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A001

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTY OF FINGER MILLET (Eleusine coracana) ON SOME SELECTED CLINICAL BACTERIA

Jeremiah Ogbeba

Federal Polytechnic Bauchi , Bauchi, Nigeria

Abstract

Finger millet in northern Nigeria was subjected to phytochemical screening using standard procedures. The agar well method was used to test the antibacterial activities of methanolic and aqeous (combined) extracts of the grain on Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The result of the antimicrobial activity as indicated by zone of inhibition ranged from 1-8mm for different extract concentrations. The finger millet extract showed zones of inhibition of 8mm against Pseudomonas aeruginosa at a concentration of 100mg/ml, 3mm at 50mg/ml and 2mm at 25mg/ml concentrations. The inhibition zones of Escherichia coli at extract concentrations of 100mg/ml,50mg/ml,25mg/ml, 12.25mg/ml and 6.125mg/ml were 4mm, 3mm, 3mm, 6mm and 1mm respectively, and for Staphylococcus aureus were 5mm,2mm, 1mm at 100mg/ml, 50mg/ml and 12.25mg/ml respectively. The zones of inhibition against all the tested isolates at 100mg/ml was not significantly different from those of 50mg/ml ($p=0.160$), 25mg/ml ($p=0.067$) and 12.5mg/ml ($p=0.160$), but significantly higher than 6.125mg/ml ($p=0.05$). Although S. aureus and S. typhi also did not differ significantly in their susceptibility to the varying concentrations of the extract ($p=0.157$), but susceptibility by S. typhi was significantly lower than those of E. coli ($p=0.007$) and P. aeruginosa ($p=0.015$). The qualitative phytochemical analysis indicated the presence tannin/phenol, flavonoids, alkaloid, saponin, glycosides, terpenoid and steroids in finger millet. The quantitative phytochemical revealed total phenolic content (6.57 mg/100g) and total flavonoid content (0.224 mg/100g). The overall results indicate that finger millet are potent antimicrobial preparations at least invitro and also have high nutritional value.

A002

Chaga Mushroom (Inonotus obliquus) inhibits growth of both lung adenocarcinoma (A549) cells and Aspergillus fumigatus

Magda Al-Tuwaijri[1], Jehane Ibrahim[2,3]

[1]Faculty of Applied Science - Umm Al-Qura University, Makkah, Saudi Arabia. [2]Adham university college - Umm Al-Qura University, Makkah, Saudi Arabia. [3]Faculty of Science - Cairo University, Makkah, Saudi Arabia

Abstract

Lung tumors and infections remain the most common leading cause of mortality worldwide. Chaga mushroom (Inonotus obliquus) has long been considered the king of medicinal mushrooms which constitutes an inexhaustible source of active compounds which can affect the survival of tumor cells. It has been used widely for centuries as a dietary supplement and tea.

In this sense, the aim of this study was to investigate in vitro cytotoxic capacity of water extracts of I. obliquus (Chaga mushroom) against the human lung cancer A549 cell line after 72 h incubation. In addition, the extracts were screened for antifungal activity on Aspergillus fumigatus species, a life-threatening cause of invasive pulmonary aspergillosis. The cytotoxic and the antimicrobial effects were performed using the MTT assay and the minimum inhibitory concentration (MIC) test, respectively.

Owing to the noticeable effect on antiproliferation of hot-water extracts, especially those from I. obliquus, the extract could be of great potential to be used as an alternative cancer therapy. However, it was not proven to have antifungal effect against A. fumigatus fungi.

A003

NUTRITIONAL QUALITY AND MICROBIAL DENSITY OF SWEET POTATO FLOUR FORTIFIED WITH SOYBEAN AND CRAYFISH FLOURS

Tesleem IBRAHIM

Rufus Giwa Polytechnic,, Owo, Nigeria

Abstract

Nutritional quality and microbial density of sweet potato based complementary food fortified with soybean and crayfish flours was investigated. Three different samples were produced from mixing sweet potato, soybean and crayfish at different formulation. Sample I, II, III are sweet potato: soybean: crayfish at ratio 80:15:5, 70:20:10 and 60:25:15 respectively. The formulated flours were analysed for proximate composition and microbial density. The result of proximate composition showed that the value of moisture content ranged between 6.50 - 8.50%, while ash content, crude protein crude fat, crude fibre, carbohydrate and energy value ranged from 5.50 – 9.50%, 25.10 – 28.50%, 4.20 – 6.20%, 0.13 – 0.27%, 47.73 – 56.27% and 363.42 – 370.48 Kcal/100g respectively. Its observed that as the level of inclusion of soybean and crayfish to sweet potato flour increases, there was increase in protein and crude fibre content but decrease in carbohydrate content. Also, the total bacteria count, coliform count, yeast and mould count ranged from 28-50 x 10^{-4}, 18-37 x 10^{-4} and 40-62 x 10^{-4} (CFU/g) respectively which are within the recommended limit value for microbial density in food. Therefore, sweet potato flour fortified with soybean and crayfish flour can be recommended as weaning food to reduce the incidence of malnutrition in infants.

A004

Detoxifying Potentials of Two Indigenous Adsorbents: Imarsil and Activated Charcoal in the Reduction of Aflatoxin in vegetable oils consumed in Nigeria.

