruses such as BTV replicate in both the vertebrate and arthropod host. From an infected blood meal, BTV may infect and pass through the midgut of *Culicoides* midges, disseminate and replicate in body tissues including the salivary glands, from which it can be transmitted to the ruminant host during subsequent feeding. Despite the importance of the salivary apparatus for virus transmission to ruminants from the bite of infected *Culicoides*, this structure has received relatively little attention. Here, we present a protocol that has enabled us to visualize the structure of this organ in 3-dimensions using immunofluorescence confocal microscopy. For the first time, we show viral infection of the salivary apparatus of female *Culicoides* vectors following a natural route of infection. Furthermore, our work shows that although all glands constituting the apparatus can harbor virus, the accessory glands are the primary site for BTV replication within the salivary apparatus. This uncovers a potential and previously unknown role of the accessory glands of *Culicoides* midges in arbovirus amplification and its subsequent transmission. Additionally, this technique will facilitate investigation of arbovirus infection and dissemination in different insect tissues (e.g., midgut) as well as further comparative studies of infection dynamics between different BTV strains, and other *Culicoides*-borne viral species. In conclusion, our work presents a powerful tool for the investigation of the infection and dissemination characteristics that determine the ability of a vector to support arbovirus transmission.

P721

Development of a porcine genome-wide CRISPR/Cas9 screen to identify host restriction factors against swine influenza virus

Vayalena Drampa[1], Rosemary Blake[1], Nicholas Parkinson[1], Wenflang Spring Tan[1], Paul Digard[1], Kenneth Baillie[2], Jonathan Snyder[3], Finn Grey[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]Centre for Inflammation Research, Edinburgh, United Kingdom. [3]Elanco Animal Health Incorporated, Indiana, USA

Abstract

Swine influenza virus has a significant financial impact in pig breeding, while simultaneously raising alarms over catastrophic pandemics due to its zoonotic nature. As an obligate intracellular parasite, it relies on host factors for its replication. Understanding of host-pathogen interactions is required to identify novel therapeutic targets and further design disease control. High throughput phenotypic screens can be a powerful tool in achieving this with CRISPR/Cas9 allowing systematic analysis of mammalian genomes. Here we developed a genome-wide CRISPR/Cas9 screen in pig cells in order to identify host restrictions factors against swine influenza virus. We transduced to a stable Cas9-expressing pig cell line with a pooled Genome-Scale CRISPR Knock-Out (GeCKO) library based on the pig genome. Subsequently, we infected the cells with H3N2 swine influenza virus at high multiplicity, fixed and stained for influenza membrane protein M2. Cells were sorted through flow cytometry based on M2 signal intensity. Finally, genomic DNA was harvested, sgRNA sequences were amplified by a 2-step PCR and sequenced through Next-Generation Sequencing. The quantification of loss or overrepresentation of sgRNAs can indicate a functional relevance of the genes they target. By extension, candidate genes, related to promoting or restricting swine influenza virus replication are selected based on statistical significance. These studies will provide a genome wide analysis of virus host interactions for swine influenza virus in porcine cells and the basis for additional characterisation and comparative studies with influenza A screens in human and chicken cells.

P722

Elucidating the interaction between Influenza A virus Matrix protein 1 and host Transportins during viral entry and uncoating

Carl Winn[1,2], Katja Klein[2], Othmar Engelhardt[1], Jason Long[1], Yohei Yamauchi[3]

[1]MHRA, Potters Bar, United Kingdom. [2]University of Bristol, Bristol, United Kingdom. [3]ETH Zurich, Zurich, Switzerland

Abstract

Influenza A viruses (IAV) cause a significant disease and economic burden. Various host factors are exploited by IAV during its replication cycle, including a viral uncoating host factor Transportin-1 (TNPO1) which interacts with viral protein Matrix protein 1 (M1) during virus entry. Furthering our understanding of viral-host protein interactions and the dependence of influenza virus on its host may in turn lead to novel therapeutic strategies. By elucidating molecular mechanisms of TNPOs in influenza M1 uncoating we will be able to generate and assess M1 mutant strains for their potential as a live attenuated candidate influenza vaccine.

Here, we sought to disrupt the M1-TNPO1 interaction through generation of recombinant M1 viruses by reverse genetics. We made substitutions in residues hypothesised to be important for this M1-TNPO1 interaction as determined by previous work and computational modelling. In comparison to the WT virus, one of the recombinant viruses showed reduced cell entry, with some other strains showing reduced growth kinetics and virion morphological changes. The virus affected in host cell entry contained a substitution in the non-canonical PY-NLS that TNPO1 potentially recognises. Despite growing to high titres in eggs this virus showed attenuation in mammalian cells. The virus overcame this growth defect with a second site M1 mutation which did not appear in eggs. Further work is ongoing using immunofluorescence assays to determine which step of virus host cell entry is disrupted and to elucidate potential differences in the role of TNPOs between avian and human cells.

P723

What goes UpA, must come down: an attenuation of Influenza A Viruses by the synonymous addition of UpA dinucleotides.

Luke Thorley ORCID iD, Colin Sharp, Eleanor Gaunt ORCID iD

The Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom

Abstract

The frequency and distribution of nucleotides across eukaryotic genomes are subject to compositional biases. An example of this is dinucleotide bias, whereby certain 5'-3' nucleotide pairings are preferentially encoded over others; notably, the Uracil-phosphate-Adenine (UpA) dinucleotide is mathematically underrepresented in eukaryotic transcriptomes. Interestingly, UpA is also under-represented in the genomes of RNA viruses that infect these eukaryotes. However, why UpA is underrepresented in RNA virus genomes as well as the mechanisms that dictate its suppression remain largely unknown.

Through an interdisciplinary approach of molecular virology and bioinformatics, the aim of this work is to determine the frequency and distribution of UpA within the genomes of RNA viruses and to determine the mechanism(s) by which they are maintained. We have characterised the distribution and frequency patterns of UpA in influenza A virus (IAV) genomes using bioinformatics approaches. UpA suppression is non-uniform and varies between viral genome segments. The introduction of UpA dinucleotides into segment 1 or segment 5 of the IAV genome through synonymous mutation results in a significant attenuation of virus replication. By ruling out defects in the transcription and translation of the mutant constructs, we have demonstrated that this attenuation is likely due to a cellular process. Candidate genes that may be responsible for UpA sensing are currently being tested.

P724

“Developing a new vaccine candidates using a modified HBcAg-VLPs including SpyTag Technology as an Antigen-Capturing system”[NS1] [NS1]Suggest this one, slightly modified

Jehad Alzahrani, Martin Stacey, David Rowlands, Nicola Stonehouse

School of Molecular and Cellular Biology, Astbury Centre for Structural and Molecular Biology, University of Leeds, Leeds, UK, Leeds, United Kingdom

Abstract

Virus-like particles (VLPs) technology could offer one of the safest platforms for the rapid development of new vaccines. VLPs are highly immunogenic, yet lack the genetic information required to cause an infection. Additionally, VLPs can be modified as generic vaccine platforms to present a variety of different antigens.

We are studying a novel and flexible VLP presentation platform based on a fused dimer of the hepatitis B core (HBc) antigen, which incorporates an antigen-capturing system. This system is based on SpyTag technology which includes the formation of a spontaneous and covalent iso-peptide bond when SpyTag interacts with its protein partner (SpyCatcher), thus creating particles which can capture multiple copies of the target antigen at their surfaces. The multimeric display of antigen proteins at the surface of recombinant VLPs can significantly improve their immunogenicity.

Here, we have produced HBc VLPs, modified to display SpyCatcher or SpyTag, in the yeast Pichia pastoris and determined their capacity to bind SpyTag-linked proteins. We have produced the glycoprotein G of respiratory syncytial virus (RSV) as a target antigen in mammalian cells. Through the generation of this VLP-RSV-G antigen complex, we have established potential new steps for the production of a new vaccine against RSV.

P725

Interrogating the viral diversity of British rodents

Jack Hill, Joseph Chappell, Theocharis Tsoleridis, Andrea Sartorius, Patrick McClure, Jonathan Ball

University of Nottingham, Nottingham, United Kingdom

Abstract

As humans and rodents are driven into closer proximity due to an expanding population and environmental changes, they interact more frequently, leading to an increased risk of zoonotic disease spillover. By increasing our understanding of the rodent viral profile, we will be able to better predict and respond to rodent to human zoonotic spillover events.

Gut and liver samples were taken from 140 small rodents across five species and three sites in Wales. RNA was extracted from these samples, and next generation sequencing (NGS)) libraries were produced, pooled, and sequenced via Illumina NovaSeq, providing over three billion reads. The reads were then examined for the presence of viruses, and identified viruses were confirmed via conventional end-point PCR.

67 viruses have been identified in the NGS data and confirmed via follow-up PCR so far, representing nine viral families. *Picornaviridae*, *Astroviridae*, *Adenoviridae* and *Parvoviridae* are the most frequently confirmed viruses, but *Reoviridae*, *Picobirnaviridae*, *Paramyxoviridae*, *Flaviviridae* and *Polyomaviridae* have also been identified. Other viral families, such as *Coronaviridae* and *Hantaviridae* have been identified in the NGS data but not yetconfirmed by follow-up PCR.

This is the most in depth investigation into the viral profile of British wildlife to date, and provides an estimate of the prevalence of many potentially pathogenic viral families in these species. This data may allow the identification of novel viruses in these animals, but should be informative about the risks of zoonotic spillover from UK wildlife into humans.

P726

The production of recombinant SBV-N protein for use in an edible algal vaccine

Jonathan Scarfe, Janet Daly, Ellen Nisbet

University of Nottingham, Nottingham, United Kingdom

Abstract

This project aims to produce an algal vaccine for use in agriculture. Schmallenberg virus (SBV) causes congenital malformations and abortions in ruminants, characterised by arthrogryposis-hydranencephaly-syndrome. Edible algal vaccines offer a novel, pragmatic and cost-effective solution for the prevention of such viral diseases. Codon optimised and his-tagged DNA encoding the SBV nucleoprotein (SBV-N) was precisely inserted into the *Chlamydomonas reinhardtii* (a green alga) plastome via glass bead transformation. Selection was based on phototrophic rescue of the cell wall deficient Δ*psbH* TN72 strain (Wannathong et al., 2016). Transformant colonies were screened by PCR, confirming SBV-N DNA integration in all transformant colonies. Reverse transcription PCR was used to confirm RNA transcription. Finally, western blot analysis confirmed the expression of SBV-N protein. These results show that SBV-N is being successfully expressed in the *Chlamydomonas reinhardtii* plastid. Future research is now being focused on expressing viral proteins of other viruses, such as tilapia lake virus and rotavirus, following the same method. The immunogenicity of such proteins will be analysed to investigate their suitability as vaccine candidates.

P727

The lost bandavirus: Exploration of the molecular interactions underpinning Bhanja bandavirus infection in mammalian cells

Andrew T Clarke ORCID iD, Mazigh Fares ORCID iD, James I Dunlop ORCID iD, Alain Kohl ORCID iD, Benjamin Brennan ORCID iD

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Bhanja bandavirus (BHAV) is a tick-borne pathogen that emerged in 1954 in India. BHAV has since been detected across Eurasia and Africa with reports of febrile and encephalitic illness in young ruminants and humans. Despite its close relationship with other highly pathogenic bandaviruses, BHAV has remained much neglected with no information regarding the virus/host molecular interactions that facilitate infection in the vertebrate host. Our work here focuses on unravelling the specific functionalities of the non-structural protein (NSs) of BHAV, which acts as a virulence factor in other members of the Phenuiviridae family. We have examined the recently identified nucleotide sequences of several isolates of BHAV. These viruses were isolated from different host species and upon examination of their genomes, disparate homology within the NSs protein was observed. Using in vitro interferon assays, we breakdown the differential ability of these NSs proteins to antagonise the anti-viral RIG-I and JAK/STAT signalling pathways. To further study BHAV NSs function in vitro and in vivo, we have developed a reverse genetics system for the BHAV isolate IG690 and have recovered recombinant wild-type and fluorescently tagged NSs deletant viruses. Collectively, this work provides a first insight into the immune evasion strategies of BHAV, while showing the development of vital biological tools to study this much overlooked tick-borne virus.

P728

Combining experimental data and predictions to determine RNA-RNA interactions in viruses

Daniel Desirò, Lee Sherry, Michael Knight, Alexander Borodavka

University of Cambridge, Cambridge, United Kingdom

Abstract

Several regulatory mechanisms of viral replication are known to be guided by functional intra-molecular long-range RNA-RNA interactions (LRIs). In contrast, inter-molecular RNA-RNA interactions and their contribution to viral replication remain challenging to predict and validate. Recent experimental evidence indicates that such interactions may play a vital role in the assembly of viral genomic RNAs in viruses with segmented genomes, and they are pivotal for the selective assembly and packaging in segmented RNA viruses of the *Orthomyxoviridae* and *Reoviridae* families. We use experimental structure probing and RNA cross-linking tools, including SHAPE-MaP and SPLASH methods to identify such RNA-RNA interactions. We show that these methods can generate various biases and spurious results that need to be further carefully analysed and experimentally validated. Here we present a new approach to detect, analyse, and validate inter-molecular RNA-RNA interactions in segmented RNA viruses by combining RNA structure probing tools and other experimental methods with state-of-the-art RNA structure prediction algorithms. Using SPLASH data from several rotavirus strains, we show that our approach significantly improves the signal-to-noise ratio and allows to reliably identify inter-molecular RNA-RNA interactions. Our method can also be extended to intra-molecular long-range RNA-RNA interactions, and it can be used for other RNA viruses.

P729

Assessing the effect of inter-isolate variation on the function of RVFV virulence determinants

Philippa Hollinghurst ORCID iD[1,2], Victoria Sy[1], Simon Gubbins[1], Daniel L. Horton[2], Kevin Maringer[1], Isabelle Dietrich[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Background

The increasing magnitude of Rift Valley fever virus (RVFV) outbreaks raises concerns over the emergence of more virulent variants. To examine the putative molecular mechanisms that underpin emerging RVFV virulence, known virulence determinants of an isolate from the Kenyan 2006 outbreak were compared with an ancestral isolate obtained during an interepidemic period in 1983.

Methods

A reverse genetics system was used to generate recombinant viruses by cloning the glycoproteins or NSs protein of the desired isolates into a RVFV strain ZH-548 backbone. Cell culture assays were used to examine inter-isolate differences in the modulation of the IFN-β response and replication kinetics in human, bovine, ovine and mosquito cells, as quantified by RT-qPCR and growth curve analyses.

Results

Recombinant RVFV that expresses the NSs protein or glycoproteins of the Kenya 2006 or 1983 strains were successfully rescued. The replication kinetics of these viruses were found to resemble that of RVFV ZH-548. The NSs-recombinant viruses were competent in abrogating the IFN-β response relative to an NSs-deficient comparator and trends in the inhibition of ISG transcription across time suggests that modulation may occur in an isolate specific manner.

Conclusions

The recovered recombinant viruses provide a useful tool for examining RVFV determinants and inter-isolate variation within the context of infection. The replication kinetics of these viruses in representative host cell lines supplements our knowledge of RVFV host adaptation. Quantification of the effect of RVFV NSs proteins on the IFN-β response could suggest inter-isolate variation in the efficiency of immunomodulation by the NSs protein.

P730

Understanding off-target growth defects introduced to influenza A virus by synonymous recoding.

Colin Sharp[1], Beth Thompson[1], Peter Simmonds[2], Paul Digard[1], Eleanor Gaunt[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

Abstract

CpG dinucleotides are under-represented in the genomes of most RNA viruses. Increasing CpG content through synonymous recoding of a range of RNA viruses reliably causes replication defects and highlights CpG enrichment as a strategy for the development of live attenuated vaccines, as we have shown with influenza A virus (IAV) mutants enriched for CpGs in either genome segment 1 or 5. The replicative defect in the segment 1 mutant could be rescued by depletion of the cellular protein ZAP (zinc-finger antiviral protein), which is known to bind CpG motifs and mediate CpG-associated viral attenuation. In contrast, the segment 5 CpG mutant was ZAP-insensitive and triggered an interferon response not evident in the ZAP-sensitive segment 1 mutant, suggesting the introduction of a growth-restricting mutation unrelated to the CpG enrichment. The specific mutation responsible for the segment 5 defect was mapped by stepwise reversion of overlapping regions in the CpG-enriched virus to wild-type. This, along with further single nucleotide reversions successfully narrowed the recoded region responsible for attenuation of the CpG-enriched virus down to two nucleotides; these were also sufficient to confer a growth defect upon introduction to wild-type virus. Identifying that small numbers of nucleotide changes can be responsible for the growth defects in a virus designed to have a formidable barrier to wild-type reversion highlights the importance of understanding the processes underlying the attenuated replication. The lessons from this study can also inform improved recoding designs in the future.

P731

Mosquito Cell Hormone Signalling Affects Rift Valley Fever Virus Proliferation

Lucas Wilson[1,2], Victoria Sy[1], Isabelle Dietrich[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom

Abstract

The hormones 20-Hydroxyecdysone (20E) and Juvenile Hormone (JH) are an essential part of arthropod development. Previous studies in the fruit fly *Drosophila melanogaster* and the mosquito *Anopheles gambiae* have demonstrated that 20E has a priming effect on immunity, upregulating specific antimicrobial peptides (AMPs) when infected with bacteria. Contrarily, JH antagonises 20E action and is a potent inhibitor of AMP expression in bacteria-challenged insects. Interestingly, a recent study has shown that 20E downregulates rather than primes immune signalling in adult *Aedes aegypti* mosquitoes. The effects of hormonal dosing on arboviral replication in vector mosquitoes remain poorly understood.

We firstly used RT-qPCR to confirm that 20E does not prime immunity in Ae. aegypti Aag2 cells in accordance with in vivo findings. We then assessed if 20E and JH treatment of mosquito cells affected the growth of the arbovirus Rift Valley fever virus (RVFV) and found that JH dosing significantly increased viral titre. Next, we will silence 20E and JH signalling using dsRNA and again quantify RVFV proliferation. We will expand our study to include hormone treatments of adult mosquitoes to examine the modulation of antiviral pathways, followed by RVFV infection.

A better understanding of how 20E and JH affect the induction of mosquito immune genes could assist in controlling the spread of RVFV and similar arboviruses, preventing economic and personal losses which are especially damaging to developing nations.

P732

Peste des petits ruminants virus (PPRV) non-structural proteins direct interaction with NFkB p65 (Rel A) protein and its resultant effect on cytokines involved in immune and inflammatory response.

JUHI JAIN, Rajeev Kaul

University of Delhi South Campus, New delhi, India

Abstract

Introduction: Peste des petits ruminant (PPR) is the contagious disease of sheep and goats with a high morbidity and mortality rate, despite the availability of an efficacious vaccine. PPRV known to prompt immune modulation.This study has been conducted to understand the mechanism behind it.

Methods: Foremost LCMS was carried out to identify the cellular protein interacting with the PPRV C, and PPRV V protein. To validate that, co-immunoprecipitation and GST-Pull Down assay were performed. To monitor the effect of viral proteins on cellular protein translocalization, immunofluorescence assay was performed. Moreover, to study the viral protein's impact on transcriptional activation of cellular protein and ,interferon induction, a Dual-luciferase reporter assay was used. At last, RT- qPCR was performed to quantify the transcript level of different cytokines involved in the immune and inflammatory responses.

Result:Our proteomics data has shown that the PPRV non-structural proteins, directly interact with the NFkB p65 subunit (RelA) which regulates the immune and inflammatory responses. The PPRV V protein interacts with RHD domain of NFkB p65 and inhibit its translocalization into the nucleus and significantly downregulated its activation by 40%. However, the ISRE-luciferase assay indicated significant dose dependent downregulation via PPRV C but 89% via PPRV V. Our RT-qPCR data showed the significant downregulation of IL2,8 by both protein while Bcl2 and C-flip by PPRV V only.

Conclusion: This study established the role of PPRV C and PPRV V in downregulating the NFkB p65 activation and ISRE induction ,thus affecting the immune and inflammatory responses of cell.

P733

Visualisation of the bunyavirus RNA synthesis machinery in action

Nicholas Smith[1], Amelia Shaw[1], Owen Byford[1], Francis Hopkins[2], George Heath[1], John Barr[1], Juan Fontana[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Linköping University, Linköping, Sweden

Abstract

The Bunyavirales order of segmented negative sense RNA viruses comprises some of the most serious human pathogens in existence, including 3 listed by the WHO as priority pathogens. Their RNA genome is always associated with two viral proteins, nucleoprotein (NP) and the RNA-dependent RNA polymerase (RdRp) to form a ribonucleoprotein (RNP) complex; this complex is the bunyavirus RNA synthesis machinery. A detailed understanding of RNP structure would reveal fundamental details of bunyavirus processes, identifying targets for antiviral strategies. However, the inherent flexibility of this complex has resulted in conflicting models describing RNP architecture. Through applying a variety of electron microscopy techniques, we recently showed that the RNP of the prototypical bunyavirales member, Bunyamwera virus (BUNV), exhibits a helical architecture. This subsequently facilitated the generation of a pseudo-atomic model of the helical RNP, which in turn allowed residues important for RNP formation to be identified.

We aim to build upon this work through investigating the RNPs of other bunyaviruses, such as members of the Arenaviridae family. We will purify RNPs from these viruses and characterise their RNPs using cryo-EM and high-speed atomic-force microscopy (AFM). AFM has been successfully used to both visualise dynamic biological processes, like RNA synthesis from RNPs, as well as to provide structural information such as the handedness of helices. Combining this approach with the cryo-EM methods developed for characterising BUNV RNPs, we will determine the structure of arenavirus RNPs, including the resident RdRp, and identify fundamental details of bunyavirus processes including viral RNA synthesis and RNP assembly.

P734

Generation of filovirus pseudotyped lentiviruses, long-term stability testing and application to serological screening of wild bat sera.

Martin Mayora-Neto ORCID iD[1], Gabor Kemenesi ORCID iD[2], Fanni Foldes[2], Edward Wright ORCID iD[3], Nigel Temperton ORCID iD[1], Peter Soema[4], Rimko ten Have[4], Ivo Ploemen[4], Simon Scott ORCID iD[1]

[1]Universities of Kent & Greenwich, Chatham Maritime, United Kingdom. [2]University of Pecs, Pecs, Hungary. [3]University of Sussex, Falmer, United Kingdom. [4]InTraVacc, Bilthoven, Netherlands

Abstract

Pseudotyped viruses (PVs) are hybrid particles containing the 'core' of one virus and 'envelope' (including surface glycoproteins) from a study virus. PVs have wide application in cell tropism, receptor, antiviral, vaccine and serological studies. Recently, we utilised Lloviu filovirus (LLOV) PVs for antibody screening of sera from 83 Miniopterus schreibersii bats from Hungary, prior to the first isolation of the virus (Kemenesi et al, 2022). Later testing of >270 further samples (from 2016-22) revealed LLOV seropositivity at ~17%.

PVs are conventionally stored at -80oC and transported on dry ice. This 'cold-chain' requirement can be limiting, particularly if used in 'field' studies or low resource laboratories. Consequently, we investigated stability of filovirus PVs (LLOV, EBOV, RAVV) after lyophilisation, under various conditions (-20, +4, +22.5, +37oC) for up to 2 years. Following lyophilisation using a bench-top freeze dryer (LabConCo FreeZone), PVs were stored, reconstituted and viability measured as cell transduction titre. Results showed that at -20oC or even 4oC titre was retained at ≥90%. At 22.5oC titre was >85% after a month, whereas at 37oC (<25 or 95% humidity) or 22.5oC titre was negligible after 6 months. We also tested the impact on stability of employing a pilot scale freeze-dryer (Telstar Lyobeta) which subjected samples to additional drying steps. Data showed >85% titre retention even after 37oC for a month.

Functionality of lyophilised PVs was assessed in antibody neutralisation tests, revealing good functionality after storage for 1.5 years at 4oC and 1 month at even 37oC, utilising lab or industrial equipment respectively.

P735

Thapsigargin (TG) -mediated post-translational modifications are antiviral against Influenza A virus (IAV)

Sarah Al-Beltagi[1], Toshana L. Foster[1], Kenneth H. Mellits[2], Kin-Chow Chang[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Nottingham LE12 5RD, UK., Nottingham, United Kingdom. [2]School of Biosciences, University of Nottingham, Sutton Bonington, Nottingham LE12 5RD, UK, Nottingham, United Kingdom

Abstract

Ubiquitination and glycosylation are critical post-translational modifications (PTMs) that modulate viral infectivity and the host immune responses to infection. The key virus-host interactions and mechanisms involved in the process of PTM are potential targets for antiviral development.

TG, a sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) pump inhibitor, has been shown to be a broad-spectrum host-centric antiviral against IAV, coronavirus OC43, SARS-CoV-2 and respiratory syncytial virus (RSV). TG has been shown to target the replication cycle of IAV post-translationally.

The aim of this research was to investigate the antiviral effects of TG on IAV ubiquitination and glycosylation. Using immunoprecipitation techniques, we found that TG deubiquitinated viral M2 protein compared to control. Further, TG treatment induced a change in the sub-cellular localization of M2 viral protein in cells. We observed that TG also induces the deglycosylation of haemagglutinin (HA) and nucleoprotein (NP) IAV viral proteins, using glycosylation enrichment assays. Furthermore, TG inhibited M-gene vRNA (negative strand RNA) production that would lead to reduced viral ribonucleoprotein (vRNP) production. These findings imply inefficient viral genome packaging, thus infectious virus production defect.

Given the role of M2, HA and NP in IAV morphogenesis and release, we visualized IAV particles from TG-treated and control cells using transmission electron microscopy. We observed changes in virus morphology as a result of TG treatment, likely linked to the PTMs induced by this antiviral compound.

In summary, TG induces its host-centric antiviral defense mechanisms through PTM of IAV viral proteins, supporting its potential as future therapeutic against IAV infection.

P736

CRISPR/Cas9-based Generation of Recombinant Infectious Laryngotracheitis Virus Expressing the F and HN Protein of Newcastle Disease Virus

Julianne Vilela ORCID iD, Mustafa Atasoy, Muhammad Munir

Lancaster University, Lancaster, United Kingdom

Abstract

Newcastle disease (ND) is a highly contagious avian disease that has a substantial impact on the global poultry industry. In this study, we developed a new recombinant virus rILTV-F-HN using the infectious laryngotracheitis virus (ILTV) vector, expressing the fusion (F) protein of the genotype VII Newcastle disease virus (NDV) circulating in Egypt, and HN protein of the Newcastle disease virus (NDV) strain ZJ1. Using clustered regularly interspaced short palindromic repeat CRISPR/Cas9 gene-editing technology, the F and HN protein expression cassettes were sequentially inserted in the unique short (US) US2 and the unique long (UL) UL0 intergenic locus of the ILTV genome, respectively. The rILTV-F-HN virus, which expressed the F and HN proteins stably in vitro, showed similar growth kinetics to the wild-type ILTV (wtILTV) virus. The F and HN protein expression of the rILTV-F-HN virus was detected by Western blotting, indirect immunofluorescence assay (IFA), and flow cytometry assay. In conclusion, this novel rILTV-F-HN vaccine candidate can be used to better protect the poultry industry against future NDV infection. Further, we have developed a CRISPR/Cas9 system that lays the groundwork for future efforts to fully protect chickens against other emerging and re-emerging infectious diseases.

Session Topic: DNA viruses

P737

Merkel cell polyomavirus and Merkel cell carcinoma

Piruz Shadbash

Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Islamic Republic of

Abstract

Merkel cell polyomavirus (MCPyV) causes a very invasive but rare type of skin cancer called Merkel cell carcinoma (MCC). This virus is responsible for about 80% of MCC. Clonal integration of MCPyV DNA into the host genome is a causative factor in most MCC tumors. Early exposure to MCPyV is thought to occur in childhood based on VP1 serological testing. Primary infection with MCPyV causes no obvious signs or symptoms. MCPyV is the only polyomavirus directly associated with human cancer. Excessive sun and ultraviolet (UV) radiation exposure, reduced immunity, and advanced age are major risk factors for SCC skin cancer. Immunosuppression through immunosuppressive therapy after HIV infection or organ transplantation or autoimmune disease may also increase the likelihood of developing MCC. This article focuses on the biological properties of Merkel cell polyomavirus and Merkel cell carcinoma.

P738

Investigating the Phage, Host, Human cell interactions of T4

Daniel White

Coventry, Coventry, United Kingdom

Abstract

Bacteriophages are viruses that can efficiently infect their bacterial hosts and show promise in the treatment of bacterial infections. Recent phage research has shown that phages both interact with and are internalised by human cells and may become more efficient in targeting their host bacteria in the presence of human cells. Previous work has identified OmpC and LPS as the primary receptors for T4 with its host, E.coli, yet no receptor has been characterised between T4 and human cells. Our work aims to investigate the interactions between phage, bacterial host and human cells, by identifying receptors and genes involved and theorise the benefits to these interactions.

Confocal microscopy utilising a recombinant GFP tagged T4 phage showed the internalisation of phage by human cells and that phages are packaged into visible structures located close to the nucleus. Having shown this, a His-tagged T4 tail fibre was produced in an attempt to extract human cell receptors that may be involved with this internalisation process.

In parallel, an E.coli knockout mutant library was screened for T4 resistance to identify genes essential for T4 infection of its host, with a number genes being identified that have not previously been associated with phage resistance.

Using these results we aim to investigate the mechanisms by which T4 interacts with both its host and human cells, and the dynamics of this triangular relationship.

P739

Structural characterization of the interactions between Herpes Simplex Virus Glycoprotein C and the Complement Protein C3b

Moisés H. Rojas Rechy ORCID iD[1], Gary Cohen[2], Harvey M. Friedman[2], Juan Fontana[3]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Pennsylvania, Philadelphia, USA. [3]University of Leeds, Leeds, USA

Abstract

Herpes simplex virus (HSV) is a ubiquitous virus that can result in oral and ocular inflammation and even in fatal encephalitis (HSV-1), or in genital infections (HSV-2). HSV serotypes have developed several mechanisms to evade the immune response of the host. One of these mechanisms evades the humoral innate immune response by utilizing the glycoprotein gC, which binds to the complement protein C3b, which is key to the complement response. However, the molecular details of how gC binds to C3b, and the structure of gC remain unknown. To address this, recombinantly-expressed ectodomains of glycoproteins gC1 from HSV-1 and gC2 from HSV-2 were purified and incubated with C3b to create a complex. Complex formation was monitored using mass photometry. Subsequently, the interaction of the proteins was visualized by electron microscopy. Both gC1 and gC2 rapidly formed 1:1 complexes with C3b (within less than 5 min), which are stable for at least 30 minutes. We have imaged the 1:1 complexes by cryo-EM, and obtained a 3D map of C3b by itself, and of C3b in complex with gC1 and gC2. We are currently working in obtaining high-resolution three-dimensional cryo-EM maps to generate atomic models of both complexes. Comparing both models will allow us to understand how gC inhibits the innate immune system by binding the C3b protein. We are also interested in analyzing the differences in the binding between gC1 and gC2 to C3b. Overall, this research will provide a potential target to tackle the burden caused by HSV.

P740

In vivo and *in vitro* haemadsorption of African Swine Fever Virus Georgia 2007/1

Laila Al-Adwani, Christopher L. Netherton, Priscilla Y.L. Tng

The Pirbright Institute, Pirbright, United Kingdom

Abstract

African Swine Fever virus (ASFV) causes a contagious and lethal disease of domestic pigs and wild boars. It is a large double stranded DNA virus that replicates in the cytoplasm of macrophages. ASFV threatens food security and leads to huge economic losses. The Georgia2007/1 isolate is currently spreading throughout Europe and Asia.

Virulent ASFV demonstrates an ability to bind red blood cells (RBC) to the surface of infected cells, which is known as the haemadsorption phenomenon, and this is modulated by the CD2v transmembrane protein. Here we examine the *in vitro* and *in vivo* haemadsorption of Georgia2007/1 in relation to heterologous strains.

Viral titres in different blood fractions were determined by quantitative PCR in samples obtained from pigs at different time points after infection with Georgia 2007/1. Virus was detected in all blood fractions with the highest titre seen in the RBC fraction (80.9 ± 9.9%) five days post challenge. This demonstrates higher virus association with RBC fractions in whole blood *in vivo*. Haemadsorption was observed in cells transiently expressing CD2v and macrophages infected with ASFV, and the association of RBC and virus *in vitro* with blood samples from naïve pigs are compared to that observed *in vivo*.

This demonstrated the preferential association of Georgia2007/1 to RBCs, indicating that haemadsorption occurs *in vivo*. These results resemble previous work with genotype I E75 strain and may be associated with virus immune evasion capabilities.

P741

The mechanical transmission of lumpy skin disease virus in cattle by haematophagous arthropods incorporates insect-specific characteristics

Henry Munyanduki ORCID iD[1], Beatriz Sanz-Bernardo[1], Najith Wijesiriwardana ORCID iD[1], Ismar Haga[1,2], Petra Fay ORCID iD[1], Chris Sanders[1], John Atkinson[3], Carrie Batten ORCID iD[1], Luke Alphey ORCID iD[1,4], Karin Darpel ORCID iD[1], Simon Gubbins ORCID iD[1], Pip Beard ORCID iD[5,1]

[1]Pirbright Institute, Pirbright, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom. [3]MSD Animal Health, London, United Kingdom. [4]University of York, York, United Kingdom. [5]Keele University, Keele, United Kingdom

Abstract

Lumpy skin disease virus (LSDV) is a poxvirus that causes skin nodules, fever, weight loss and death in cattle. LSDV is endemic in Africa and currently at epidemic proportions in the Middle East and Asia, causing widespread and substantial loss to the cattle industry. LSDV is transmitted mechanically by haematophagous arthropods. Previous work in the lab has found that the virus is rapidly acquired by a range of insect species from skin nodules on affected calves, and retained on the mouthparts of arthropods for up to 8 days, suggesting an unusual form of mechanical transmission. This project used a bovine experimental model of LSD to examine the transmission of LSDV by *Aedes aegypti* mosquitoes, *Stomoxys calcitrans* stable flies, and *Culicoides nubeculosus* midges. We found that *Ae. aegypti* and *S. calcitrans* were very efficient at transmitting LSDV from cutaneous nodules on a donor calf to a naïve recipient calf when transfer of the insects occurred immediately. As few as 36 virus-positive insects were sufficient to cause disease in a recipient calf. *Ae. aegypti* but not *S. calcitrans* or *C. nubeculosus* were also able to transmit LSDV after a delay of 4 days between donor and recipient feeding. This indicates that different vector species may play different roles in the transmission of the virus, and provides quantitative data that can be used in the modelling of LSD spread.

P742

The KSHV protein ORF11 drives formation of novel paraspeckle-like condensates to aid replication and virion production

Elena Harrington, Katherine Harper, Adrian Whitehouse

University of Leeds, Leeds, United Kingdom

Abstract

Paraspeckles are nuclear-localised membraneless organelles composed of several core paraspeckles proteins around a ncRNA scaffold, with putative roles in RNA processing, RNA retention and splicing. Due to their dynamic nature and variability between cell types their full role is unknown, however, they have been implicated in several diseases including Alzheimer's and viral infection. We have investigated the formation of virus-induced paraspeckle-like condensates during Kaposi's Sarcoma-Associated Herpesvirus (KSHV) infection. Like all herpesviruses, KSHV has two distinct phases of its life cycle: lytic replication and latency. The KSHV protein ORF11 is expressed early during lytic replication and associates with large paraspeckle-like condensates that form through liquid-liquid phase separation. These novel condensates differ from canonical paraspeckles in structure, size and contents, with their formation essential for successful viral replication. TMT-LC/MS uncovered protein interaction profiles in these condensates that are distinct from canonical paraspeckle-associated proteins thus suggesting a unique role for these structures in driving KSHV lytic replication and virion production.