Oni Eniola, Oluwafemi Flora, Kareem Sarafadeen, Omemu Adebukunolami

Federal University of Agricuture, Abeokuta, Nigeria

Abstract

Food contamination with aflatoxin is more prevalent in tropical regions where environmental conditions such as high temperature and humidity prevail, which favour the growth of toxigenic fungi and accumulation of these toxins in food and feeds represent a major threat to human and animal health. In lieu of the previously known adsorbents, adsorption studies of Aflatoxin (AF) were performed using inexpensive, readily available and local adsorbents *Imarsil* and activated charcoal (AC). Fifteen edible oils were purchases from open markets in Nigeria and screened for aflatoxin using High Performance Liquid Chromatography (HPLC). Thirteen out of the fifteen vegetable oil samples were positive to aflatoxin at the following concentration (172, 123, 195, 142, 46, 107, 96, 116, 22, 33, 228, 17 and 4) ng/kg while two had no detectable AF. At six different concentrations (0.5, 1, 1.5, 2, 2.5 and 3%) of *Imarsil* and activated charcoal (AC) with contact time of 1,2 and 3hours at room temperature (37°C), the aflatoxin-adsorbing capabilities depend on the adsorbent concentrations and contact time. *Imarsil* demostrated 100 % adsorption efficiency within one hour. At AF contamination rates of 96-228ng/kg, activated charcoal was not effective while *Imarsil* had 100 % removal efficiency within 3 hours with a significant reduction ($p< 0.05$) observed at the highest contamination rate and adsorbent concentration. AC demonstrated very mild adsorption activity. Results from this study indicated that Industrial incorporation of *Imarsil* into the oil refining process would reduce greatly the menace of aflatoxicosis. Hence, the use of *Imarsil* should be encouraged.

A005

Starter culture development using selected strains of Bacillus spp. Associated with "kantong" production in Ghana

Felix Kwashie Madilo[1], Richard Kwame Glover[2], Lawer Terlabie[3]

[1]University of Ghana, Accra, Ghana. [2]3International Council for Science, Regional Office for Africa, Pretoria, South Africa. [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Abstract

Technological properties of *Bacillus* spp. involved in fermenting *Ceiba pentandra* seeds into "kantong" a condiment prepared and consumed in the northern part of Ghana were invested so as to develop starter cultures with the predominant species for commercial production of "kantong". The *Bacillus* species which predominated 205 *Bacillus* strains isolated from 11 stages of "kantong" production includes: *Bacillus amyloliquefaciens, B. safensis, B. altitudinis, B. thuringiensis, B. pumilus, B. megaterium, B. cereus, B. circulans, B. coagulans, B. firmus, B. subtilis*, and *B. licheniformis.* These predominant species were assessed for some technological properties such as proliferation at different temperatures and pH; substrate utilization preferences including "kantong" formulated media (i.e. 48-hrs sample, dried pellet and the final product) and inhibitory activity against 12 selected pathogenic and spoilage microorganisms. The strains proliferated at different temperatures between 10°C and 55°C and pH of 2 to 9. Substrate utilization preferences were Nutrient Agar with 5-9% Sodium Chloride (NA/ NaCl C, and ordinary Nutrient Broth [NB], Nutrient Agar (NA), Potato Dextrose Agar (PDA), Tryptone Soya Agar (TSA), MacConkey Agar (MCA) and "kantong" formulated media. All strains exhibited inhibitory activity against one or more pathogenic and spoilage organisms. *Salmonella typhimurium, staphylococcus aureus, E. faecium* and *Proteus vulgris* were the most susceptible indicator microorganisms. Many strains qualified as potential candidates for selection and development as starter cultures to be used in the large scale commercial production of "kantong" of consistent and acceptable organoleptic quality.

A006

Carbapenemase-producing *Enterobacteriaceae* (CPE) isolated from pigs in China

Man-Ki Tong, Melissa Chun-Jiao Liu, Eileen Ling-Yi Lai, Kin-Hung Chow, Pak-Leung Ho

The University of Hong Kong, Hong Kong, Hong Kong

Abstract

Background:

The increasing prevalence of CPE is a global concern in public health. CPE were found to be present in livestock. Food animals act as a reservoir for NDM-producing bacteria. However, there is limited information about CPE in food animals. Here we screen the carbapenemase-producing bacteria in pig samples.

Objective:

To investigate the prevalence of carbapenem resistance in swine from China.

Methods:

A total of 138 rectal swabs from pigs imported from China were collected in Hong Kong between June 2017 and Oct 2018. Bacterial identification was conducted by MALDI-TOF for all isolates. Carba-NP test and disc diffusion method were performed to detect carbapenemase and determine the antibiotic susceptibility. Identification of carbapenemase gene and replicon type of plasmid, and further characterization of isolates were performed through PCR and next-generation sequencing respectively.

Results:

Twenty-one CPE isolates including *Escherichia coli* (*n*= 20) and *Enterobacter cloacae* (*n*= 1) were isolated from 20 pigs, which were resistant to carbapenem (meropenem, ertapenem and imipenem). The prevalence rate of carbapenemase producers was 14% (20/138). All isolates were positive in carba-NP test and harboured carbapenemase gene bla_{NDM}. Two-third of IncX3 (14/21) plasmid appeared in bla_{NDM}-producing isolates. Different resistance patterns were discovered among NDM-carrying isolates, but all of them were susceptible to fosfomycin and azithromycin.

Conclusion

Our data show that the prevalence of carbapenem-resistance Enterobacteriaceae among swine in China during 2017 to 2018. It is also observed that NDM carbapenemases is still circulating in pigs over times.

A007

Characterisation of the interaction of *Pseudomonas putida* and *Psuedomonas tolaasii* with *Trichoderma aggressivum* - co-pathogens of *Agaricus bisporus*

Dejana Kosanovic[1], Helen Grogan[2], Kevin Kavanagh[1]

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

Abstract

Button mushrooms (*Agaricus bisporus*) are consumed globally, but the industry is facing major problems controlling mycopathogens. Green mould disease is caused by *Trichoderma aggressivum* which colonizes mushroom compost and reduces yield. Two *Pseudomonas* species are associated with mushroom compost: *Pseudomonas putida* which stimulates mushroom pinning while *Pseudomonas tolaasii* produces a toxin, tolaasin, that has a negative effect on crop production. The aim of this work was to characterize *T. aggressivum* – *Pseudomonas* interactions as these may be an important factor in the development of green mould disease.

The effect of supernatants *P. tolaasii* and *P. putida* on *T. aggressivum* growth was assessed. *P. tolaasii* inhibited growth but *P. putida* stimulated growth. Tolaasin production was identified in *P. tolaassii* cultures and reached a peak at 120 hours. Fluorescent microscopy was performed on treated hyphae and showed that exposure to *P. tolaasii* supernatant decreased mycelial formation while increasing the abundance of conidia compared to *P. putida* and control groups. Label free proteomic analysis of changes in the abundance of *T. aggressivum* proteins following exposure of cells to *P. tolaasii* or *P. putida* supernatant was performed. Results indicate that exposure to *P. tolaasii* supernatant lead to an oxidative stress response, while exposure to *P. putida* supernatant lead to an increase in proteins associated with growth and development.

These results indicate that exposure to *P. putida* can stimulate the growth of *T. aggressivum* and this interaction may be an important factor in increasing green mould disease in mushroom crops and so reduce yield.

A008

The use of microbiological methods to reduce aflatoxin M_1 in cheese

Bruna Gonçalves, Jennifer Henck, Rômulo Uliana, Eliana Kamimura, Carlos Oliveira, Carlos Corassin

University of São Paulo, Faculty of Animal Science and Food Engineering, Pirassununga, SP, Brazil

Abstract

Studies have shown evidence of human exposure to aflatoxin M_1 due to the consumption of contaminated milk and dairy products (mainly cheeses). This poses a great risk to public health, since milk and milk products are frequently consumed by a portion of the population considered immunosuppressed, children and the elderly. Knowledge of the negative impacts of aflatoxins on health and economics has led to investigations of strategies to prevent their formation in food, as well as to eliminate, inactivate or reduce the bioavailability of these toxins in contaminated products This study evaluated the effect of microbiological methods using lactic acid bacteria on aflatoxin M_1 (AFM_1) reduction in Minas Frescal cheese (typical Brazilian product, being among the most consumed cheeses in Brazil) spiked with 1 µg/L AFM_1. Inactivated lactic acid bacteria (0,5%, v/v de *L. rhamnosus* e *L. lactis*) were added during the cheese production process. Nine cheeses were produced, divided into three treatments: negative controls (without AFM_1 or lactic acid bacteria), positive controls (AFM_1 only), and lactic acid bacteria + AFM_1. Samples of cheese were collected on days 2, 10, 20 and 30 after the date of production and submitted to composition analyses and determination of AFM_1 by high performance liquid chromatography. The reductions of AFM_1 in cheese by lactic acid bacteria at the end of the trial indicate a potential application of inactivated lactic acid bacteria in reducing the bioavailability of AFM_1 in Minas frescal cheese without physical-chemical and microbiological modifications during the 30-day experimental period.

A009

Impact of air pollution on buff-tailed bumblebee (*Bombus terrestris*) behaviour and their gut microbiome

Hannah Sampson, Julie Morrissey, Julian Ketley, Eamonn Mallon

University of Leicester, Leicester, United Kingdom

Abstract

Bumblebees play a major role in global pollination. Consequently, their health is of high importance for global food security. Yet, recent population estimates show that their numbers are declining. This decline has been attributed to habitat loss, infection and use of pesticides. An important factor for bee health that contributes to population survival is gut microbiome composition. The bee gut microbiome is functionally comparable to the human gut microbiome as they both provide protection from pathogens, are specific to the host and help break down food. Without a balanced gut microbiome, the health of the bee is threatened through increased infection and mortality. The bee gut microbiome is relatively simple, being dominated by 8 core bacterial species providing a convenient study system. Previous published data shows that air pollution has an impact on bacteria. Therefore, our hypothesis is exposure to air pollution causes an imbalance in the bee gut microbiome. To test this, we exposed bees to black carbon (BC), a major component of air pollution particulate matter, and assessed the effects on bee behaviour and microbiome composition. Bees treated with BC showed a significant reduction in viable bacterial cells in their faecal community. This supports the hypothesis that air pollution can cause an imbalance in the bee gut microbiome, thus adversely influencing bee health and pollinator populations.

Global food security: the challenges for microbiology
Presentations: Monday and Tuesday evening

A010

Integrated phenotypic and genomics analysis to elucidate differences in stress resistance and virulence of *Listeria monocytogenes* strains

Alexandros Stratakos[1], Mark Linton[1], Patrick Ward[2], Umer Ijaz[3], Pam Scates[1], Jane McBride[1], Ozan Gundogdu[4], Nicolae Corcionivoschi[1]

[1]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [2]Auranta, Dublin, Ireland. [3]University of Glasgow, Glasgow, United Kingdom. [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Listeriosis is an important food-borne disease responsible for high rates of morbidity and mortality. *L. monocytogenes* has been the cause of several food-borne outbreaks and product recalls throughout the world. It can adapt and survive in a wide range of stress conditions which makes it difficult for food producers to eradicate. The goal of this study was to use phenotypic assays and whole genome sequencing to elucidate possible links between food related stress resistance and virulence phenotypes in *L. monocytogenes* strains originating from different sources. Four *L. monocytogenes* isolates from sweetcorn and one isolate from a food processing environment (control) were sequenced and evaluated for the ability to survive in acid (pH 3.5, 15 min), in the presence of a commercial antimicrobial mixture (2% v/v, 90 min), heat (60^{o}C, 5 min) and hydrogen peroxide (420 mM, 15 min). Results showed that the strains had different resistance levels to the above stressors with the environmental strain being more susceptible to heat and the commercial antimicrobial. Also, results showed that the four sweetcorn isolates were more virulent than the environmental isolate as they had significantly higher attachment and invasion capacity onto HCT-8 cells ($P<0.05$). Pan-genome analysis revealed that the four isolates fall within a class associated with recent outbreak strains. Single Nucleotide Polymorphisms (SNPs) analysis was performed on the five genome sequences and subsequent cluster analyses on the resulting whole genome SNP matrix revealed differences between the strains.