P743

HBcAg VLPs modified to present Affimers for antigen capture can be adapted to simultaneously present two different peptides.

Khalid Alluhaybi ORCID iD[1], Kaniz Fatema[1,2], David Rowlands[1], Nicola Stonehouse[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

VLPs self-assemble from multiple subunits of viral proteins and can be produced in different expression systems. VLPs contain no viral genetic material and may be used in multiple applications such as vaccine development. HBcAg VLPs have been extensively utilised to present different antigens at the surface of the particles. Here, we modified the HBcAg protein by genetically fusing an anti-SUMO Affimer at the N-terminus. The resulting VLP is capable of binding SUMO-tagged proteins for antigen presentation purposes. In a preliminary investigation, the ability of the anti-SUMO Affimers-HBcAg protein to assemble as VLPs and present tagged antigens was investigated. The interaction between HBcAg VLPs and SUMO-tagged proteins was monitored by ELISA and sucrose density gradient analyses. Experiments were designed to study the VLP decoration with a single peptide or with two different peptides. We found that these VLPs could present two different peptides on the same particle. However, competition studies showed that saturation binding by one SUMO-tagged peptide reduced subsequent binding of a second SUMO-tagged peptide. We are aiming to generate a second-generation capturing system for developing VLPs that can present different peptides simultaneously at different positions at the surfaces of the particles.

P744

The impact of HCMV mediated ICOSL dysregulation on DC function

Nia Cwyfan Hughes, Lauren Kerr, Ceri Fielding, Edward Wang, Richard Stanton

Cardiff University, Cardiff, United Kingdom

Abstract

Human Cytomegalovirus (HCMV) is a highly prevalent virus infecting numerous cell types, including Dendritic Cells (DCs). Little is known about the consequences of HCMV infection for DC function. Proteomics of purified HCMV infected DCs identified 99 host proteins that were downregulated at least 3-fold by HCMV infection. One of these was ICOSL, a secondary co-stimulatory molecule for T-cells.

To assess the consequences of ICOSL targeting, an assay was developed where CD4+ T-cell activation, examined by measuring proliferation, was dependent on DC interaction. Immature DCs were infected by co-culturing with infected/uninfected HFs for 24h, then DCs purified using a custom MACS pipeline. DCs were then co-cultured with naïve CD4+ T-cells which were pre-stimulated with CD3, and incorporated a proliferation dye. Cell division was assessed over 7 days.

Robust proliferation of CD4+ T-cells was seen following incubation with mock-infected DCs, however this was prevented following HCMV infection. To ensure that the inhibition was not due to viral induced apoptosis of the DCs the experiment was repeated in the presence of untreated DCs, irradiated DCs or Z-Vad (caspase inhibitor). Minimal differences were observed indicating that inhibition was more likely due to viral modulation, rather than target cell death. Finally, mutant viruses which could not modulate ICOSL were tested. A transient partial recovery was observed in the mutant virus infections at day 5 which disappeared by day 7 of co-culture.

This indicates that HCMV expresses proteins that promote survival by downregulating ICOSL which modulates DC function to reduce the induction of T-cell immunity.

P745

Separation of *T*=3 and *T*=4 assembles of hepatitis B capsid VLPs by liquid chromatography.

Pedro Martín-Reyes

University of Leeds, Leeds, United Kingdom

Abstract

Hepatitis B core antigen (HBcAg) is the capsid protein of hepatitis B virus and self-assembles into a VLP which can be modified to form a platform or scaffold to display foreign antigens. HBcAg VLPs are assembled from HBcAg dimers, with 90 (*T* = 3) or 120 (*T* =4) . Dimers assembling to form particles approximately 30 and 34 nm in diameter, respectively. The physicochemical properties of these VLPs can be used to perform a separation via chromatography, for example using net charge, size and hydrophobicity.

In this work, the VLPs were expressed in ClearColi, which is a modified *E. coli* low in endotoxin. The techniques used were cationic and anionic exchange which separates the VLPs from other contaminants based on their charge, and multimodal chromatography, which combines ion exchange and size exclusion chromatography in a single step.

This purification approach resulted in purer material than that obtained by density gradient centrifugation an allowed the separation of *T*=3 and *T*=4 particles.

P746

Detection of conformation-specific p72 polyclonal antibody responses in low virulent African swine fever virus immunized pigs.

Egle Pauletto, Christopher L. Netherton, Priscilla Y.L. Tng

The Pirbright Institute, Pirbright, United Kingdom

Abstract

African swine fever virus (ASFV) is a large double-stranded DNA virus causing acute hemorrhagic disease in swine and affecting the global swine industry; no effective vaccines or treatments are available. Among the over 150 viral proteins that ASFV genome encodes, many are still uncharacterised. The structural protein p72 requires a chaperone, pB602L, for its correct folding. We sought to characterise conformation-specific antibody responses to p72 in the sera of low-virulent ASFV vaccinated pigs challenged with virulent ASFV using a luciferase-linked antibody capture assay (LACA) and nanoluciferase tagged p72 (p72-Nluc).

Proper p72-Nluc conformation and expression ratio of p72-Nluc/pB602L was first determined by co-transfection in Veros and immunofluorescence staining with a conformation specific antibody. High levels of correctly folded proteins were found at 1:1 ratio. Lysates from HEK293T transiently expressing p72-Nluc or p72-Nluc/pB602L were incubated with pig sera. Antigen-antibody complexes were captured on protein-A-coated microplates and detected with coelenterazine.

Higher levels of p72 antibodies were detected with p72-Nluc/pB602L than p72-Nluc due to increased levels of p72-Nluc folding modulated by pB602L. Anti-p72 antibodies were first observed in most animals 7 days post-vaccination (dpv) and was the highest in the animal that developed chronic ASF on 21dpv. Antibody levels increased after peak viral titres were reached in animals that survived viral challenge.

Hence co-expression of pB602L is essential to chart antigen- and conformation-specific humoral responses to p72 with LACA. This strategy can potentially be applied on other ASFV proteins involved in viral assembly to improve exogenous ASFV protein expression and characterise antigen specific antibodies.

P747

Isolation and characterization of a novel *Klebsiella pneumoniae* phage and its anti-biofilm efficacy

Ban Abdulsattar ORCID iD, Ali Alkinani, Nadal AL-SARYI ORCID iD

Mustansiriyah University, Baghdad, Iraq

Abstract

The emergence of multidrug-resistant MDR (MDR) *Klebsiella pneumoniae* is one of the most increasing threats to public health that is involved directly in life threatening infections. With the need for effective and safe alternatives, the interest in phage therapy is increasing. Bacteriophages are a promising antimicrobial alternative that can be used to treat infections caused by MDR *K. pneumoniae*. We isolated a novel phage named ΦAYH from the sewage of Baghdad Medical City using a host from the same site. Phage ΦAYH was classified as being in the order *Caudovirales*, belonging to the *Podoviridae* family. Physical and chemical stability is assessed using the agar overlay method. The ΦAYH maintained temperature stability (-20-50) and pH (5-11). The phage host range was assessed. In addition, the phage host *K. pneumoniae* and 28 pathogenic *K. pneumoniae* isolates were tested for antibiotic resistance by the VITEK® 2 system and genotypic detection for resistance genes. The phage host was sensitive to the most antibiotics tested, expect for amoxicillin, while the pathogenic *K. pneumoniae* isolates showed resistance to the most antibiotics tested. Furthermore, biofilm formation was tested for all isolates including the host isolate. These isolates showed different ability to form biofilm, as the following: 68.97 % as weak, 27.59% as moderate, and 3.44% as strong biofilm producers. Furthermore, the phage was tested for anti-biofilm activity including the phage host. Together these results demonstrate that ΦAYH can be a promising candidate that could be used in phage therapy applications against *K. pneumonia*.

P748

Characterisation of novel mutations in African swine fever isolates taken from an outbreak in Lithuania between 2018-2019.

Tom Foster, Lynnette Goatley ORCID iD, Chris Netherton

The Pirbright Institute, Woking, United Kingdom

Abstract

African swine fever (ASF) is a lethal haemorrhagic disease affecting domestic pigs and wild boar with mortality rate as high as 100%. ASF poses a persistent and serious threat to food security and the livestock industry worldwide with this situation compounded by the lack of a commercially available vaccine against the disease.

ASF is caused by African swine fever virus (ASFV), a large double-stranded DNA virus, it has a 170-193kbp genome which mutates slowly making molecular epidemiology studies difficult. In this project, we characterised 18 Indels and SNPs in ASF-positive samples taken from an outbreak in Lithuania between 2018 and 2019.

Live virus from 13 samples was cultured, the viral DNA purified and sequenced using the Illumina platform. The resulting data was assembled into complete genomes and aligned to a well characterised isolate. We chose the 7 novel SNPs/InDels to investigate further, we then designed PCR primers to amplify these mutation sites to allow for sequencing by the Sanger method to confirm these were not artifacts of Illumina sequencing. Subsequently, the DNA of the remaining ASF-positive samples were searched for these same mutations using the same PCR primers and Sanger sequencing. The data was utilised to plot the spread of ASF throughout Lithuania.

P749

Investigating the regulatory interactome of HPV-upregulated miR-18a and its output in cancer progression

Joseph Cogan, Molly Patterson, Katherine Harper, Adrian Whitehouse, Andrew Macdonald

University of Leeds, Leeds, United Kingdom

Abstract

Human Papillomaviruses (HPV) are responsible for almost all cervical cancer cases and over half of head and neck squamous cell carcinomas (HNSCC). Due to the absence of targeted therapeutic treatment of HPV-driven malignancies, there is an urgency to understand the mechanisms exploited in HPV-host interactions. Infection of High-risk HPV (HR-HPV) drives tumorigenesis through a range of mechanisms that manipulate host cell signalling, including regulation of host miRNA expression.

miRNAs are small non-coding transcripts, with critical roles in regulation of cellular function, predominantly through direct post-transcriptional repression of target mRNAs. Each miRNA has the potential to regulate hundreds of target genes, and a great array of miRNA-mRNA interactions remain to be discovered. The integration of computational genomics into biomedical research has led to the development of a broad range of tools dedicated to the prediction of miRNA-mRNA interactions, improving the efficiency of miRNA target discovery.

miR-18a is described as an oncomiR and is upregulated in HPV+ cervical cancer cell lines, compared with HPV- cells; as well as cervical cancer tissue compared with adjacent normal tissue. It is known that miR-18a directly suppresses several tumour suppressors, consistent with neighbouring members of the miR-17-92 polycistronic cluster. Bioinformatics analysis indicated 280 potential direct targets of miR-18a with over-representation of cancer-associated biological pathways, including tissue remodelling, regulation of mitotic cell cycle, and ERBB signalling. Moreover, many of these targets inversely correlated with miR-18a expression. Our study interrogates the miR-18a interactome and aims to explore the large-scale role of aberrant miR-18a activity in cancer progression.

P750

The functional linkage of EBNA2 and EBNA3 in B and T cell infection

Wiyada Wongwiwat, Christine Styles, Claudio Elgueta, Stephanie Tye, Paul Farrell, Rob White

Imperial College London, London, United Kingdom

Abstract

A major element of Epstein-Barr virus (EBV) genetic diversity – type 1 vs type 2 – is concentrated in the EBV nuclear antigens EBNA2 and EBNA3 (but not intervening regions of the genome). Type 2 EBV is less efficient at B cell transformation, prevalent in equatorial regions, and reported to infect T cells as well as B cells. EBV strains with a mixture of type 1 and type 2 sequences are rare. We hypothesised a functional linkage between EBNA2 and EBNA3 of the same type.

We generated recombinant B95-8-based EBVs carrying different combinations of type 1 and type 2 EBNA2 and EBNA3 loci, and compared these with type 1 (B95-8) and type 2 (Jijoye) BAC-cloned EBVs. During B cell infection, EBVs with a type 1 EBV showed better transformation efficiency from about day 8 post infection. Pre-activating B cells with CD40L prior to EBV infection disproportionately improved the transformation by viruses with a type 2 EBNA2. Attempts to infect adult T cells did not produce appreciable GFP expression or T cell proliferation. Cord Blood T cells could be infected by both type 1 and type 2 EBVs.

Preliminary transcriptome analysis of LCLs carrying these viruses allows type-dependent attribution of regulation to either EBNA2 and EBNA3. LCLs expressing a type 2 EBNA2 exhibited elevated expression of EBV lytic genes. Finally, some host genes were regulated differently when the EBNA2 and EBNA3 types were matched, compared to when they were mismatched, implying that certain co-operative EBNA2 and EBNA3 functions require type-specific co-ordination.

P751

Utilising human induced pluripotent stem cells (iPSCs) to investigate HSV-1 infections in neurones

Daniel Nash ORCID iD[1], Alex Nicholson[2], Janet Deane ORCID iD[2], Colin Crump[1], Stephen Graham ORCID iD[1]

[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom

Abstract

Herpes simplex virus (HSV)-1 is a neuroinvasive human pathogen that persists in the sensory ganglia of infected hosts and can cause severe disease when it spreads to the central nervous system. Herpes simplex encephalitis is the most common viral encephalitis, with severe sequelae despite the availability of direct-acting antivirals, and HSV-1 infection has been linked to the development of Alzheimer's disease. Induced pluripotent stem cell (iPSC) technology allows culture of authentic human neurones in vitro, facilitating the study of neuronal infection and providing an experimental platform for developing new prophylaxes and antiviral therapies. We are studying HSV-1 neuronal infection using modified human iPSCs that express neurogenin-2 (NGN2) under the control of a doxycycline inducible promoter, which facilitates their complete and rapid differentiation into mature human glutamatergic cortical neurones. This integrated, inducible, and isogenic (i3) neurone system allows for the rapid production of neurones at scales suitable for proteomic, biochemical, and functional analysis. We will use these cells to monitor the kinetics of wild-type and mutant HSV-1 replication and cell-to-cell spread. We will also use quantitative proteomics to study how wild-type and mutant HSV-1 isolates change the neuronal whole-cell proteome during infection. These proteomic experiments will identify key innate immune restriction factors that are either upregulated or downregulated upon infection, and may illuminate proteomic similarities between HSV-1 infection and Alzheimer's disease and related dementias.

P752

A novel antiviral effector pathway revealed by spatial proteomics

Marisa Oliveira[1,2], Ben Ravenhill[1,2], George Wood[3], Betty Chung[3], Mike P. Weekes[1,2]

[1]Cambridge Institute for Medical Research, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Abstract

Cellular sensors and their signalling components form a crucial first step in the recognition of intracellular pathogens, determining the outcome of infection by orchestrating effective immunity. Cytoplasmic-to-nuclear transition of terminal signalling components such as IRF3 and NF-kB underpins sensing pathways for viruses, bacteria, fungi and parasites. The discovery of related, novel signalling pathways will provide key new insights into intrinsic immunity and transform our understanding of how pathogen recognition triggers immune gene expression. A detailed mechanistic understanding of known sensing/signalling components has led directly to the development of vaccine adjuvants, insights into autoimmune disease and treatments for chronic inflammation and cancer. Many viral signalling proteins exhibit subcellular relocalisation upon activation, triggering expression of interferon and antiviral genes. To identify novel sensing/signalling components, we have developed innovative proteomic methodologies to globally quantify subcellular protein redistribution in virusinfected cells. Using Sendai virus infection of human fibroblasts, we identified CREB Regulated Transcription Coactivators-2 and 3 (CRTC2/3) a key novel 'hits' that translocate from cytoplasm to nucleus in addition to IRF3/NF-kB. CRTC2/3 also translocate during DNA virus infection, suggesting a common, novel cytosolic nucleic acid response. We have now identified a subset of CRTC2/3-dependent genes induced by viral infection. These include IL-11, an understudied cytokine acting via the same intracellular pathway as IL-6. Excitingly, IL-11 stimulation leads to expression of proteins playing important antiviral roles. By combining advanced proteomics with transcriptomics and cell biology, we have thus identified a new arm of the signalling and effector response to foreign nucleic acids.

P753

An improved strategy for cloning Epstein-Barr virus genomes.

Anrong Li, Amr Bayoumy, Mohammed Ba abdullah, Luke Granger, Rob White

Imperial College, London, United Kingdom

Abstract

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus with a dsDNA genome of around 175 kb. EBV can transform resting B cells to become lymphoblastoid cell lines (LCLs) where it persists as an often multi-copy circular episome. However, because producing infectious EBV from authentic strains is challenging, lab strains are mutated or cancer derived, so may exhibit atypical biology.

We aim to develop a strategy for reliably cloning and modifying authentic EBV strains that produce authentic infectious progeny. The Kanda lab used a Cas9 expression vector to cut EBV and promote homology-directed repair to insert a BAC between the BVLF1 and BVRF1 ORFs, which was selected by outgrowth in antibiotics. We found this strategy produced unstable BAC clones, and did not work for cloning authentic strains from spontaneous LCLs.

The following modifications improved the capture of Jijoye EBV, while minimising the time for cloning, avoiding antibiotic selection, and improving BAC stability: i) Recoding the BVRF1 and BVLF1 coding sequences avoided duplicated sequence destabilising the BAC clones; ii) improved electroporation protocol; iii) Incorporating the Cas9 target sequence to linearise the capture vector increased capture of EBV genomes; iv) Using a biotinylated EBNA1 DNA-binding domain to pull-down EBV genomes depleted unused capture vector from genomic DNA extracts, allowing recovery of cloned Jijoye-BACs from cells 4 days after transfection. We are currently testing this protocol in spontaneous LCLs, assessing whether ribonucleoprotein transfection is better than the cas9 expressing plasmid, and whether loxP sites allow excision of the BAC from the infectious EBV.