A011

Distance-decay patterns overshadow effects of long-term fertilization and tillage on microbial community structure in agricultural soils

Claire Brown, Thorunn Helgason, Calvin Dytham, James Moir

University of York, York, United Kingdom

Abstract

Community profiling is one of the most utilised tools in microbial ecology today. The relationships between microbial communities and their environment affect ecosystem function in fields spanning from medicine to agriculture; and understanding the community dynamics of a microbial community is key to understanding the complex effects of human intervention. In this study, we look at the effects of long-term tillage and fertilization regimes in soils from an agricultural block-designed field trial set up in 2001. By studying the microbial community composition, absolute microbial abundance and diversity of denitrification functional genes in the context of environmental data, we were able to address the question of how specific land management histories affect the diversity and distribution of bacteria and denitrification genes within agricultural soils. It was found that microbial communities appear to be largely unaffected by land management history, and cluster predominantly by spatial location within the field, despite lack of significant environmental variation. In this well-established agricultural field trial, Euclidean distance is the major identifiable determinant of microbial community dissimilarity (as well as dissimilarity in microbial abundance).That ecological drift, rather than physicochemical factors can be the major determinant of genetic potential may have consequences for attempts to understand nutrient availability in agricultural systems. Additionally, the overwhelming variation caused by spatial distance indicates that block designed experiments may not always have sufficient statistical power to identify any effects of human treatment.

A012

Incorporation of *V. vulnificus* into marine snow for oyster uptake and *in vivo* bacterial competition assays.

Cam Hubert, Steve Michell

University of Exeter, Exeter, United Kingdom

Abstract

Vibrio vulnificus is a significant human pathogen found in high numbers in oysters. Despite the environmental prevalence of *V. vulnificus*, clinical cases are uncommon. We hypothesised that *in vivo* competition between *V. vulnificus* and other strains/species resulted in the killing of hypervirulent strains, reducing clinical incidence. To assess this, we have developed an oyster model into which we can ensure ingestion of high quantities of *V. vulnificus* using a defined 'marine snow'. Marine snow describes the aggregation of naturally occurring phytoplankton, bacteria, debris and other organic materials. Our marine snow substrate is comprised solely of the diatom, *Thalassiosira pseudonana.*

Bottles containing artificial seawater, 10^9 *T. pseudonana*, *V. vulnificus* culture and hyaluronic acid were rotated at 16 rpm for 24 hours to generate aggregates. *V. vulnificus*-containing marine snow was added to beakers holding individual oysters. Following 24 hours uptake, oyster stomachs were excised and homogenised in PBS. The resulting suspension was serially diluted for plate-counts and DNA extracted for downstream qPCR analysis.

Diatom-based aggregates present a controllable and reproducible model for incorporating *V. vulnificus* into marine snow. Using this methodology, we demonstrate greater uptake of *V. vulnificus* by oysters than any current study. This has potential applications for future *in vivo* work studying a range of microorganisms in oysters, such as other human pathogens or those of interest to aquaculture. Future work will undertake *in vivo* bacterial competition assays to determine the role that intra-/inter-species competition has on the ecology of *V. vulnificus* and whether this impacts the clinical incidence.

A013

Can footbathing prevent the colonisation of *Dichelobacter nodosus* and help towards the control of lameness in flocks?

Hayley Marshall[1], Lis King[2], Fiona Lovatt[1], Adam Blanchard [3], Peers Davies[4], Sabine Tötemeyer[1]

[1]University of Nottingham , Nottingham, United Kingdom. [2]AHDB, Kenilworth, United Kingdom. [3]Nottingham Trent University, Nottingham, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom

Abstract

Ovine footrot is a highly contagious disease, primarily caused by the Gram negative bacterium *Dichelobacter nodosus.* Recent studies indicate *D. nodosus* load is greatest on the interdigital skin of sheep with interdigital dermatitis, the initial clinical presentation of footrot. Widely endemic in the UK, footrot can cause severe lameness and has a great economic impact costing the industry between £24-80 million per year. The aim of these studies were to investigate the impact of a 2% glutaraldehyde footbath on *D. nodosus* colonisation of the ovine interdigital skin when applied once (study A) or weekly (study B).

Study A: Six sheep had the interdigital skin of each hoof swabbed before, immediately afterwards and at intervals over four weeks after a single footbath. Study B: Six sheep were swabbed before and twenty-four hours after each footbath and *D. nodosus* load was compared against a control group of six sheep. Swab samples were analysed by qPCR to determine prevalence and load of *D. nodosus* for both studies.

A single footbath had no effect on low levels of *D. nodosus*. In contrast, a weekly footbath significantly reduced the load of *D. nodosus* after six weeks of application. These data provide novel insight into the role footbaths play in the prevention of *D. nodosus* colonisation. These finding coupled with on-going research will enable the development of footbathing protocols for farmers to use to control the prevalence of lameness. In turn, this would improve animal welfare and productivity and ultimately contribute to improving UK food security.

A014

Sheep feet as a vehicle to transmit antimicrobial resistant bacteria in the environment

Hayley Marshall[1], Jessica Hardwick[1], Eoin Boyle[1], Dov Stekel[1], Lis King[2], Fiona Lovatt[1], Adam Blanchard[3], Peers Davies[4], Sabine Tötemeyer[1]

[1]University of Nottingham , Nottingham, United Kingdom. [2]AHDB, Kenilworth, United Kingdom. [3]Nottingham Trent University, Nottingham, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom

Abstract

The overuse of antibiotics in both human and animal medicine has intensified selective pressure on bacteria, promoting multiple mechanisms of resistance, spreading rapidly through horizontal gene transfer. Antimicrobial resistant bacteria, transferred from animals to humans, impact on future food security and human health. The UK sheep industry represents the largest in Europe and tetracycline is the most common antibiotic used to treat infectious diseases such as footrot.

E. coli isolated in the presence and absence of tetracycline from ovine interdigital skin swabs were whole genome sequenced and the antibiotic sensitivity was tested by disk diffusion assays. Antibiotics of veterinary importance (spectinomycin, neomycin), critically important (fluoroquinolone & carbapenem) and highly important to human health (2nd generation cephalosporin) based on WHO (World Health Organisation) classification were tested.

All of the *E coli* isolates were multidrug resistant (3-4 classes, including sulphonamides and aminoglycosides) forming 11 resistance profiles, with two dominant ones: Profile 1 with resistance to tetracycline, streptomycin, spectinomycin and sulphatriad; Profile 2 with resistance to tetracycline, streptomycin, spectinomycin, sulphatriad and intermediate level for imipenem.

Although isolates found on the ovine interdigital skin were not resistant to antibiotics important to human health, they are multi-drug resistant to antibiotics used in the sheep industry. In particular, resistance to spectinomycin is of concern as it is used to treat neonatal lambs with colibacillosis (watery mouth). This study highlights the importance of responsible use of antibiotics to slow the spread of resistance and to maintain effective treatment.

A015

Application of Plasma Activated Water for Decontamination of Alfalfa and Mung Bean Seeds

Bernardino Machado-Moreira[1,2], Brijesh Tiwari[3], Florence Abram[2], Catherine Burgess[1]

[1]Teagasc Food Research Centre, Ashtown, Dublin, Ireland. [2]Functional Envirnmental Microbiology, National University of Ireland Galway, Galway, Ireland. [3]Food Chemistry and Technology, Teagasc Food Research Centre, Ashtown, Dublin, Ireland

Abstract

Microbial contamination of fresh produce is a major public health concern, with the number of microbial disease outbreaks increasing in recent years. The consumption of sprouted beans and seeds is of particular concern, as these foodstuffs are generally consumed raw, and are produced in conditions favourable to growth of pathogenic microorganisms, if they are present in seeds prior to sprouting.

This work aimed to evaluate the activity of plasma activated water (PAW) as a disinfecting agent in Alfalfa (*Medicago sativa*) and Mung Bean (*Vigna radiata*) seeds, during seed soaking. Each seed type was inoculated with *Escherichia coli* O157, *E. coli* O104, *Listeria monocytogenes* or *Salmonella* Montevideo, and treated with PAW for one hour, three hours or overnight. Seeds treated for one and three hours were subsequently soaked in water to replicate commercial practices. Microbial counts for each pathogen were determined after treatment and soaking.

Observed reductions in alfalfa seeds range from a 0.86 log reduction in *S.* Montevideo concentration after overnight treatment, to a 1.13 log decrease in *E. coli* O104 levels after three hours of treatment and soaking. For mung bean seeds, observed results range from a 1.17 log decrease in the levels of *S.* Montevideo after overnight treatment, to a 2.48 log reduction in concentration of *E. coli* O104, after three hours of treatment followed by seed soaking.

These results demonstrate the potential for PAW to be used in the inactivation of pathogenic microorganisms on sprouted seeds and beans, ensuring the safety of the products for consumers.

A016

Characterisation of recent clinical Salmonella Dublin isolates from bovine abortions.

Jemma Franklin, Stijn Brussen, Sabine Tötemeyer

University of Nottingham School of Veterinary Medicine and Science, Nottingham, United Kingdom

Abstract

Salmonella enterica serovar Dublin is one of the most common bacterial causes of abortion in cattle in the UK. Despite its prevalence, little is understood about the progression of the disease from initial infection to abortion in pregnant females. Characterisation of strains implicated in bovine abortion will provide insights into the behaviour of the bacteria, and aid in our knowledge of bacterial dissemination and disease progression.

This study aims to describe the growth, virulence and antibiotic sensitivity of 15 circulating isolates derived from bovine abortions in 2017.

Sensitivity to antibiotics commonly used in the beef and dairy industries (tetracycline, streptomycin, chloramphenicol, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole and nalidixic acid) was measured by disc diffusion assays. Virulence was investigated by infecting bovine caruncular epithelial cells (BCEC) for 2 and 24 hours. All assays were performed alongside the well-characterised strain 2229 as a comparison.

Consistent with current UK surveillance findings, all 15 clinical strains were sensitive to all antibiotics tested in this study. All *S.* Dublin isolates were able to infect BCECs and replicate over the course of 24 hours, and initial studies suggest differences between strains.

Ongoing survival assays in serum will aid in our understanding of systemic dissemination. Taken together, these studies will provide insights into the progression of the infection leading to colonisation of the placentome and subsequent abortion.

A017

Investigating the effect of alkaline stress on biofilm formation by *Salmonella enteritidis*

Katerina Papakonstantinou[1], Georgios Efthimiou[2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Hull, Hull, United Kingdom

Abstract

Bacterial biofilm formation is an important survival strategy in multiple environments. It is affected by the attachment surface, the bacterial strain and the surrounding environment. In *Salmonella enteritidis*, a biofilm-forming foodborne pathogen, the molecular biofilm regulators act as on-off controls between the sessile and the planktonic population, while the exact underlying formation mechanism still remains unclear. The aim of this project is to study the effect of alkaline environment on the formation of *Salmonella* biofilms and to examine the architecture of biofilm produced under alkaline conditions, by use confocal microscopy. Neutral pH was found to be the optimal pH for *Salmonella* biofilm formation, while pH 10 significantly reduces it (p-value=0.015). However, cell viability remains high at pH 10, which suggests that the pathogen can easily survive the alkaline stress. Biofilm morphology at pH 7 is characterized by thick cell clusters, whereas at pH 10 it is characterised by thin layers of individual cells. These findings can help us understand how *Salmonella enteritidis* survives under highly alkaline conditions, potentially leading to the design of new and more effective disinfection strategies involving highly alkaline detergents.

A018

Identification of parasite-derived antimicrobial peptides to tackle emerging antimicrobial resistance.

Allister Irvine, Sharon Huws, Louise Atkinson, Angela Mousley

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a serious threat to Agri-Food sectors worldwide where traditional antibiotics are failing. The discovery of novel antimicrobials is critical to the sustained future control of agricultural diseases. Antimicrobial peptides (AMPs) hold potential as novel antimicrobials to combat AMR, with a number of AMPs in various stages of clinical trials. Invertebrates, which lack an adaptive immune system, combat microbial challenge through the activity of endogenous AMPs. This is particularly relevant to parasitic helminths which live in microbial rich environments, however knowledge of their AMP armoury is limited.