P754

GENERATING AFRICAN SWINE FEVER VIRUS PSEUDOTYPES TO STUDY CELL ENTRY

George Hooper[1], Edward Wright[1], Christopher Netherton[2], Thomas Foster[2]

[1]University of Sussex, Falmer, United Kingdom. [2]Pirbright Institution, Pirbright, United Kingdom

Abstract

Outbreaks of African swine fever (ASF) in domestic pigs are highly lethal and can spread rapidly through the herd. The exceptionally high mortality rate is in part because the virus replicates to high titres before the host can mount an effective immune response. The One Health threat is compounded by the fact that currently there are no approved treatments or vaccines, although, several are in development. Therefore, the virus has caused the deaths of millions of pigs and massive economic losses, crippling the pig industry and threatening biodiversity.

The causative agent is a large, complex DNA virus that is vectored by long lived soft bodied ticks. Our understanding of ASFV has improved thanks to modern technological advances, however crucial questions remain unanswered, such as which viral and cellular factors are involved in cell entry. We have sought to address this by attempting to generate pseudotyped viruses (PV) to study this stage of replication.

Based on genomic analysis and published data, a set of ASFV proteins potentially involved in cell entry were cloned. Their expression was assessed by western blot and their incorporation onto the surface of PV by infection studies.

ASFV pseudotypes would make it possible to identify host cell and viral factors involved in cell entry. This information is important for researchers designing the much needed treatments and vaccines to limit the impact this disease has in domestic pigs and on economies worldwide.

P755

CIAlign, a software tool for processing multiple sequence alignments, and its application to microbial data.

Katy Brown ORCID iD, Charlotte Tumescheit ORCID iD, Andrew Firth ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

Many applications of multiple sequence alignments (MSAs) involve working with sequences which are not ideal. Sequences can be incomplete, contain errors or be highly divergent with many mismatches. This is very common when working with high throughput sequencing data. Microbial data can be particularly prone to these types of issues, for example when working with highly divergent viral alignments, data from non-model organisms or with complex mixed metagenomic or microbiome datasets. MSAs based on these sequences often contain many gaps and areas of low quality alignment. It's still common to manually edit MSAs before performing further analysis but this method is time-consuming and not easily reproducible.

CIAlign is a command line tool we have developed to clean, interpret and visualise multiple sequence alignments (MSAs). The original version of the tool, released in 2022, includes functions to remove non-majority insertions, poorly aligned sequence ends, highly divergent sequences and short sequences from MSAs. It can also be used to visualise alignments efficiently and to produce sequence logos, distance matrices and quality control plots. We have now added a number of additional functions. For example, CIAlign can identify and isolate conserved regions of an alignment, generate position weight matrices and output various measures of sequence conservation and alignment quality.

P756

Comprehensive quantitative temporal proteomic and interactome analysis of mpox

Xinyue Wang[1,2], Martin Potts[1,2], Yongxu Lu[3], Samuel Richardson[4], Charlotte Cook[4], Dagmara Bialy[4], Pip Beard[5], Bryan Charleston[4], Geoffrey L. Smith[3], Jonas D. Albarnaz[1,2], Michael P. Weekes[1,2]

[1]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom. [2]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [3]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [4]Pirbright Institute, Woking, United Kingdom. [5]School of Life Sciences, Keele University, Staffordshire, United Kingdom

Abstract

Mpox is a zoonotic disease caused by monkeypox virus (MPXV), which is endemic in Africa. However, since May 2022, a multi-country outbreak of mpox has affected >80,000 individuals with sustained human-to-human transmission. In the UK, 3,725 cases (98.6% males) have been infected by the end of November 2022, affecting mostly men who have sex with men. Although prophylactic and therapeutic measures, including immunisation with the smallpox vaccine and antiviral chemotherapy with Tecovirimat, have been implemented, those measures are not widely available and accessible in most countries. Resistance to Tecovirimat has been detected and reinforced the urgent need to study mpox infection in human cells in order to develop novel therapies. The outcome of infection is partly determined by the interplay between host antiviral restriction factors (ARFs), which can inhibit viral entry, replication or exit from an infected cell, and their antagonism by one or more viral proteins. Many viruses have evolved to subvert cellular degradation pathways to degrade ARFs. Here, we will present a quantitative temporal proteomic analysis of MPXV infection in human fibroblasts, including the use of proteasome inhibitors to identify degraded ARFs. We will also present the interactome so far of all 191 MPXV proteins by affinity purification-mass spectrometry, which we are using in particular to uncover viral proteins inducing the degradation of host factors. Overall, this study will provide deeper insights into how MPXV interacts with human antiviral defences, and may ultimately offer routes for generation of novel therapeutics against mpox.

P757

Ackermannviridae phage host range engineering

Tomás Kappes-Reveco ORCID iD, Jessica Bergman, George P. C. Salmond

University of Cambridge, Cambridge, United Kingdom

Abstract

Bacteriophages are considered the most abundant biological entities on earth and powerful evolutionary driving forces in their bacterial hosts. Tailed bacteriophages have been intensively studied, and they rely on tail spikes or tail fibres to recognise their cognate host receptors when adsorbing to bacteria. This adsorption step is clearly crucial for the infection process, and correspondingly, it is also a key factor dictating host range specificity.

Ackermannviridae phages are a recently reclassified family of tailed phages previously included in the Myoviridae family. They include virulent phages with dsDNA genomes of ~150Kb, and some can exhibit generalised transduction capacity. Representative members of this family have been isolated on different Gram-negative bacteria. The most common hosts for these viruses are members of the Enterobacteriales order, including animal and plant pathogens such as Salmonella, Escherichia, Klebsiella, Shigella and Dickeya. Although there has been limited analysis of Viunalikevirus receptors, the primary receptor of the defining example of this viral group (the Vi01 phage) in Salmonella Typhi has been defined as a capsular polysaccharide (CPS).

We investigated phage-host interactions between the enterobacterial CPS and Salmonella phage Vi01 adsorption and replication. Using a synthetic vector, we heterologously expressed the Vi01 receptor in a range of Gram-negative bacterial species. This system enabled promiscuity of phage infection and transduction capacity in particular bacterial strains across multiple species and taxonomic orders.

P758

Streptococcus suis as a Model for the Phage-Based Biocontrol Agents

Emmanuel Osei ORCID iD[1,2,3], John Kenny ORCID iD[1,2], Jennifer Mahony ORCID iD[2,3], Reuben O'hea[3], Edgar Garcia Manzanilla ORCID iD[1]

[1]Teagasc Food Research Centre, Fermoy, Ireland. [2]APC Microbiome Irelalnd, Cork, Ireland. [3]University College Cork, Cork, Ireland

Abstract

Streptococcus suis is a major pathobiont of pigs, and a significant source of economic losses in the pig sector and has proven recalcitrant to vaccine development. Phages are increasingly considered as a suitable measure to reduce bacterial infections and contamination in the food industry. We aimed to isolate and characterise virulent phages against *S. suis* and explore the therapeutic potential of *S. suis* prophages using novel synthetic biology approaches.

To date, only one phage has been characterised against *Streptococcus suis*. We screened pig saliva and post-mortem tissue samples collected from 20 farms across Ireland against a panel of 25 *S. suis* strains including the commonly reported zoonotic serotypes 1/2, 2, 9 and 14. Three novel phages were isolated which infect pathogenic strains of multiple *S. suis* serotypes. Further characterisation of these phages was performed, including host range and temperature and pH stability, efficiency of plating, growth kinetics, genome sequencing and electron microscopy. Moreover, genomics approaches predicted 501 prophages from 133 *S. suis* genomes. Of these, 71 full-length prophages were identified, which encode genes representing a template for the development of therapeutic phages or enzybiotics.

Further characterisation (mass spectrometry, determination of RBP, interactions in simulated swine nasopharyngeal microbiome) of the newly isolated phages is underway. This data will be combined with engineering approaches to generate lytic phages from the predicted prophages, and to explore phage host interaction in *S. suis*. Overall, we report phages that have the potential to be applied in the development of phage-based therapeutics to combat swine infections.

P759

The development and optimization of a simple screening assay for in vitro anti-MPXV activity

Diogo Correa Mendonca, Nicole Upfold, Marion McElwee, Sergi Molina Arias, Agnieszka Szemiel, Arvind Patel

University of Glasgow, Glasgow, United Kingdom

Abstract

In May 2022 the WHO declared Mpox disease as an international public health emergency, as the virus spread across Europe, North and South America. Currently, two FDA-approved drugs, Cidofovir and Tecovirimat, are approved for the emergency treatment of Mpox . Both target the virus directly and resistance to these compounds is well described, making the discovery of new drugs desirable.

As part of an Mpox drug discovery strategy we tested three different assays: a) Cytopathic effect assay (CPE), b) CellTiter-Glo assay (CTG) and c) MTS assay and compared their effectiveness in measuring MPXV induced viral effects on the cell.

The CPE assay proved to be highly sensitive and was able to detect an initial viral inoculum of 160 PFU/well. The CTG assay was not able to differentiate between infected and uninfected cells and the MTS assay could only distinguish the highest viral inputs (20000 and 4000 PFU/well) from mock-infected cells.

We then analysed eight different host-targeting compounds plus Tecovirimat as an assay control, in Vero E6 cells infected with MPXV at an MOI of 0.1. Tecovirimat had an IC50 of 2nM in the CPE assay, which was comparable to the IC50 values reported in the literature. Ibrutinib, a tyrosine kinase inhibitor, was active against MPXV with an IC50 of 3.9 µM.

Further screening will be carried out with a larger library of host-targeting compounds to identify possible compounds using the CPE assay which may contribute to the discovery of new anti-MPXV drugs.

P760

TRIM25, ZAP and KHNYN restrict VACV and are targeted for proteasomal degradation

Yongxu Lu ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

E3 ubiquitin ligase TRIM25 has important roles in innate immunity, such as the restriction of viral RNA expression via co-operation with ZC3HAV1V (ZAP). ZAP targets viral RNAs with high CpG in the cytoplasm to inhibit virus replication. TRIM25 also regulates the function of ZAP's binding partner KHNYN, a ribonuclease that is implicated in degradation of RNA targeted by ZAP. VACV infection induces the degradation of TRIM25, ZAP and KHNYN in a proteasome-dependent manner, suggesting these factors might have anti-VACV activity. To test this we made HEK293T and HeLa cell lines lacking these proteins separately, and found that VACV plaque size and infectious virus titre are enhanced in all these cells. Complementation of the missing protein reversed this phenotype and, conversley, overexpression of ZAP or KHNYN reduced VACV plaque size and virus yield. To investigate how VACV mediates degradation of these proteins we used VACV mutants lacking blocks of genes from near the genomic termini. One mutant, vSSK2, was unable to degrade KHNYN suggesting the virus protein responsible for KHNYN degradation is located near the right end of the genome. The mechanism of VACV restriction by KHNYN, TRIM25 and ZAP are being investigated.

P761

Small animal modelling of OvHV-2: towards a Vaccine for Malignant Catarrhal Fever.

Eleanor Bentley[1], Mohammed Al-Saadi[1], Joseph Angel[1], Rosalie Fabian[2], Anja Kipar[2], James Stewart[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Univeristy of Zurich, Zurich, Switzerland

Abstract

Ovine herpesvirus-2 (OvHV-2) is the causative agent of sheep associated malignant catarrhal fever (MCF), a generally fatal disease with a wide range of hosts including but not limited to cattle, buffalo and bison. Sheep largely present asymptomatically so act as a reservoir for OvHV-2. Viral transmission to MCF susceptible hosts in mixed farming systems and thereby sporadic MCF is a constant threat to food production. No specific treatments exist and empirical treatments are largely ineffective. Currently there is no vaccine licensed for the prevention of OvHV-2, although the urgent need is recognised. Large animals are physically and financially impractical for modelling disease in vivo, however the susceptibility of rodents to OvHV-2 has been previously described.

Syrian golden hamsters were infected with lymph cells from a hamster previously infected with OvHV-2. Body temperature and behaviour of the hamsters was monitored. Hamsters were culled following a sustained increase in temperature, and/or clinical signs.

A second cohort of hamsters was given either the OvHV-2 vaccine, WT MVA or PBS, then challenged with OvHV-2. Again, they were monitored daily and culled following a sustained increase in temperature and/or clinical signs. Tissues and blood were collected for histopathological examination, OvHV-2 quantification by qPCR and antibody titre by ELISA.

Hamsters readily became infected with OvHV-2 when inoculated with lymphatic tissue cells. Multifocal T-cell infiltrates were observed in multiple tissues. OvHV-2 viral load varied across different tissue samples. We observed no ittle protective effect from the vaccine to OvHV-2 infection.

Session Topic: Positive strand RNA viruses

P762

Comparison of the virological and immunological responses during mild and moderate porcine respiratory coronavirus *in vivo* infection

Isabella Atkinson, Veronica Carr, Sarah Keep, Albert Fones, Erica Bickerton, Elma Tchilian

The Pirbright Institute, Woking, United Kingdom

Abstract

The spectrum of outcomes following SARS-CoV-2 exposure is wide and largely determined by the host response. Currently mouse, ferret, hamster and non-human primate models have been developed to study SARS-CoV-2 but these are not natural host species for infection. In contrast pigs are natural hosts for several coronaviruses. Similarly, to SARS-CoV-2, infection can be mild or asymptomatic, but in some instances can lead to severe lung damage. To determine what leads to a clearance of infection or tissue damage, a porcine respiratory coronavirus (PRCV) model was developed to compare the immune responses to strains of PRCV which cause mild or severe lung pathology. Outbred and Inbred Babraham pigs were inoculated with either PRCV strain 135, previously shown to cause severe lung pathology, or with ISU-1, that causes mild lung pathology. Blood was taken weekly and staged *post mortem* performed at days 1, 5, 11 and 21. The presence of PRCV specific antibodies in sera and bronchoalveolar lavage fluid (BAL) was examined by ELISA and neutralization. We also used overlapping peptides in the inbred Babraham pigs to define the epitope specificity of the T cell response and determine if the specificity is different after infection with pathogenic and apathogenic PRCV. The long-term aim of this work will be to understand the coronavirus genomic changes that result in enhanced disease and to understand local and systemic protective immune responses to respiratory coronaviruses.

P763

Three amino acid changes within the spike protein of infectious bronchitis virus confer replication in Vero cells.

James Kirk, Phoebe Stevenson-Leggett, Sarah Keep, Erica Bickerton

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Infectious bronchitis virus (IBV) is an economically important coronavirus of chickens, causing reductions in egg production and quality. Live attenuated vaccines for IBV are created using serial passage in embryonated hens' eggs, this is expensive and time consuming. The molecular method of attenuation is unknown and vaccines can revert to virulence, demonstrating a need for rationally designed vaccines not reliant on the supply of embryonated eggs.

Most IBV strains exhibit restricted tropism *in vitro*, unable to replicate in continuous cell lines. Beaudette, an attenuated laboratory strain, exhibits extended tropism and can replicate in Vero cells, a mammalian cell line, licensed for vaccine production.

We have previously identified amino acid residues within the spike (S) protein involved with Beaudette's extended tropism known as the Beaudette specific motif (BSM). The three amino acid changes Pro687Arg, Arg689Lys and Phe692Leu were made in the S protein of M41-K, a molecular clone of the pathogenic strain M41-CK, generating the recombinant IBV M41K-BSM. The growth kinetics of M41K-BSM were assessed in primary chicken kidney (CK) cells and Vero cells.

In CK cells, M41K-BSM replicated similarly to Beau-R, a molecular clone of Beaudette-CK, and M41-K. In Vero cells, unlike M41-K, M41K-BSM exhibited sustained productive replication over a 96-hour period demonstrating that the BSM is enough to confer Beaudette's ability to replicate in Vero cells. Titres however, were lower suggesting other factors are involved. This demonstrates the potential for *in vitro* vaccine propagation which is more efficient and cost effective than serial passage in embryonated eggs.

P764

Proteolytic cleavage orchestrates the formation and function of the astrovirus replication complex

David Noyvert, Valeria Lulla

University of Cambridge, Cambridge, United Kingdom

Abstract

Astroviruses are positive-sense, single-stranded RNA viruses infecting both avian and mammalian species. Classical human astroviruses are common, enteric pathogens, affecting young children and presenting mild gastroenteritis symptoms. Recently, two novel strains of human astroviruses, MLB and VA, were shown to possess extraintestinal pathogenicity and demonstrate neurotropic features, with reported infections of the central nervous system. The replication cycle of astroviruses begins with translation of non-structural polyproteins nsp1a and nsp1ab from the VPg-linked genomic RNA. The polyproteins are cleaved by the viral serine protease, releasing proteins for genomic and subgenomic RNA replication to initiate structural protein synthesis. Cleavage sites for nsp1a have been predicted but not experimentally confirmed and the subsequent processing products have not been characterised. We aim to map the cleavage sites to study the processing dynamics in HAstV1 and MLB2 astrovirus strains. We combine mass spectrometry with the detection of processed and full-length virus-specific products at different stages of infection to experimentally validate nsp1a processing. To investigate the importance of the identified functional elements in the virus life cycle, we use astrovirus replicon and reverse genetics systems. This work advances our understanding of astrovirus non-structural polyprotein cleavage and may inform on future drug targets and vaccine candidates.