This project has identified > 2,000 putative AMP-encoding genes in parasitic helminths highlighting an untapped source of novel antimicrobials. *In-silico* mining approaches and computational AMP prediction tools were employed to search for AMPs within genome/predicted protein datasets, and to predict antimicrobial activity. The distribution of AMPs was mapped across 96 nematode and 31 flatworm species revealing a pan phylum profile. Glycine Rich Secreted Peptides (GRSPs) dominated the parasitic helminth AMP profile. Further analysis of helminth AMPs will characterise antimicrobial activity and explore the potential of parasite-derived AMPs against pathogenic microorganisms.

A019

Food Inspection of The Maltese Cheeselet using Hyperspectral Imaging. *The 'Food Inspection using Hyperspectral Imaging' (FIHI) project is financed by the Malta Council for Science and Technology, for and on behalf of the Foundation for Science and Technology, through the FUSION: R&I technology Development Programme.*

Sholeem Griffin, Owen Falzon, Kenneth Camilleri, Vasilis Valdramidis

University of Malta, Msida, Malta

Abstract

The island of Malta has a rich heritage, which is evident in the rustic appeal of this island. *Ġbejna* forms an integral part of the Maltese food heritage. This artisan product is made from sheep or goat milk curds and aged for several months to develop its distinctive taste. During the ageing process, the cheese can become spoiled by fungi and unsafe for human consumption. This is a significant public health risk and a financial liability for producers. Conventional microbiology techniques do not detect these slow-growing pigment-less fungi early, allowing occasional distribution of contaminated products.

We propose the use of hyperspectral imaging to detect these fungi during the early stages of cheese production. In contrast with a typical digital camera, which compiles the light signal into three broad wavelength bands; red, green and blue, a hyperspectral camera records numerous narrow and contiguous wavelength bands reflected from an object. This produces a series of images, each corresponding to the reflected electromagnetic energy in the respective narrow band of wavelengths. This image series may, in turn, be used for early fungal detection and identification.

To test this hypothesis, a model cheeselet was produced to conduct compatibility and stability studies, through measurements of colony forming units, water activity, moisture levels, pH, protein and sugar content. The *ġbejna* model was then challenged with fungal strains isolated from commercial ġbejna and imaged using a hyperspectral camera. An algorithm is under development to differentiate contaminated samples from uncontaminated samples using image analysis and multivariate statistics.

A020

Microbiome & resistome of the gastrointestinal tract of broiler chickens

Sarah Delaney[1,2], Charley McCarthy[1], Richard Murphy[2], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Ireland. [2]Alltech, Dunboyne, Ireland

Abstract

Antibiotics are used extensively in agriculture as therapeutics, and in some countries, for prophylaxis and as growth promotors. Alarmingly, there is a potential resistance transmission pathway from animals to humans through food. We analysed the microbiome and resistome of sixteen broiler chickens, which are raised for meat production. DNA was extracted from caecal samples taken at days 27 and 34 posthatch from broilers, half of which had their diets supplemented with a mannan rich fraction. Paired end sequencing was performed using an Illumina HiSeq 4000. The data was analysed by MGnify to generate taxonomic read files. The microbiome was analysed using Calypso software and the antibiotic resistance genes (ARGs) identified using the ARGs-OAP pipeline. The main phyla detected in all samples was Firmicutes and Bacteroidetes. Clostridia was the main class detected and Clostridiales the main order. Faecalibacterium, Lactobacillus and Bacteroides were the main genus detected, which are common in the broiler caecal microbiome. There was an increase in Bifidobacterium in the treated group. Principle component analysis showed that both time points cluster together. Rarefaction analysis confirms that a sufficient sequencing depth was obtained. Tetracycline resistance comprised the greatest proportions of ARGs present, followed by the aminoglycoside, macrolide, vancomycin, beta-lactam and bacitracin classes. Further analysis of the sequences will allow for full characterisation of the resistome between treatment groups. The antibiotic resistance residues in food animals may have the potential to disseminate antibiotic resistance to the human community via the food chain.

A021

Surveillance of Shiga-toxin producing *Escherichia coli* in Irish sheep

Siobhán McCarthy[1,2], Guerrino Macori[1], Catherine Burgess[1], Séamus Fanning[2], Geraldine Duffy[1]

[1]Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland. [2]University College Dublin, Belfield, Dublin 4, Ireland

Abstract

Shiga-toxin producing *Escherichia coli* (STEC) is a foodborne zoonotic pathogen of significant public health concern. Ruminant animals are considered the primary reservoir of STEC. STEC predominantly colonises the lower gastro-intestinal tract, termed the recto-anal junction (RAJ). The number of STEC shed in the faeces of ruminants can vary widely with some animals, termed 'super-shedders' (>Log_{10}4 CFU/g faeces), high risk carriers of the pathogen. The objective of this study was to sample a large cohort of Irish sheep, with quantitative and qualitative analysis of each sample for STEC. RAJ swab samples (N=410) were collected over a 9-month period from an ovine slaughtering facility. Each swab was enriched in 30ml of modified Tryptone Soya Broth with Novobiocin at 41.5^{o}C for 5 hours and subjected to a quantitative real-time PCR assay to detect and enumerate serogroups O157 and O26 in super-shedding animals. Incubation was allowed to continue for 24 hours and shiga-toxin prevalence was assessed using a targeted qualitative real-time PCR assay. Eight O157 strains were isolated, of which six were super-shedding strains. The incidence of *stx*, O157 and O26 positive swabs was 49.3%, 1.95% and 0.24% respectively. The prevalence of *stx1*, *stx2* and *stx1/stx2* virulence factors in isolated strains was 15.9%, 8.8% and 22.4%. Additionally, the occurrence of *stx1/stx2* in combination with *eaeA* in strains was found to be significant according to Pearson's correlation and a paired T-test. In conclusion, these results underline the risk Irish sheep pose as a potential source of STEC infection.

A022

Analysis of phenotypic traits which may impact long term survival of different *Escherichia coli* pathotypes

Jennifer Gray[1,2], Sonia Rani[1,2], Geraldine Duffy[2], Seamus Fanning[1], Catherine Burgess[2]

[1]University College Dublin, Dublin, Ireland. [2]Teagasc, Dublin, Ireland

Abstract

Shiga toxin producing *E. coli* (STEC) is a foodborne pathogen which causes severe, debilitating, and sometimes fatal, illness. Ireland consistently has one of the highest incidence rates of human STEC infection in Europe. Cattle are one of the primary reservoirs for STEC, excreting the pathogen in their faeces. The amount of pathogen excreted varies greatly, with animals shedding >log10^4 cfu/g faeces being termed "super-shedders". Human infection can occur from faecal contamination of meat, dairy, fresh produce or drinking water. STEC can survive for extended periods in soil, slurry and water, although the exact means is unknown. The objective of this study was to examine phenotypic traits potentially relevant to extended environmental survival in two strain banks: (1) clinical and bovine STEC, in comparison with non-STEC, isolated from the production environment and (2) *E. coli* O157:H7 of known shedding status. The strain banks were assessed for biofilm-forming abilities and the ability to adhere to the muscle component collagen-I, using a 96-well crystal violet assay, where the absorbance of bound cells indicated biofilm formation levels or adherence to collagen. Extracellular components involved in attachment were assessed using Congo red agar and pellicle formation was also examined. Phenotypic traits potentially related to extended environmental persistence were observed more frequently in non-STEC. However, these traits were also observed in some STEC isolates, showing phenotype is strain dependent indicating a risk for enhanced environmental survival of some STEC isolates. The shedding status of *E. coli* O157:H7 is not dictated by the investigated characteristics alone.

A023

Perceptions towards antimicrobial use and resistance in the UK pork supply chain

Hollie Bradford

Queens University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial Resistance (AMR) occurs when micro-organisms develop the ability to counteract antimicrobial drugs through previous exposure. The past decade has seen an increased prevalence of AMR bacteria due to widespread antimicrobial use (AMU). A substantial share of antimicrobial consumption is attributed to animal production, particularly within the pig industry as it is recognised to be a high user of antimicrobials. Considerable research has focused on the scientific mechanisms of AMR; however, limited literature exists regarding the perceptions of food producers towards AMR.

Four databases; Web of Science, PubMed, ScienceDirect and Google were used to search keywords; “UK,” “pork,” “supply” and “chain” to identify relevant papers. Each paper was inspected to ensure that a pork supply chain was illustrated. Interviews were conducted respectively with professionals working in the pork sector to verify the chain and uncover perceptions towards AMU and AMR.

Results verified the accurate mapping of the pork chain, enabling professionals to highlight areas of AMU. Stakeholders perceived antimicrobials as useful for the treatment of diseases however, opinions varied regarding the transfer of AMR to humans and the effects this may have on health.

To combat the problem of AMR and high use of antimicrobials within the pig industry, it is necessary to identify the key stages of AMU and to uncover stakeholder perceptions along the pork chain. Results will be verified using surveys and an intervention will be designed to enhance knowledge and understanding of AMR to influence a change in behaviour and thus, positively impact farmers on-farm practices.

A024

Proteomic Analysis of Three Ubiquitous *Phytophthora* Species Threatening Global Forest Ecosystems

Jamie McGowan[1], Eoin O'Connor[1], Richard O'Hanlon[2], Rebecca A Owens[1], David A Fitzpatrick[1]

[1]Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland. [2]Agri-Food and Biosciences Institute (AFBI), Belfast, Co. Antrim, United Kingdom

Abstract

Phytophthora are a genus of microbial, filamentous eukaryotes that morphologically resemble fungi but belong to the Oomycete class. *Phytophthora* species include some of the most destructive pathogens of plants, including many economically important crops and forest species. They represent one of the biggest threats to worldwide food security and natural ecosystems. *Phytophthora* are notorious for secreting large arsenals of effector proteins which facilitate infection by degrading host cell components, exploiting host nutrients, dampening host immune responses and inducing necrosis. Compared to other taxonomic groups, there is a paucity of OMICs data available to study *Phytophthora* species. To this end, we have used an LC-MS/MS strategy to perform the first large-scale profiling of the secretomes of three *Phytophthora* species that are an increasing threat to global forest ecosystems: *Ph. chlamydospora*, *Ph. gonapodyides* and *Ph. pseudosyringae*.

Together, *Ph. gonapodyides* and *Ph. chlamydospora* represent the two most widespread *Phytophthora* species, having been found in a wide range of habitats globally. *Ph. pseudosyringae* has been identified as the cause of oak and beech decline across Europe and America. Here, we use mass spectrometry to characterise the secretome of these *Phytophthora* species by identifying proteins secreted into different growth media. We detect a number of important effector families including proteins involved in the breakdown of plant cell wall carbohydrates (CAZymes) and toxin families such as necrosis-inducing proteins. Our results provide important insights into understanding the molecular mechanisms of *Phytophthora* infection.

A025

Biotechnological approach to produce riboflavin enriched iru - using riboflavin overproducing Bacillus subtilis

Afolake Olanbiwoninu[1], Anna Greppi[2], Christophe Lacroix[2], Christoph Jans[2]

[1]Ajayi Crowther University, Oyo, Nigeria. [2]Institute of Food, Nutrition and Health, Zurich, Switzerland

Abstract

Dietary deficiencies are major cause of malnutrition in the developing world, particularly vitamins deficiencies such as riboflavin, which lead to various health disorders. Traditional plant fermentation and their indigenous starter cultures such as Bacillus subtilis might provide solutions to bioenrich riboflavin.. We aimed to investigate this idea on the example of B. subtilis derived from iru an alkaline fermented condiment playing an important role in rural Nigeria. After initial isolation, identification and safety assessment, roseoflavin exposure was used to obtain riboflavin overproducing mutants. These were further analysed for functional characteristics. Bàcillus species' (n-123) were isolated from iru. Initial riboflavin production in supportive growth medium ranged from 50.3 - 479.0 ug/L for 27 out of 123 strains evaluated. Subsequent gràdual exposure to 200 mg/L roseoflavin increased riboflavin production in the three best producing strains from 350 ug/L to 542 ug/L, 479 ug/L to 580 ug/L and 362 ug/L to 618 ug/L. This increased riboflavin in lab-scàle iru fermentation by over 150 percent to 0.12 -0.14 mg/g and near the recommended daily intake while retaining desired proteolytic and esterase activity. This research provides important proof of concept for the bioenrich the of traditional B. subtilis based plant fermentations used across sub-Saharan Africa and possibly other areas globally.