P765

Variation in antibody responses following prime-boost-boost vaccination regimes against avian coronavirus

Phoebe Stevenson-Leggett ORCID iD, Kristel Ramirez, Marie Di Placido ORCID iD, John Hammond, Erica Bickerton ORCID iD

The Pirbright Institute, Woking, United Kingdom

Abstract

Infectious bronchitis virus (IBV) is a highly contagious Gammacoronavirus causing substantial losses in the global poultry industry due to reductions in egg production and meat quality. Live-attenuated vaccines are used to protect against IBV but they offer poor cross-protection between the many different co-circulating strains and newly emerging serotypes of IBV. There is a drive to develop more cross-protective vaccines.

Chickens were vaccinated with heterologous strains from three serotypes of IBV, at 21-day intervals, in two different orders: H120:CR88:QX (Group 1) or CR88:H120:QX (Group 2). Total serum IBV antibodies were measured by ELISA and neutralising antibodies against a range of IBV strains were assessed by neutralisation assay in cells and ex vivo tracheal organ cultures.

A significant boost in total IBV antibody levels was observed following two vaccinations, but not after three, along with a high level of variation between individual animals in Group 1. Neutralising antibodies against each vaccine strain were detected in the majority of birds in both groups, with more variation observed in antibody titres against H120 and QX compared to CR88. Cross-neutralisation of an additional heterologous strain (Ark99) was observed in one bird after three vaccinations. Overall, vaccination with heterologous strains of IBV induced a neutralising antibody response with high levels of variation between birds. Antigen-specific B cells will be isolated from these birds and antibodies will be sequenced using tools and techniques developed by Pirbright Antibody Hub for antigen-specific antibody discovery in multiples species to identify epitopes involved in cross-protection, informing better IBV vaccine design.

P766

Arrayed interferon-stimulated gene expression screening reveals genes with candidate antiviral activity against HCoV-OC43 infection

Emma L. Davies, Arthur Wickenhagen, Matthew L. Turnbull, Douglas G. Stewart, Suzannah J. Rihn, Massimo Palmarini, Sam J. Wilson

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

The COVID-19 pandemic, as well as the SARS and MERS outbreaks, highlight the consequences of coronavirus transmission within the human population. Since the presence and timing of a functional interferon (IFN) response is important in controlling coronavirus infection, identifying interferon-stimulated genes (ISGs) with antiviral activity can provide insights into genetic risk factors associated with coronavirus disease severity and into the barriers to CoV zoonosis. To identify ISGs that inhibit wild-type coronaviruses, we optimised an arrayed ISG expression screening protocol that involves immunostaining of the dsRNA replication intermediate with quantification of virus infection by image cytometry. The endemic coronavirus HCoV-OC43 was screened against a library of >500 human, >300 macaque and >250 bovine genes encoded by lentiviral vectors. This revealed ISGs with known antiviral activity against coronaviruses, including HCoV-OC43, as well as ISGs not previously shown to be involved in HCoV-OC43 infection. The ability of these hits to cause cytotoxicity and/or stimulation of IFN-stimulated response elements was determined to exclude non-specific inhibitors of HCoV-OC43. Further investigation into the specificity and mechanism of action of these ISGs of interest will be carried out. Understanding the mechanisms by which ISGs specifically inhibit CoVs could provide new information on interactions of both endemic and emerging coronaviruses with the innate immune system and can help inform future therapeutic approaches.

P767

Formulation and Dose Validation of Foot and Mouth Disease virus Vaccine in Buffalo Calves using Endemic Field Strains

Qaiser Akram, Ahsan Naeem Muhammad Naeem, Waqas Ahmad

university of veterinary and Animal sciences, Lahore, Pakistan

Abstract

Foot and Mouth Disease (FMD) is a viral infection of cloven hoofed animals and A, O and Asia-1 are its most prevalent serotypes in Asia. Mass vaccination is the only possible option. Hence, the present study was conducted, keeping the poor economic countries situation, by collecting and identifying local field strain, preparation of vaccine and dose validation by analyzing antibody response at various doses. Vesicle samples from field were collected, viruses were isolated and strains were identified through PCR (A, O and Asia-1 were identified actually). BHK-21 cell line was grown in roller culture bottles. Virus of each strain was cultivated on monolayer of BHK-21 cells. Binary ethylene amine was used to inactivate viruses and emulsified in Montanide ISA-70. Each strain was divided into 3 $TCID_{50}$ categories i.e. $1x10^{6.2}$, $2x10^{6.2}$ and $3x10^{6.2}$. Trivalent vaccine of each $TCID_{50}$ category was formulated and inoculated in animal of respective group. Booster doses were given after 30, 60, 90 and 180 days. Complement fixation test was applied to get humoral response or antibody titer by geometric mean in $\log_2$ form In the experiment, each of the virus serotypes (O, A and Asia-1) showed high infectivity titer (more than 10^7 units of $TCID_{50}$) on BHK-21 cells. $2x10^{6.2}$ vaccine dose is considered promising and its booster given after 2 months provide complete humoral protection for 6 months.

Key words: Serotypes, Foot and Mouth Disease, Formulation, Immunogen, Validation

P768

Investigating the interaction between IBV accessory protein 3a and the interferon signaling cascade

Anna-Mariya Anastasova[1], Diana Attasseril[1], Ross Hall[1,2], Nicole Doyle[1], Helena J. Maier[1]

[1]The Pirbright Institute, Pirbright, United Kingdom. [2]The Francis Crick Institute, London, United Kingdom

Abstract

Infectious bronchitis is a highly contagious disease of domestic fowl that causes significant economic loss to the global poultry industry. The disease is caused by infectious bronchitis virus, a member of the Coronavirus family. A better understanding of the molecular biology behind the virus is required to reduce the consequences of the disease. The viral genome of IBV encodes for five accessory proteins, with accessory protein 3a implicated in both stimulating and inhibiting interferon expression. The mechanism of action of this viral protein is still unknown. We aim to investigate the interplay between host and viral proteins in IBV infection. Using a luciferase assay, we have shown that 3a acts upon various stages in the interferon signaling cascade in a dose-dependent manner. We have also identified host proteins which interact with both 3a and the interferon response. CAND1 has become the research target due to its regulatory role in the cascade. We investigated the interaction between 3a and CAND1 in the cell and the role of CAND1 in 3a regulation of interferon induction. The interferon signaling cascade is the primary response to viral infection. Identifying research targets that counter the effect of viral proteins on the cascade, such as CAND1, is a vital step forward in combating IBV.

P769

Investigating the role of non-canonically transcribed sgmRNAs during avian coronavirus replication.

Harry Francis, Sarah Keep, Isobel Webb, Erica Bickerton

The Pirbright institute, Woking, United Kingdom

Abstract

Coronaviruses translate their structural and accessory proteins from subgenomic mRNAs (sgmRNAs) produced via discontinuous transcription. The model of discontinuous transcription relies on identical transcription regulatory sequences (TRS) located at the 5' end of the genome (TRS-L) and at the 5' end of each structural or accessory gene (TRS-B). Sequence similarity between the TRS-L and TRS-B is thought to be necessary to produce sgmRNA. Previous work on infectious bronchitis virus (IBV), identified two sgmRNAs denoted 4b and 7 produced from TRS-Bs with far less similarity to the TRS-L, termed non-canonical TRS-Bs. We have focused our research on sgmRNA 4b which encodes two proteins, 4b and 4c. In this study we investigated the conservation of 4b and 4c between strains of IBV and identified similarities in the amino acid sequence. We deleted sgmRNA 4b from the pathogenic IBV M41 to investigate the effects of 4b and 4c on viral replication and clinical disease. Experiments looking at replication in vitro showed that replication of the 4b and 4c deletion mutant was comparable to the parental unmodified IBV. Studies in vivo showed that chickens infected with the 4b and 4c deletion mutant presented with clinical signs later than those infected with unmodified IBV suggesting a role for both 4b and 4c during in vivo infection. Our results suggest that the presence of sgmRNAs transcribed from non-canonical TRS-Bs offer a mechanism to increase the repertoire of accessory proteins generated, ones that may play yet undefined roles in IBV pathogenesis and in vivo replication.

P770

Evidence for transmission of Japanese Encephalitis virus (JEV) genotype I in British mosquitoes and the potential effect of climate change

Luis M. Hernandez-Triana ORCID iD, Sanam Sewgobind ORCID iD, Insiyah Parekh, Arran J. Folly, Nicholas Johnson ORCID iD, Karen L. Mansfield ORCID iD

Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

Japanese encephalitis virus (JEV) is the principal cause of human viral encephalitis in Asia and can cause reproductive disease in pigs. As a result of climate change, higher UK average temperatures and increased flooding has enabled mosquito populations to flourish, increasing the risk for establishment of previously undetected viruses in UK mosquitoes.

In this study, we assessed the vector competence of UK Culex pipiens mosquitoes for JEV genotype I. Artificial blood-feeding was used to expose groups of mosquitoes to JEV (strain UVE/JEV/ 2009/LA/CNS769), maintained at either 21°C, representative of current average summer temperatures, or 25°C, representative of a projected increase. At 14 days post-infection (dpi), virus infection and dissemination were analysed by specific RT-PCR and confirmed by virus isolation. Transmission potential was assessed by detection of virus in saliva.

At 21°C, the blood feeding rate was 60.5% (26/43), however no specimens demonstrated virus infection, dissemination or transmission at 14 dpi. At 25°C, the blood feeding rate was 58.6% (36/62). Virus infection was detected in 13% mosquitoes (5/36)) and dissemination in 50% of these specimens. Additionally, virus was detected in 66% of mosquito saliva samples in which dissemination had occurred, confirming potential for virus transmission in this species.

Culex pipiens is a competent vector for JEV lineage I. However, this appears constrained by temperature with transmission occurring at the higher temperature of 25°C. This data provides further evidence for the impact of climate change on the risk to animal and human health in the UK.

P771

Finding host proteins required for coronavirus replication organelle formation and function

Nicole Doyle[1], Anna-Mariya Anastasova[1], Pippa Hawes[1], David Matthews[2], Andrew Davidson[2], Helena J. Maier[1]

[1]The Pirbright Institute, Pirbright, Woking, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Avian infectious bronchitis virus (IBV), is a gammacoronavirus which infects poultry and is a significant pathogen to the poultry industry, causing a substantial economic impact. As in all positive strand RNA virus infections, IBV rearranges host cell intracellular membranes, forming replication organelles. Replication organelle formation is a crucial and highly conserved step in the viral life cycle. In previous work, we showed that sites of coronaviral RNA synthesis are located within membrane-bound compartments in the cell, however relatively little is known about the cellular proteins involved in the formation and function of the replication organelles. To further our understanding, we fractionated infected and mock-infected cells, enriching for replication organelles and therefore the host cell proteins located at membranous replication organelles. Mass spectrometry analysis of cellular proteins found associated with the membrane fraction has identified proteins involved in the antiviral response in addition to proteins involved in the regulation of transcription, translation, membrane transport, cell signalling and cytoskeletal components. Work is underway to validate findings, to determine the role of identified proteins in the viral life cycle, as well as to compare these findings across other coronavirus genera. Adding to our knowledge of the replication and host cell interactions of these viruses is imperative to effectively find ways to manage them.

P772

Fluorescent replicons and trans-encapsidation systems for the investigation of Enterovirus entry and replication. Mamma Mia - Here we glow again: Enterovirus Trans-encapsidation assays.

Philippa Hall ORCID iD, Natalie Kingston ORCID iD, Nicola Stonehouse, David Rowlands

University of Leeds, Leeds, United Kingdom

Abstract

Enteroviruses (EVs) are a family of human and animal pathogens, which pose a significant threat to global health, with infections resulting in a range of clinical manifestations from hand, foot and mouth disease to severe neurological complications, such as acute flaccid myelitis. Notably, a number of large outbreaks caused by EVA71 and EVD68, and the re-emergence of the prototypal poliovirus (PV), have highlighted the need to better understand this family of viruses. Currently, licensed antiviral therapies are lacking against EVs. Therefore, developing tools to study EV lifecycles may help to identify novel targets for the development of future antiviral compounds.

To this end, we have generated full-length EVA71 and EVD68 replicons, whereby the structural protein coding region (P1) was removed and replaced with a GFP reporter gene, thus allowing real-time monitoring of EV replication utilising live cell imaging. Using these constructs as the basis of our system, we subsequently established a trans-encapsidation assay, whereby viral structural proteins are provided in trans. Combining these structural proteins with a fluorescent replicon allows us to evaluate aspects of the viral lifecycle in real-time. Importantly, this approach provides improved levels of biosafety as the structural proteins are absent from the viral genome. We are currently exploiting these tools to study EV entry and replication and as a screening platform for potential antiviral compounds.

P773

The impact of the T16A and A26F Mutations in the Envelope Protein of an Avian Coronavirus on Viral Replication is Strain Dependent.

Isobel Webb[1,2], Sarah Keep[2], Holly Everest[2], Graham Freimanis[2], Helena Maier[2], Andrew Davidson[1], Erica Bickerton[2]

[1]University of Bristol, Bristol, United Kingdom. [2]The Pirbright Institute, Woking, United Kingdom

Abstract

Infectious bronchitis virus (IBV) is a Gammacoronavirus which infects the epithelial surfaces of poultry. The IBV genome encodes four structural proteins, Spike (S), Nucleocapsid (N), Membrane (M) and Envelope (E). In infected cells, the E protein is present as both a monomer and a pentameric ion channel, these two forms can be selected for via either an A26F or a T16A mutation, respectively. Using reverse genetics, both the T16A and A26F mutations were generated in the E protein of both a non-pathogenic IBV, Beau-R, and a pathogenic IBV, M41-K. M41K-A26F was not replication competent, unlike BeauR-A26F, which was rescued successfully but replicated to a lower level than Beau-R in some cell systems. This indicates that the A26 residue is essential for viral replication in M41-K but not Beau-R. Whole genome sequencing identified non-synonymous mutations in the S and M proteins of some M41K-T16A isolates. Predictive structural modelling found that the mutations in the S protein did not impact the structure, but the M protein mutation is predicted to create an alpha helix in a region which is important for E and M interaction. The replication kinetics of BeauR-T16A was comparable to Beau-R in primary chick kidney cells, tracheal organ cultures and in embryonated eggs. Isolates of M41K-T16A which lack the mutations in S and M genes did not replicate comparably to M41-K in these cell systems. Taken together, this suggests that M41-K is less tolerant of the T16A and A26F mutations than Beau-R.

P774

A novel in-cell micro neutralisation assay for the human coronavirus OC-43: Allowing rapid and automated quantification of neutralising effect of antibodies and antiviral compounds.

Jim Chadwick ORCID iD[1,2], William James ORCID iD[2], Sai Liu[2], Tiong Tan[3]

[1]The Pirbright Institute, Guildford, United Kingdom. [2]Sir William Dunn School of Pathology - University of Oxford, Oxford, United Kingdom. [3]MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

Abstract

Human coronavirus (HCoV) such as OC43 can result in respiratory infection and disease known to occur more frequently on a seasonal basis during winter. These HCoV species contribute to 15%–30% of common cold cases in human adults. As an endemic Betacoronavirus, OC43 emerged from zoonotic transfer and has recently gained attention as a model species for research on SARS-CoV-2 due to sharing aspects of cell pathology. OC43 has worldwide circulation and global economic impact as the disease requires on average three-day period for recovery.

To date, published quantification methods have been based upon utilising cell-based cytopathic effect, ELISA neutralisation or qPCR methods. Presented is a novel alternative method for automated quantification of viral foci formation, providing an in-cell approach to both titrate and examine the effect of antibodies and antiviral compounds with inhibitory effects on viral infection. In proof-of-concept experiments, a SARS-CoV-2 derived anti-spike HmAB[EB1] OC2, with a known neutralising effect to OC43, reduced the formation of viral foci via the assay.

These experiments demonstrate that we have developed a faster, less subjective assay using a modified cell line to enhance viral susceptibility, providing an alternate platform of quantification via focus formation or reduction.

P775

mTOR dysregulation in human nasal epithelial cells infected by the seasonal coronavirus NL63

Riaz Ali Somji[1], Charalampos Rallis ORCID iD[1], Efstathios S Giotis ORCID iD[1,2]

[1]The University of Essex, Colchester, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Human coronavirus (HCoV) NL63 is a single-stranded positive sensed RNA virus implicated in 4.7% of all collective respiratory diseases. The majority of the cases result in acute respiratory illnesses. It's potential to cause severe disease in children and the immunocompromised has shown the importance to investigate potential approaches for anti-NL63 therapy. In addition, it is proposed that HCoV-NL63 can be used as a safer surrogate for COVID-19 as both coronaviruses interact with the host cell angiotensin converting enzyme-2 (ACE2) receptor. Recent studies have shown that COVID-19 utilises host metabolic pathways such as the mechanistic target of rapamycin (mTOR) pathway. Over-activation of the pathway can promote viral replication, while mTORC1 (mTOR complex 1) can promote viral replication of COVID-19 in human kidney epithelial cells and 3D culture air-liquid interface human nasal epithelial cells (NEC-3D) cultures. Here, we present evidence of mTOR activation within human epithelial cells Caco-2[RC1] following HCoV-NL63 infection, and the effects of mTOR inhibitors in reducing HCoV-NL63 viral replication in Caco-2 cells and NEC-3D cultures.

P776

Differential responses of innate immunity triggered by seasonal coronaviruses OC43 and 229E on primary and continuous cell line

Tukur Baba Abdullahi[1], Nelson Fernandez[1], Efstathios Giotis[1,2]

[1]University of Essex, Colchester, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

The human coronaviruses (HCoV) strains OC43 and 229E cause one-third of common colds and hospital-acquired upper respiratory tract infections in premature new-borns. The outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has rekindled interest on HCoV as surrogate viruses for understanding both SARS-CoV-2 and CoV future infection. Normally, viral infections trigger anti-viral innate immunity host defences and inflammatory mechanisms orchestrated by the release of type I interferon (IFN) and the activation of NF-κB. IFN induces the production of an array of interferon-stimulated genes (ISGs), which inhibit virus replication. Current knowledge on the host innate immune responses to seasonal coronaviruses is rudimentary. We performed a detailed RNA-seq study designed to dissect, at transcriptional level the modulation of the type I IFN host response induced by the alpha HCoV-229E and beta HCoV-OC43 viral strains. 3D culture air-liquid interface of nasal epithelial cells (NEC-3D) isolated from a pool of human donors were infected with either virus (at MOI:0.1), mock infected or stimulated with recombinant IFN-α. The cells were screened with bulk RNA-sequencing (PE150-NovaSeq) at 24 and 72 hours post infection. Both viruses triggered a diminished type I IFN response. In addition, an induction of NF-κB-driven pro-inflammatory transcripts by each virus was observed. By comparing this data with in-house transcriptomic data obtained from SARS-CoV-2-infected NEC-3D as well as with studies using dual-luciferase reporter systems revealed distinct epithelial and immune cell responses for 229E and OC43 viral strains. Taken together, these data points to a divergent disease course of CoV compared to SARS-CoV-2 infection.

P777

SARS-CoV-2 Delta variant protects against longitudinal homologous and heterologous variant re-infection and transmission in a mild/asymptomatic ferret model

Amanda H. Seekings ORCID iD, Rebecca Shipley, Shweta Shukla, Alexander M.P. Byrne, Nadia Chew, Victoria Gould, Keeler Arrowsmith, Amber Martyn, Lorraine McElhinney, Joe James, Sharon M. Brookes

Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

SARS-CoV-2 infection causes varied clinical presentation in humans and animals ranging from asymptomatic to severe disease. Infection and transmission dynamics have been studied yet the extent primary infection protects against longitudinal re-infection with a homologous or heterologous variant remains poorly defined. The consequence of SARS-CoV-2 re-infection, ability for onward transmission and the role of humoral immunity on SARS-CoV-2 virus evolution was investigated in a mild/asymptomatic ferret model. Ferrets were directly infected with either SARS-CoV-2 Delta or Omicron (BA.1.17) variant and then re-infected after five months with the homologous or heterologous virus. Clinical, virological and immunological parameters were assessed. Upper respiratory and gastro-intestinal shedding was observed from all directly inoculated previously naïve ferrets with higher shedding titres from Delta infected ferrets compared to Omicron. Neutralising antibody (NAb) titres calculated as inhibition concentration 50% (IC_{50}) were high in Delta infected ferrets (>90) and this correlated with protective immunity against homologous and heterologous re-infection. Ferrets directly infected with Omicron resulted in lower NAb titres (≤90) with limited re-infection following homologous challenge. However, ferrets infected with Omicron with very low NAb titres (≤8) prior to re-infection were not protected against heterologous challenge and resulted in direct-contact transmission. This study demonstrated that NAbs can be sustained for up to five months in ferrets and provides protective immunity against re-infection with a heterologous variant. This supportive evidence for longitudinal immunity against re-infection and transmission contributes to our understanding on the threats of novel SARS-CoV-2 variants with regards to prior exposure, 'herd' immunity and vaccination strategies.

P778

Low pH induced exposure of buried rhinovirus capsid epitopes is reversible

James Kelly ORCID iD, Giann Dellosa, Joseph Newman, Toby Tuthill

The Pirbright Institute, Pirbright, United Kingdom

Abstract

The capsids of non-enveloped viruses such as rhinoviruses in the picornavirus family, are dynamic structures that undergo a series of conformational changes. These are often triggered by specific environmental cues such as receptor binding or exposure to low pH. Conformational changes include the transient or irreversible exposure of buried epitopes (N-termini of VP1and VP4) and release of the viral genome. Together these changes facilitate binding of the capsid to endosomal membranes and proposed formation of a pore in the endosomal membrane which delivers the viral genome into the cytoplasm.

The buried N-termini of VP1 and VP4 are thought to be transiently exposed, this is known as capsid breathing. Previous studies show blocking antibodies that target VP1 and VP4 N-termini prevent infection, indicating their exposure is essential for infection. We have investigated the exposure of these protein termini and the capsid breathing process of rhinovirus 16 (RV16) using receptor trapping ELISAs and thermofluor assays. This revealed that incubation of RV16 at low pH exposes VP1 and VP4 N-termini and that exposure of these epitopes can be reversed by neutralising the pH. This effectively mimics the breathing process.

A greater understanding of capsid breathing could aid development of novel rhinovirus therapies that inhibit the function of VP1 and VP4.

P779

Probing the building blocks of foot-and-mouth disease virus replication by enrichment of replication complexes

Connor Hayward, Samuel Dobson, Morgan Herod, David Rowlands, Juan Fontana, Nicola Stonehouse

University of Leeds, Leeds, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV) is a pertinent pathogen, which continues to pose a threat to the economic stability of nations regardless of their economic status. FMDV has a positive-sense (+ve) RNA genome that is replicated via a negative-sense (-ve) intermediate in virus produced organelle or "replication complex", that are assembled from viral RNA and proteins in complex with host cell factors. However, the precise composition of the FMDV replication complex remains undefined, and a better understanding of the organisation and composition of this complex could provide novel therapeutic targets. Understanding the composition of the FMDV replication complex and the host factors has remained challenging due to the technical difficulties in isolating such complex from infected cells.

To address this, we have used gradient purification to separate FMDV replication complexes over time during. We have demonstrated the co-sedimentation and subsequent floatation of key viral proteins (such as the viral polymerase) by western blot analysis. Using our recently developed Click Chemistry tagged-RTqPCR method we have measured the relative enrichment of viral and host RNA's in fractions containing these key viral proteins. With this approach we have able to show differential detection of both +ve and –ve viral RNA associated with different viral proteins. Work is ongoing to probe the host protein composition and transcription competency of these fractions using radiolabelled nucleotides. These data together will build a more complete picture of FMDV replication.

P780

Development of a high-throughput imaging assay to investigate opsonisation by FMDV-specific antibodies

Matthew Nagel-Smith, Amina Yasmin, Andrew Shaw, Donald King, Toby Tuthill, Joseph Newman

The Pirbright Institution, Pirbright, United Kingdom

Abstract

Background: Neutralising antibodies can confer protection against disease by preventing viruses from infecting new host cells. Non-neutralising antibodies are found to have significant anti-viral potential via various effector functions. e.g., opsonisation, mediated by the constant fragment (Fc) region of the antibody. In this study we investigated the ability of foot-and-mouth-disease virus (FMDV)-specific antibodies to mediate opsonisation.

Methods: Immune complexes (IC) were formed by mixing virus with neutralising or non-neutralising antibodies. Cultures of a murine-macrophage-derived-cell-line (RAW264.7) on coverslips or in 96-well plates were exposed to IC, virus only or antibody only. RAW264.7 don't express the receptors required for normal virus entry but express Fc receptor (FcR), therefore, uptake of virus and development of CPE are used as an indication of IC formation and uptake via FcR. Uptake of virus into cells was analysed by immunofluorescence confocal microscopy and development of cytopathic effect (CPE) was determined using the Sartorius Incucyte imaging platform.

Results: Uptake of virus and CPE in RAW cells was observed only when cells were exposed to IC virus with FMDV specific antibodies indicating opsonising activity of the antibodies. These assays will facilitate analysis of opsonising activity of polyclonal sera from vaccinated and/or challenged animals to correlate opsonising activity with protection from disease.

Conclusion: Neutralisation of virus by antibody is important but it doesn't quantify other important functions of antibodies such as opsonisation. This high-throughput assay provides a rapid approach for identifying and determining the opsonising potential of FMDV-specific antibodies and their role in disease protection.

P781

First isolation of tick-borne encephalitis virus from *Ixodes ricinus* ticks in the UK

Mollie Curran-French ORCID iD[1,2], Maya Holding[1,2], Gillian Slack[1], Roger Hewson[1,3]

[1]UKHSA, Porton, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]LSHTM, London, United Kingdom

Abstract

The flavivirus tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis, a potentially fatal viral disease of the central nervous system. TBEV is endemic throughout the Far-east, Asia and central and northern Europe, with evidence of western spread.

TBEV was first identified in the UK in 2019, through a deer serosurveillance survey, RT-PCR screening of Ixodes ricinus ticks collected from seropositive deer and subsequent genome sequencing of TBEV +ve tick homogenates. Live virus has yet to be recovered, as large-scale surveillance studies inactivate any live viruses in tick homogenates during high-throughput testing. Therefore, follow up targeted tick collection at potential UK TBEV locations was carried out with tick homogenisation in PBS to enable potential virus isolation at containment level 3.

Using A549 cells we were able to isolate infectious TBEV from UK ticks. Isolation of UK strains will allow us to update current TBEV UK sequences to include UTR regions, confirm robustness of current diagnostic assays and produce virus stocks of current circulating strains for use in pathogenicity studies and intervention testing, including accessibility to the wider scientific community through EVAg and NCPV programmes. Studies are underway to compare plaque phenotypes and growth curves of TBEV UK Hampshire and the well-defined European strain TBEV Neudorfl. Detailed genomic information will support work to reanimate viruses from cDNA and enable reverse genetics studies; identify changes in quasispecies caused by mammalian and tick cell host switching and investigate possible recombination events between TBEV and closely related and cocirculating louping ill virus.

P782

High-throughput proteomic analysis of the Dengue virus replication complex

James Bazire ORCID iD, Maia Kavanagh Williamson, Andrew Davidson

University of Bristol, BRISTOL, United Kingdom

Abstract

Dengue virus (DENV) is the causative agent of Dengue fever, a tropical disease of global importance. Although the properties of proteins encoded by the DENV positive sense RNA genome are well established, less is understood concerning their interaction with host cell proteins. DENV intracellular replication is intimately associated with the ER membrane and results in the induction of an ER membrane derived replication complex containing both viral and host proteins. This study aimed to identify host proteins recruited to the replication complex in human Huh-7 liver cells, either infected with DENV or stably expressing a DENV replicon.

A heavy membrane fraction (containing the virus replication complex, termed “16K fraction”) was isolated from DENV, DENV replicon and mock infected cells and analysed by tandem mass tagging and quantitative high-throughput mass spectrometry. 7200 proteins were identified and analysis showed that 400 proteins were increased in abundance at least 1.5-Fold ($p<0.05$) in the 16K fraction of DENV infected cells compared to the mock infected. The proteins increased in amount were predominantly mitochondrial transporters (notably multiple members of the SLC25A small molecule transporter family) and respiratory proteins. In cells transfected with the replicon 320 proteins were increased 1.5-Fold or more compared to the mock, and these proteins had roles predominantly associated with secretory pathways and ER function. Combined, these results highlight potential differences in host proteins involved in replication in DENV infected and replicon expressing cells and demonstrate the power of mass spectrometry for analysing the virus-host interaction.

P783

Investigating the role of neutrophils in dengue virus (DENV) infection

Isaac Dowell ORCID iD, Borko Amulic, Andrew Davidson

University of Bristol, Bristol, United Kingdom

Abstract

DENV causes approximately 400 million infections annually and places substantial strain on healthcare systems in affected tropical and sub-tropical regions. Severity and symptoms vary substantially between patients, from asymptomatic to the potentially fatal Severe Dengue (SD). The primary feature of SD is an increase in vascular permeability, resulting in plasma leakage from blood vessels to the surrounding tissue. Neutrophils are the most numerous immune cells in the blood and have a significant role in innate immunity. Neutrophils contribute to vascular leakage in inflammatory diseases and varied disease severity in some viral diseases. This project will investigate the hypothesis that neutrophil activation during DENV infection affects disease progression by modulating either a) viral replication or b) inflammation and endothelial permeability.

Proteomic data produced using serum from patients with different grades of dengue disease severity was analysed and a number of pathways linked to neutrophil activation identified that are differentially decreased in patients with different disease severities. The response of neutrophils to DENV and DENV-infected cells will be characterised through a number of established in vitro assays, which assess the neutrophil response mechanisms, including the production of Reactive Oxygen Species, the release of antimicrobial granule proteins and the formation of Neutrophil Extracellular Traps (chromatin nets that sequester and destroy microbes). Understanding the interplay between the neutrophil response and DENV infection could be important for understanding and treating SD cases, as well as for understanding the role of neutrophils in viral infection in general.

P784

Conserved regions of RNA in Hepatitis E virus

Vianca Shah, Jordan Skittrall, Emma Beniston

Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Abstract

Background: Hepatitis E virus (HEV) infects a wide range of mammals and causes over 20 million human infections each year, leading to 70,000 deaths and 3,000 stillbirths. HEV is taxonomically distinct from other human-infecting viruses; therapeutic options are limited and complete prevention impractical.

Methods: We analysed 482 publicly available HEV genomes from human infections, using a computational workflow designed to find previously unknown regions of high nucleotide conservation in viral genome alignments and to make structural and functional predictions about the biology of such regions.

Results: We identified six regions with high conservation. All identified regions have previously been described, ranging from overlapping open reading frames to regions thought to be cis-acting elements important for viral replication. We characterise the extent of the regions required to be conserved and make predictions of conserved structure.

Conclusions: Our workflow's ability to replicate known regions of conservation provides reassurance of its overall ability to find conserved regions. By providing additional characterisation of conserved regions, we move closer to understanding what it is about these regions that makes them key to the viral lifecycle. We therefore understand better how it may be possible to disrupt these regions, and therefore which aspects to pursue as candidate drug targets.

P785

Understanding the Role of Norovirus Non-Structural Proteins in Virion Assembly and Stability

Jake Mills ORCID iD, David Rowlands, Morgan Herod

University of Leeds, Leeds, United Kingdom

Abstract

Human noroviruses are a prevalent cause of gastroenteritis that contribute >200,000 deaths and are estimated to cost >£40 billion worldwide per annum. There is currently no efficacious vaccine or approved anti-viral therapy to treat norovirus infection, and a greater fundamental understanding of the virus life-cycle is required to develop new approaches to disease control.

The norovirus single-stranded positive-sense RNA genome is enclosed within a capsid comprised of the two viral structural proteins, VP1 and VP2. During the viral life-cycle, nascent viral genomes must assemble with these two capsid proteins to generate infectious virions to spread infection. However, how this process is coordinated is not understood. During a previous study using the murine norovirus (MNV) model system, we generated a series of heat-stable viruses by in vitro evolution. Next generation sequencing of these viral populations revealed multiple mutations in VP1, but intriguingly, mutations in membrane-associated non-structural proteins (e.g. NS3) were also observed. Reintroduction of these identified mutations individually into an infectious clone impaired viral recovery, suggesting the non-structural substitutions are required in conjunction with changes in the capsid for virion stability. We are currently dissecting the function of these mutations on the RNA and protein level to understand the roles of non-structural proteins in virion assembly.

P786

Construction and evaluation of an expression plasmid for porcine respiratory and reproductive syndrome virus 1 (PRRSV-1) GP3

Bhavya Harika Gowraram[1], Arun Thandam Parambil[1], Simon Graham[2], Yashar Sadigh[1]

[1]Coventry University, Coventry, United Kingdom. [2]The Pirbright Institute, Pirbright, United Kingdom

Abstract

The Arteriviridae family is known to cause various diseases in mammals. Porcine reproductive respiratory syndrome viruses (PRRSV) have gained attention during the last 20 years by drastically harming the global swine industry. With the prevalence of this viral illness, there is a need for novel vaccines that have the potential to combat the rapidly mutating PRRSV. Therefore, efforts are being conducted to develop a protein subunit vaccine based on GP3 glycoprotein of PRRSV.

Molecular cloning was performed to amplify ORF3 of PRRSV-1 and clone it into pTYB21 vector. Successful clones were verified using Sanger sequencing. Bacterial strain E. *coli* ER2566 was used for protein expression studies, protein expression optimisation and purification were conducted using chitin-based IMPACT protein purification technology (NEB).

A portion of GP3 gene was identified based on its amino acid sequence immunogenicity and subsequently amplified and cloned into pTYB21 protein expression plasmid using DNA ligase method. The results of Sanger sequencing were examined to determine DNA sequence integrity by comparing the data with the analysed sequence of PRRSV from the NCBI database. Subsequently, protein expression was conducted to facilitate optimal protein expression conditions by using IPTG, and 160 mM was observed to be the optimum concentration for expression. Protein purification is ongoing.

The protein expression plasmid with GP3 was constructed, and the optimisation of protein expression is ongoing. Following the successful completion of protein purification, an in vivo experiment to assess immunogenicity may be designed.

Keywords: Porcine reproductive and respiratory syndrome virus, protein purification, molecular cloning, pTYB21, IMPACT kit.

P787

Virus-like particles expressed in *Pichia pastoris* as a novel polio vaccine – a role for myristoylation?

Jessica Swanson[1], Lee Sherry[2,1], Keith Grehan[1], Kirsty Sawtell[1], David J Rowlands[1], Nicola J Stonehouse[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Poliovirus (PV) is the causative agent of poliomyelitis which can lead to paralysis and death. With the successes of both the oral and inactivated polio vaccines (OPV and IPV respectively) there has been a >99% reduction in cases of paralytic poliomyelitis. Despite this, there are still challenges for the eradication of PV due to the risk of reintroduction of the virus from both vaccines. Virus-like particle (VLP) based vaccines provide an attractive alternative to both vaccines as they retain the structure of PV but lack viral genome, thus removing the risk of PV reintroduction. Our previous work has shown that our stabilised PV VLPs expressed in the yeast *Pichia pastoris* are predominantly in the native (D) antigenic confirmation and offer good protection in animal models.

Myristoylation is a post-translational modification of the N-terminus of the capsid precursor protein, VP0, which has been shown to play a role in viral capsid assembly. Through mass spectrometry analysis of *P. pastoris* derived PV VLPs, it was observed that only approximately a third of the copies of VP0 are myristoylated.

To investigate whether the reduced level of myristoylation affects assembly of VLPs and the ratio of D and C antigenic particles produced, mutations designed to enhance myristoylation in yeast were introduced at the N-terminal of VP0. The mutated VLPs were expressed in *P. pastoris* and the resulting particles characterised. We will present evidence of the importance of myristoylation in the production of stabilised VLPs as novel vaccine candidates.

P788

Cloning and Expression of glycoprotein 2 (GP2) of porcine respiratory and reproductive syndrome virus 1

Arun Thandam Parambil[1], bhavya harika Gowraram[1], simon graham[2], Yashar sadigh[3]

[1]Coventry university, coventry, United Kingdom. [2]The Pirbright Institute, Pirbright, United Kingdom. [3]Coventry University, coventry, United Kingdom

Abstract

Background: Porcine reproductive and respiratory syndrome (PRRS) has been one of the most economically significant swine diseases worldwide for over two decades. An array of PRRS vaccine products is available in various regions of the world. However, despite extensive efforts, little progress has been made to improve the efficacy of the PRRS vaccines. The main challenge to this is the continual evolution of PRRS viruses (PRRSV). With the aim of producing a novel protein subunit-based vaccine candidate, we, cloned and expressed PRRSV-1 GP2.

METHOD: PRRSV-1 GP2 was amplified using PCR from a pool of viral cDNA. For cloning, the expression vector pTYB21 was used. Protein expression was induced in E. coli and purification using IMPACT kit (New England Biolabs) is ongoing. IMPACT kit uses a novel expression system based on chitin, chitin-binding protein, and intein domain.

RESULT: Bioinformatics was performed to identify a suitable portion of GP2 which contains immunological epitopes. The encoding area of PRRSV-1 ORF2 was amplified, and DNA ligase-based cloning was performed. Successful clones were checked with Sanger sequencing. Data were compared with the published sequences of PRRSV. Protein expression was checked using SDS-PAGE. To optimise protein expression, different concentrations of IPTG were used and 160 mM concentration was determined as the best concentration for expression. Further work is in progress to purify GP2.

CONCLUSION: A predicted antigen domain of PRRSV-1 GP2 has been expressed in bacteria. Upon successful completion of protein purification, an in vivo study may be performed to assess its immunogenicity.

P789

Isolation of PRCV spike-specific porcine B cells following infection with PRCV

Emily Briggs[1,2], Basudev Paudyal[1], Marie Di Placido[1], Joe Newman[1], Brigid Veronica Carr[1], Erica Bickerton[1], Elma Tchilian[1]

[1]The Pirbright Institute, Pirbright, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Coronaviruses represent a significant burden on public health, causing three major epidemics since 2003. Vaccination has been the primary strategy for control of SARS-CoV-2, however monoclonal antibodies present a promising therapeutic tool in treatment of vulnerable individuals. A model for SARS-CoV-2 disease in the pig utilizing infection by pathogenic strains of porcine respiratory coronavirus (PRCV) has been developed and will be used to generate monoclonal antibodies against the PRCV spike protein.

The antibody response following PRCV infection in pigs was characterized by ELISA. The PRCV spike sequence from the PRCV ISU-1 strain was cloned, expressed, purified and biotinylated. The biotinylated spike was then used to isolate antigen specific B cells by FACS from the infected animals for subsequent single-cell amplification and sequencing of antibody heavy and light chains using Illumina Next Generation Sequencing. In parallel, the antibody repertoire in blood and lung from PRCV infected animals is being analyzed over the time course of infection to determine if it differs between local and systemic tissues. Whole repertoire amplification will facilitate clustering of antibody sequences and identification of antibodies of interest from the single cell dataset, which will be further cloned and expressed to confirm binding specificity. Future studies will determine the specificity and epitopes of the isolated mAbs and their functional activities. These studies will provide insights into coronavirus infections and how to generate and administer novel efficacious therapies against coronaviruses in humans.

P790

Pharmacological inhibition of host cell lipid metabolism impairs a late step of the MERS-CoV replication cycle

Maria Soltsioti ORCID iD, Anja de Jong, Ronald Limpens, Patrick Wanningen, Heidi de Gruyter, Montserrat Barcena, Marjolein Kikkert, Eric Snijder

Leiden University Medical Center, Leiden, Netherlands

Abstract

Coronaviruses hijack host cell metabolic pathways and resources to support their replication. They induce extensive host endomembrane remodelling to generate viral replication organelles and exploit host membranes for assembly and budding of enveloped progeny virions. Because of the overall significance of host membranes, we sought to understand the role of host cell lipid metabolism factors in the coronavirus-infected cell. Initially, we screened compounds targeting enzymes of the de novo lipogenesis pathways for their ability to inhibit MERS-Coronavirus replication. Our results show that pharmacological inhibition of acetyl-CoA carboxylase enzyme (ACC), a key factor in fatty acid biosynthesis, impaired viral progeny formation by 3log10, while intracellular viral RNA levels remained largely unaffected. Moreover, the intracellular production of viral structural proteins was not affected, but preliminary confocal microscopy data suggested that their trafficking was altered. Electron microscopy also showed that the assembly or egress of progeny virions was severely disrupted. The inhibitory effect of the compound could be reversed by palmitic acid supplementation, while pharmacological inhibition of fatty acid synthase and palmitoyltransferase enzymes (operating downstream of ACC) also inhibited viral progeny formation. We postulate that our results reflect a need for palmitoylation of the MERS-CoV Spike protein, which appears important for interaction with other viral structural proteins. This would contradict observations on SARS-CoV-2 and SARS-CoV by others, proposing that Spike protein palmitoylation is not necessary for interaction with the Membrane protein. Our results also highlight the potential of ACC as a candidate target for the development of host-directed antiviral treatment.

P791

In vitro transcription of RNA virus genomes to study possible sites of post transcriptional modification using nanopore direct RNA sequencing.

Shichun Gu, Rachel Milligan, Andrew Davidson, David Matthews

University of Bristol, Bristol, United Kingdom

Abstract

Viral genomic and mRNA is frequently modified post transcriptionally and such modifications are known to aid viral replication and even evasion of innate immune responses.

We have been making extensive use of direct RNA sequencing to analyse the transcriptome of a number of different viruses including SARS-CoV-2, Adenovirus and Respiratory Syncytial Virus (RSV). For some time it has been know that it is possible to analyse the data from direct RNA sequencing and discern the locations of post transcriptional modification events in the RNA. In order to maximise the sensitivity of this kind of analysis, we have been looking at different ways to generate in vitro transcribed (IVT) RNA so that it can be sequenced on a nanopore device. Subsequently the resulting raw signal can be compared to the signal generated by viral RNA extracted from infected cells. Areas where the IVT signal and the viral RNA signal differ are likely to be regions where the viral RHA has been modified. We highlight regions on the SARS-CoV-2 genome that are likely modified on both the classic SARS-CoV-2 genome as well as the more recent delta strain. In addition we show that there are differences in the distribution of post transcriptional events between these two VoCs. Allied to this we also pinpoint locations on RSV mRNA that are highly likely to be modified post transcriptionally.

P792

Hepatitis E virus infection of primary human brain cells

Ruth Haverty, Nicola F Fletcher

University College Dublin, Dublin, Ireland

Abstract

Hepatitis E virus (HEV) is a major cause of viral hepatitis worldwide, and it is the only zoonotic hepatitis virus. While many HEV infections are associated with acute disease that resolves without treatment, immunocompromised individuals or those with pre-existing liver disease can progress to chronic hepatitis. Additionally, both acute and chronic HEV infections may lead to a number of extrahepatic manifestations. Neurological disease is the most frequent extrahepatic HEV-associated condition, and the most commonly described include Guillain-Barré Syndrome, neuralgic amyotrophy, and encephalitis.

However, pathogenesis of neurological disease in individuals with HEV infection is unclear. Neurological disease may be caused by direct infection of the nervous system or indirect sequelae of peripheral infection. To investigate these possible mechanisms, we tested a range of primary human brain cells and cell lines representing microvascular endothelium, astrocytes, pericytes, choroid plexus epithelium and neurons for their ability to support HEV infection in vitro. HEV infected multiple human brain cell types. These results may suggest that HEV may be capable of invading and infecting cells within the human central nervous system, which has implications for therapeutic strategies.

P793

Phylogenetic analysis of Bovine coronavirus spike coding sequences reveals variation compared to the Mebus strain used to design some vaccines

Tristan Russell ORCID iD, Gerald Barry ORCID iD

University College Dublin, School of Veterinary Medicine, Dublin, Ireland

Abstract

Bovine coronavirus (BoCoV) is an aetiological agent of respiratory and enteric diseases in cattle, including winter dysentery and neonatal calf diarrhoea. Vaccines are deployed to control diseases caused by this virus, but recently there have been reports of diminished vaccine protection. This could be caused by currently circulating isolates drifting away from the BoCoV Mebus strain used to design the vaccine most widely used in Ireland. Analysis of selective pressures acting on multiple BoCoV protein coding sequences revealed higher levels of positive selection acting on haemagglutinin esterase and spike, which are two viral proteins recognised by the host immune system. Pressure to evade the host immune response could be selecting for amino acid changes in these two proteins. There is limited genetic information on BoCoV in Ireland so the spike coding sequence of BoCoV field isolates collected throughout 2022 was PCR amplified and sequenced. Alignments of these and other available spike sequences revealed variation compared to an Irish isolate of BoCoV from 2011; variation compared with recent isolates from several European and Asian countries; and variation between the isolates from this study. There are also regions of conservation within the spike coding region. Variation between the spike amino acid sequences of circulating BoCoV in Ireland and the vaccine strains could be one factor contributing to reduced vaccine protection.

P794

Semliki Forest virus replication, not viral entry, causes early autophagy inhibition

Cameron Stockwell ORCID iD, Tara Sabir, Sareen Galbraith ORCID iD

Leeds Beckett University, Leeds, United Kingdom

Abstract

Autophagy is a cellular process in which cytoplasmic cargo and debris are delivered to lysosomes for degradation in specialised double membrane vesicles called autophagosomes. The autophagy pathway is regulated by the mTOR and Beclin-1 protein complexes. Beclin-1 is essential for autophagosome nucleation, and complexes with VPS34 to promote phospholipid generation, allowing lipidation of autophagosome membranes with LC3 - an autophagy marker. Recent research has focussed on the manipulation of autophagy as a potential treatment for RNA virus encephalitis. Semliki Forest virus (SFV) is a positive-sense single stranded RNA virus from the alphavirus genus often researched as model for encephalitic Flaviviruses. Previous work from our group has shown SFV supresses autophagy during the first 8 hours of infection increasing virus titre. Others have revealed an accumulation of autophagosomes during late stage (>24 h) SFV infection in Human osteosarcoma cells which also increased virus titre. We utilised UV-inactivated virus alongside live virus to analyse the effects on LC3B, Beclin-1, and VPS34 expression using RT-qPCR, during early infection of non-neuronal and neuronal cells. Fluorescent microscopy was utilised to quantify dsRNA puncta, LC3 puncta, and VPS34 puncta. Virus titre was quantified via plaque assay. Results suggest autophagy is supressed by viral replication during the first 8 hours of infection and not by viral entry. Fluorescent imaging revealed the presence of dsRNA puncta within 10 minutes of viral entry (MOI=10) indicative of rapid formation of replication complexes. This research provides evidence that autophagy has the potential to be exploited as a treatment for neurovirulent viruses.

P795

Investigation of the Host Innate Response by Seasonal and Severe Coronaviruses

Mai Almsaud[1,2], Stuart Armstrong[1], Rebekah Penrice-Randal[1], Julian Hiscox[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia

Abstract

The emergence of human coronaviruses in the 21st century poses a serious threat to global health security. To date, MERS-CoV cases are still reported in Saudi Arabia, with mortality rate of 35%, placing this as the deadliest of CoVs.

The recent lethal HCoVs have evolved strategies to manipulate innate immunity and subvert IFN production pathways, leading to delays between the innate and severe immune pathology. However, the viral immune evasion strategies during CoV infections are not currently fully understood. MERS-CoV encodes two proteins: 4a and 4b that subvert the innate response. In SARS and SARS-CoV-2, ORF6 share similar function. This project investigates whether a set of homolog proteins in CoVs share similar strength in the IFN subversion process, or whether one set of viral proteins is better than the other, possibly accounting for differences in disease profiles.

Investigation of novel protein interactions between MERS-CoV-4a/4b and host factors was conducted through Mass Spectrometry analysis and Nanopore MinION sequencing. MERS-CoV-4a/4b protein functionality was compared to SARS, SARS-CoV-2 and HCoV-229E in controlled *in vitro* assays to measure IFN-β antagonism performance. Our results suggest different levels of inhibition of the IFN-β promoter, of which MERS-CoV-4b was the strongest. In our transcriptomic data, the expression of REL, a transcriptional factor of the NF-κB complex, was down-regulated in cells transfected with MERS-CoV-4a. The identification of virus-encoded IFN antagonists and understanding of their mechanism of action may provide novel targets for therapeutic intervention; modulating the innate immune response to disrupt viral replication and mitigate disease screening.

P796

Extracellular polysaccharides from A. platensis inhibit different emerging viruses by targeting conserved entry mechanisms

Maria Sole Regina Lancerin[1], Joseph Palmer[2], Nigel Temperton[3], Richard Sloan[1,4]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]ScotBio Naturally Pioneering, Glasgow, United Kingdom. [3]University of Kent, Canterbury, United Kingdom. [4]University of Zejiang, Zejiang, China

Abstract

Extracts obtained from the cyanobacterium Arthrospira platensis are reported to inhibit a broad range of viruses, including HIV-1, herpesviruses, influenza A, and coronaviruses. However, neither the active compound(s) within this extract, nor its mechanism of action had previously been identified.

Using lentiviral pseudotypes we have identified extracellular polysaccharides secreted by A. platensis as responsible for this activity. Complete inhibition of viral infection has been achieved towards different strains of Coronaviruses, as well as Influenza type A, and validated with replication competent lab-adapted viral strains.

Time-of-addition experiments show that the inhibition occurs during the initial phases of viral replication, attachment and entry, consistently with the expectation that A. platensis must target a process common to its numerous targets.

Furthermore, differences in potency observed against various pseudotypes indicate the mechanism of action to be related the entry pathway: lower IC50 values are observed towards viruses that fuse directly with the plasma membrane, and higher values for those which exploit endosomes. Fusion and viral attachment assays are underway to verify whether viral envelope fusion is indeed targeted, and if the route of entry (plasma membrane versus endosomal) plays a role.

A. platensis exopolysaccharides are active against unrelated viruses of pandemic potential, suggesting that the antiviral activity may extend to other emerging viruses. Moreover, they show no cytotoxicity, and A. platensis is already approved for human consumption. For these reasons, A. platensis exopolysaccharides show great pharmacological potential in contributing to the medical need for off-the-shelf antivirals in the event of a new pandemic.

P797

Understanding Rotavirus Replication in the context of Liquid-Liquid Phase Separation

Julia Acker

Cambridge, Cambridge, United Kingdom

Abstract

Many viruses require formation of specialised cytoplasmic compartments, termed viroplasms or viral replication factories, which support their replication. Rotavirus viroplasms are complex ribonucleoprotein condensates formed via liquid-liquid phase separation of the viral scaffold protein NSP5, the viral RNA chaperone NSP2 and cognate nucleic acids. In the course of viral infection, rotavirus transcripts accumulate within the host cytoplasm and are selectively recruited to viroplasms. It remains a mystery how viral transcripts, but not host RNA, partitions into replication factories. Using single molecule fluorescent microscopy, we show that selection of viral RNA is strongly dependent on their conserved terminal regions, while poly-adenylated RNA remains excluded. Here, we propose a model for viroplasm formation that includes selective uptake of eleven distinct gene segments, which may provide a selective environment for non-translated RNAs destined for packaging.

Session Topic: A Greener Future: sustainable and scalable solutions in industrial biotechnology and green pharma

P798

Upgrading bioprivileged monomers from problematic polymers

Ewan Waters, Rajesh Bommareddy

Northumbria University, Newcastle, United Kingdom

Abstract

Decarbonisation of the chemical industry is critical in satisfying objectives set out in the Paris Accord. This can be done through utilising renewable carbon sources in lieu of traditional petroleum feedstocks. Natural and waste carbon feedstocks, such as lignin and polyethylene terephthalate (PET), are highly abundant and underutilised reservoirs of carbon. Yet these polymers are problematic, as lignin and PET are both recalcitrant to efficient biocatalysis. Once degraded, the monomers produced can be classed as bioprivileged in that they can be biofunneled to produce value-added products. The facultative chemolithoautotroph Cupriavidus necator H16 is able to assimilate organic carbon substrates in addition to CO2 and H2. Resultantly, C. necator can be used as microbial chassis for production of value-added chemicals in a carbon neutral bioprocess. Herein, we will metabolically engineer C. necator to valorise lignin and PET-derived monomers. Lignin-derived guaiacol will be upgraded to muconic acid, a precursor involved in PET production. PET-derived terephthalic acid (TPA) will be upgraded to bioplastic PDCA. The efficiency of these bioprocesses will be improved through engineering of key enzymes in these pathways. Whilst use of C. necator to carry out these pathways under mixotrophic fermentation conditions will establish carbon neutrality. Through doing this, we hope to establish efficient and sustainable routes for production of conventional plastics (PET) and bioplastics (PDCA).

P799

Microbial engineering for efficient overproduction of C3 and C5 oxoacids from CO_2

Toby Davies, Rajesh Bommareddy

Northumbria University, Newcastle upon Tyne, United Kingdom

Abstract

Fuel and chemical production have been dominated by petrochemicals; this requires radical change if global sustainability goals are to be met. Thus, the removal of unsustainable carbon from these processes is vital. Metabolic engineering represents an exciting solution for the synthesis of industrially relevant chemicals from sustainable or otherwise overlooked feedstocks. This will aid in reducing the dependency on fossil fuels and petrochemical-based chemical synthesis. The facultative chemolithoautotroph Cupriavidus necator H16 is a relevant microbial chassis that is popular for biotechnological processes as it has a highly diverse metabolism and is able to switch between heterotrophy and autotrophy and metabolises a plethora of substrates as carbon sources. During autotrophy H16 utilises CO_2 as its carbon source and H_2 as the electron donor. In this project we aim to create strains of C. necator that utilise these gases for the generation of value-added compounds. In view of this, through metabolic engineering and fermentation processes we seek to show a complete bioprocess where biofuel and chemical derivatives such as isobutyraldehyde and acetaldehyde are produced in substantial amounts from CO_2 and H_2. Both autotrophic and mixotrophic growth will be tested, with the mixotrophy representing a closed bioprocess where no waste gases are produced from the degradation of fatty acids, or any feedstock whose degradation results in the production of CO_2.

P800

Microbial engineering for a healthy planet

Neil Dixon ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

Linear consumption models maintain our reliance on fossil fuel-based feedstocks and the poor treatment of our waste results in negative impact upon the climate and environment. The used of genetically engineered microbes (GEMs) can provide tractable approaches to address sustainability challenges including i) the production of energy, chemicals and materials from non-fossil fuel-based sources, ii) solutions for carbon and nitrogen fixation, iii) bioremediation of contaminated environments. Sustainable production of chemicals, materials and energy from renewable biomass and waste offers one potential alternative to the continued use of finite geological oil reserves. However, in order to compete with current petrochemical refinery processes, alternative biorefinery processes must overcome significant costs and productivity barriers. Pathway design and optimization is a major bottleneck due to the vast number of possible genetic and process variables and the metabolic burden associated with bioproduction. However, genetically encoded biosensors can provide a solution by transducing the target metabolite concentration into detectable signals to provide high-throughput phenotypic read-outs and allow dynamic pathway regulation [1]. In this talk I will present some approaches and activities from our research group towards addressing these engineering biology challenges. This will include the development of advanced biorefinery processes by consolidated production of high value chemicals directly from waste agro-industrial residues [2], microbial processes to degrade, assimilate [3], and valorise plastic and other waste feedstocks, and thirdly the development of underpinning genetic toolbox to control GEMs for sensing, regulation and production [4-6].

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P801

A ‘Pants’ pilot study: Using edible fungi to degrade semi-synthetic textiles

Liberty Hazelgrove, Suzy Moody, Rachel Harper

Kingston University, London, United Kingdom

Abstract

Background: Each year ~92 million tonnes of fabric are incinerated or sent to landfill. This releases pollutants into the environment, such as microplastics and greenhouse gases, which are known to reduce soil, water, and air quality. New sustainable methods are therefore needed to tackle the global textile waste problem.

Methods: One possible solution is mycoremediation, which uses fungi to degrade pollutants. This research investigated the possibility of growing edible fungi on semi-synthetic textile waste, such as pants, to try and develop a circular economic process with mushroom farming giving value to textile waste. Comparative bioinformatics was used to identify five species of edible fungi with enzymatic potential to degrade semi-synthetic fabrics. The fungi identified were then successfully cultured in an agar-independent manner, using semi-synthetic fabric as a substrate. At the end of the incubation period, the microcosms were analysed using metabolomics and imaging.

Results: The results revealed 5 key classes of enzyme present in every fungus, with differing proportions across the species. The fungi were successfully grown on cotton/bamboo viscose with 4% elastane. Growth rate differed between fungi, suggesting some would be more amenable to agar-free culture on a larger scale. Light microscopy and SEM were used to assess growth and textile damage. The metabolome also varied between species, giving a clear indication of which species have potential for scale up to an industrial scale.

Conclusion: Growing mushrooms on textile waste may establish a more sustainable circular economic approach to managing textile waste worldwide.

P802

The potential of mycoremediation as a novel and sustainable means of textile waste disposal

Rachel Harper, Suzy Clare Moody ORCID iD

Kingston University, Kingston, United Kingdom

Abstract

Background: Textile waste comprises natural and petroleum-derived fibres and as such presents a complex recycling challenge. Currently, the main disposal methods used are landfill or incineration, both of which cause serious environmental damage so a cleaner and more sustainable alternative is required.

Methods: A novel agar-free microcosm was developed to assess the potential of two wood decay fungi to grow on semi-synthetic textiles. The microcosms were incubated on a range of fabrics with an increasing elastane content. The fabrics were imaged regularly, with the volatile metabolome and the percentage of dye remaining in the fabric analysed at 3, 5 and 8 month timepoints.

Results: The agar-free microcosm was developed with a view to industrial scalability and demonstrated that low tech, low maintenance microbial growth on fabric was achievable. The fungi used, one brown rot and one white rot, both grew successfully on all fabrics, showing minimal impact of the increasing synthetic content. The white rot fungus was able to bioremediate the dye (Reactive Black 5) from all fabrics, while the brown rot fungus microcosms showed minimal dye loss from the highest elastane content microcosm only. Volatile analysis enabled a preliminary safety assessment of this process, and showed that volatile release was minimal in all microcosms.

Conclusion: 60% of all clothes contain some synthetic fibres, and with over 100 billion items of clothing produced every year there is an urgent need to find sustainable methods of disposal. This is an under-researched field but this study suggests fungi are worth further investigation.

P803

Purification and biochemical characterization of SM14est, a polyesterase enzyme from the marine sponge-derived *Streptomyces* sp. SM14

Clodagh M. Carr ORCID iD[1,2], Malene B. Keller ORCID iD[3], Bijoya Paul ORCID iD[3,4], Sune W. Schubert ORCID iD[3], Kristine S. R. Clausen[3], David J. Clarke ORCID iD[1,5], Peter Westh ORCID iD[3], Alan D. W. Dobson ORCID iD[1,2,6]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]SSPC-SFI Research Centre for Pharmaceuticals, University College Cork, Cork, Ireland. [3]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark. [4]Novozymes AS, Lyngby, Denmark. [5]Alimentary Pharmabiotic Center, University College Cork, Cork, Ireland. [6]Environmental Research Institute, University College Cork, Cork, Ireland

Abstract

The marine environment has been increasingly recognized as a promising source of microbial polyesterases for the enzymatic degradation of polyester plastics. Here, we present the purification and characterization of SM14est, a marine sponge-derived polyesterase that was previously identified during the genome mining of 52 *Streptomyces* spp. sequences for enzymes that degrade polyethylene terephthalate (PET) plastic.

The SM14est gene was codon-optimized for expression in *Bacillus subtilis* and the recombinant, His-tagged protein was purified by Ni-affinity chromatography. The purification of SM14est was verified by SDS-PAGE and nano differential scanning fluorimetry (nanoDSF) was employed to study protein stability. A suspension-based PET hydrolase assay was used to characterize the activity of SM14est towards semi-crystalline PET powder (particle size ~300µm).

The thermal transition midpoint (T_m) of SM14est was determined to be 55 °C in sodium phosphate buffer, pH 8. SM14est was found to perform well at 45 °C, with PET hydrolysis products detected at UV 240 nm within 1 h of incubation. The PET hydrolyzing activity of SM14est was enhanced at increasing concentrations of sodium chloride (0.1 to 1M). When product release was monitored over 7 hours with and without salt, the initial PET hydrolysis rates at an enzyme concentration of 0.5 µM were determined as 0.635 µM/min and 0.1208 µM/min, respectively, representing a 5-fold increase when sodium chloride (500 mM) is added.

Salt-tolerant polyesterases could play a role in the biological degradation of plastic waste, especially given that plastic particles and microplastics frequently contaminate marine ecosystems, together with high-salt industrial wastewaters.

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Understanding the influence of lysine to arginine ratio in driving aggregation of periplasmic proteins in Escherichia coli

Javeria Mehboob[1], Chris Lennon[2], Gwenola Ninon[2], Safwan Akram[3], Peter Ashton[1], Gavin Thomas[1]

[1]University of York, York, United Kingdom. [2]Fujifilm DioSynth Biotechnologies, Belasis Ave, Stockton-on-Tees, Billingham, United Kingdom. [3]Teesside University, Middlesbrough, United Kingdom

Abstract

Understanding and controlling protein aggregation and insolubility are important challenges for both academia and industry. Several experimental approaches and in silico predictors have been introduced to improve protein solubility and yield. However, there is no consistent framework that can be applied to readily control whether protein do or do not form protein aggregates, also referred to as inclusion bodies (IBs). To understand the drivers of IB formation, we studied a family of E. coli protein with identical folds and sub-cellular location, but with significantly different IB forming potential. These substrate-binding protein (SBP) components of peptide ABC transporters contain a strong IB former, Protein X and highly soluble close homologues, including Protein Y. We explored various experimental and in silico techniques with the aim to improve protein solubility, folding and therefore active protein yield. An in-silico solubility predictor used to compare Protein X and Y revealed the importance of the lysine to arginine (KmR) ratio as a potential contributing factor for protein X insolubility and aggregation. With this knowledge, site-directed mutagenesis experiments were designed to improve the solubility of protein X and reverse experiments were designed to create mutants to protein Y to make it more insoluble to study the effects of changing the KmR, supporting a fundamental role of surface-located Arg residues in impacting protein aggregation.

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Culture and Metagenomic analyses of solvent contaminated sites

Thomas Hender[1], Nick Tucker[2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Strathcylde, Glasgow, United Kingdom

Abstract

The biosynthesis of industrial platform chemicals has become a promising route for a sustainable alternative to petrochemicals. Limitations due to toxic effects on production strains are currently a major bottleneck. Organisms from contaminated environments which have built a natural resilience to a variety of industrially relevant compounds may hold the key to a sustainable plastics industry.

The set design department of the Royal Conservatoire of Scotland (RCS) use solvents, paints and dyes that are frequently passed through the drainage system provided an alternative to an industrial. Here we demonstrate that mixed culture-metagenomics of RCS samples can be used to screen for tolerant-organisms to certain industrially relevant compounds. We show a dramatic increase in Pseudomonas, including a potentially novel species in enrichment cultures treated with a methacrylate ester involved in the production of acrylic plastic. Assembles of a conjugative-plasmid predicted to encode drug resistance mechanisms of the ATP binding cassette super family, the resistance nodulation cell division superfamily and mercery transport proteins MerP, MerT. These assemblies have been confirmed using PCR and full genome sequencing of the isolated organism. The organism RCS09A shows increased tolerance when compared to industry standard P. putida. This mega-plasmid could be used to improve production strain tolerance and increase yields.

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Accessing novel biocatalytic solutions through (meta)genomic exploration of the marine ecosystem

David Woods, Aoife Kearney, Edel Murphy, Anita Maguire, Fergal O'Gara, F. Jerry Reen

University College Cork, Cork, Ireland

Abstract

The advent of metagenomic technologies, coupled with greater access to genomic sequences and predictive tools, has enabled the research and development community to harness bioactive potential from previously untapped sources. A particularly intriguing example of this is the Porifera phylum (marine sponges), which has delivered novel anti-infectives, anti-cancer, and anti-inflammatory therapeutics as well as biocatalysts with exciting transformation potential. Indeed, the marine sponge ecosystem has been shown to sustain a rich microbial biodiversity, the biocatalytic potential of which is largely unexplored when compared with its terrestrial counterpart. Understanding the ecological role of biotransformation within the polymicrobial communities that sustain the marine sponge ecosystem is key to advancing biocatalytic mining of this and other resources.

Using both metagenomic and genomic (in silico) mining tools we have uncovered a suite of biocatalytic activities with industrially relevant properties. These include marine ω-transaminase, lipase/esterase, amylase, nitrilase, and protease activities isolated from *Axinella dissimilis* microbial communities. Remote stereospecificity, rapid biotransformation, and the ability to accept challenging substrates with bulky R groups have been characteristic of the marine enzymes, which have proven to be suitable for heterologous expression and are stable under reaction conditions. Both transaminase and lipase candidates have also proven amenable to scaled-up production in 4 L bioreactors. Substrate profiling and molecular modelling of the lead biocatalysts has provided insights into the molecular structures that underpin these novel activities and current work is focused on genetic engineering towards further enhancement of their biocatalytic properties.

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Clostridium saccharoperbutylacetonicum N1-H4 CO_2 biocapture in Bioelectrochemical Systems (BESs) under different operational conditions.

Rosa Anna Nastro ORCID iD[1], Chandrasekhar Kuppam[2], Vincenzo Pasquale[1], Andrea Pietrelli[3], Claudio Avignone-Rossa[4]

[1]Department of Science and Technology, University Parthenope of Naples, Naples, Italy. [2]Department of Biotechnology, Vignan's Foundation for Science, Technology and Research, Vadlam-udi-522213, Guntur, Andhra Pradesh, India. [3]Laboratoire Ampere CNRS UMR 5005, Université de Lyon, UCBL, INSA, ECL, F-69621 Villeurbanne, France. [4]Laboratory of Systems Microbiology, Department of Microbial Sciences, University of Surrey, Guildford, United Kingdom

Abstract

Microbial Electrochemical Technology (MET) has shown its potential in CO_2 conversion in organic compounds through a process named microbial electrosynthesis (MES). In the present study, we investigated the ability of *Clostridium saccharoperbutylacetonicum* N1-4 to produce acetate and D-3-hydroxybutyrate from carbon dioxide in a CO_2:N_2 gas mix in non-electrogenic control coltures and in Bioelectrochemical Systems (BESs) under different operational conditions: the application of a 1.5V external voltage (MFC_1.5V), the connection to a 1000 Ω resistor (MFC_1000Ω) and Open Circuit Voltage (OCV) (MFC_OCV). We measured CO_2 assimilation rate and the concentration of metabolites produced by each system and estimated the overall energy spent in the capture process by BESs. We investigated the electronic exchange mechanism and potential effects of electrogenesys on microbial metabolism. Our results showed that *C. saccharoperbutylacetonicum* NT-1 achieved the maximum CO_2 assimilation (95.5 %) in MFC_1000 Ω i.e. when receiving the reducing power provided by the anode. MFC_1000 Ω was also the most efficient system, with 86.4 J spent per mole of CO_2. A Cyclic Voltammetry (CV) revealed a direct electron exchange at the cathode while the biosynthesis of D-3-hydroxybutyrate and the acetate was higher in MFC_1.5V and MFC_OCV respectively. We observed an increased ability to use polymers and amminoacids after a prologed utilization in BESs. The monitoring of microbial metabolism let us detect the change in the ability of bacteria to use different carbon source in BESs and opens to a better understanding of microbial physiology in presence of electrogenesis and its future exploitation.

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Heterologous production of a *Trametes polyzona* laccase in *Saccharomyces cerevisiae*

Daniela Bucchieri ORCID iD[1], Marco Mangiagalli ORCID iD[1], Immacola Serra ORCID iD[1], Fabrizio Beltrametti[2], Marina Lotti ORCID iD[1], Paola Branduardi ORCID iD[1]

[1]University of Milano Bicocca, Milan, Italy. [2]BioC-CheM Solutions Srl, Gerenzano, Italy

Abstract

In recent years, thanks to an increased awareness of the potential of biodiversity and to the versatility of biomolecular tools, the use of eco-friendly biocatalysts has increased further its biotechnological interest.

Laccases are considered versatile biocatalysts since they catalyze the oxidation of various aromatic and related compounds with the concomitant reduction of oxygen to water as the only by-product, without the need of additional cofactors.

Laccases can be exploited both for synthetic and degradative reactions and have gained a prominent role in different industrial fields for very diverse purposes, ranging from food additive and beverage processing to biomedical diagnosis, from pulp delignification to bleaching and textile dye transformations. Furthermore, their ability to transform complex xenobiotics makes them useful in enzymatic bioremediation and detoxification. For these reasons, new laccases are continuously sought as their different characteristics can match specific industrial requirements.

It is known that laccases are widely distributed in higher plants and bacteria, but mostly in white-rot fungi.

To identify novel enzymatic laccase activities of potential industrial interest, we tested several poorly characterized white-rot fungi that were screened on substrates known to be oxidized by the laccases. We focused on the best candidate, Trametes polyzona, which was further studied for laccase production. Moreover, to describe potential traits of interest of single enzymes, we overexpressed a putative T. polyzona laccase encoding gene in the yeast Saccharomyces cerevisiae. Here we will illustrate the cloning, expression and production strategies together with first results and perspectives on the potential interest of this novel laccase.

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Identification of industry-relevant genetic parts for heterologous protein production in the yeast *Kluyveromyces marxianus*

Carlos Belloch-Molina, Maria Jose Sousa-Gallagher, John P Morrissey

University College Cork, Cork, Ireland

Abstract

To achieve sustainability goals, biotechnology industries need to accelerate the design and improvement of bioprocesses for production of recombinant proteins, human therapeutics, and high-added value biomolecules. However, there are major challenges when combining the engineering and biological dimensions in bioprocess development. For instance, very often when designing the biological platform for protein expression, the final conditions in which the engineered microbial strain will grow are not fully considered. This design gap can lead to strains failing later when scaling up laboratory findings. To solve this issue, a top-to-bottom strategy is applied to develop strains suitable for bioprocesses that use the dairy side stream, whey permeate, as the growth medium. To achieve circularity, whey permeate can be converted into high-added value products by the dairy yeast *Kluyveromyces marxianus*. The main goal of this project is to identify genetic elements in *K. marxianus* (promoters, terminators and secretion tags), that will deliver high performance under industrial conditions, in order to design synthetic biology parts to apply for the production of recombinant proteins from cheese whey at the industrial scale. In this process-specific approach, *in silico* and transcriptomic approaches were applied to identify suitable parts, which are currently being tested in proof of concept and prototype systems.