A026

Identification and Characterisation of Antimicrobial Peptides across Phylum Nematoda

Sorcha Donnelly, Nikki Marks, Linda Stewart, Louise Atkinson, Angela Mousley

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial peptides (AMPs) are a key component of the innate immune system in invertebrates where they play an important role in protecting the animal from infection by various mechanisms.

Little is known about the profile and importance of AMPs to nematode biology, survival, and transmission. However, parasitic nematodes often live in hazardous environments, such as the gut, where they are exposed to a range of diverse bacterial pathogens. Therefore, they are likely to be armed with natural defence strategies that may include a battery of AMPs derived from nematode tissues and fluids, and/or nematode-associated bacterial AMPs derived from nematode microbiota, representing both cellular and humoral immune response mechanisms. *Ascaris suum* is an excellent model species due to its experimental tractability including large size, ease of tissue/fluid isolation, and susceptibility to a range tools.

The characterisation of nematode-derived antimicrobial peptides offers opportunities to exploit their role in nematode survival as a novel approach to anti-parasitic control. In addition, profiling the structures and activities of nematode- or nematode-associated bacteria derived AMPs may provide a template for the design of novel antimicrobials.

This project aims to exploit this experimental tractability of *A. suum* to identify and profile AMP expression in the pseudocoelomic fluid (PCF), to examine the antimicrobial activities of *A. suum* PCF under different environmental challenges, and to identify and functionally characterise the endogenous microbial community within the PCF.

A027

Commercial potential of plant growth promoting rhizobacteria on Amaranthus hybridus in Ede, Osun State

Oluwatosin Ajibade

Adeleke University, Ede, Nigeria

Abstract

The eco- friendly improvement of crop yield is a mammoth task that must be tackled in order to meet the ever increasing world population in need of food. Plant growth promoting rhizobacteria (PGPR) are organisms known to increase the growth of plants, by directly or indirectly facilitating crop yield by a number of mechanisms. Vegetables are stable foods rich in numerous vitamins. Low income countries get a balance diet through regular consumption of vegetables like *Amaranthus hybridus* which is regularly consumed with highly starchy food across the Southern states of Nigeria. This study investigated the effect of different bacterial suspension samples in increasing the growth parameters of *Amaranthus hybridus* through different plant growth plant growth conditions and treatments, determination of rhizospheric and endophytic bacterial colonization, RNA extraction, cDNA synthesis and qRT – PCR analyses, and Microarray hybridization. Bacterization by microorganisms tagged ADK 1, ADK 2, ADK3, ADK4, ADK 5, ADK 6 and ADK 7. The pot trial showed an increase in the growth yield of Plant affected by ADK 5 and ADK 7. A synergistic effect of ADK 5 and ADK 7 did not give an increased yield as each individual effect. An *Amaranthus hybridus* transcriptome analysis revealed that several genes showed differential expression after inoculated by ADK 7. These genes are implicated in stress response and hormone pathways. Investigations into the bacterization of indigenous vegetables by indigenously isolated bacteria is an eco - friendly agricultural practice to be promoted.

A028

on the cellular lipidome

Maia Kavanagh Williamson[1], Amjad Yousuf[1], Chris Arthur[2], Andrew Davidson[1]

[1]School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom. [2]School of Chemistry, University of Bristol, Bristol, United Kingdom

Abstract

Dengue represents the most important arthropod-borne viral disease, causing significant human morbidity and mortality worldwide, with ~350 million dengue virus (DENV) infections annually. With no antivirals or specific DENV treatment, and ongoing issues surrounding the only available DENV vaccine, research to understand the pathogenesis of this virus remains of critical importance. Lipids have been shown to play a key role in multiple stages of the life cycle of DENV and other flaviviruses.

We have developed a pipeline to conduct side-by-side lipidomic and proteomic analyses, to characterise alterations in the lipid profile between uninfected and DENV infected cells as well as to identify changes in lipid metabolic proteins, as potential antiviral targets. Infection of liver cells by DENV *in vitro* results in characteristic rearrangement of ER-derived membranes to form sites of viral replication termed 'replication complexes' (RC). Lipid extracts and protein preparations from 1) whole cells and 2) heavy membrane cellular fractions containing RC and shown to retain DENV RNA-dependant-RNA-polymerase activity, are being assessed by LC-MS/MS. Identified alterations in lipid metabolic proteins will be validated by western blot and their role in DENV infection investigated by siRNA knock-down. We have also produced Huh-7 cells stably expressing a DENV-2 replicon which are being assessed for their use as a model DENV-2 system for lipidomic studies.

Vaccines against bacterial pathogens
Presentations: Monday and Tuesday evening

A029

The identification of novel vaccine antigens and their potential role in preventing VTEC infections in children.

Conor Quinn[1], Susanna Frost [2], Siobhán McClean [1]

[1]University College Dublin, Belfield, Dublin 4, Ireland. [2]National Children's Hospital, Tallaght Hospital , Tallaght, Dublin 24, Ireland

Abstract

VTEC are a group of strains of *E. coli*, which cause severe bloody diarrhoea. VTEC infections can lead to the development of haemolytic uraemic syndrome (HUS), which is the main cause of kidney failure among children. HUS can cause other life-long complications including seizures, bowel perforation and blindness. Ireland currently has the highest incidence of VTEC infections compared to any other European country. There are no vaccines available to protect children and immunocompromised adults against VTEC infections. Due to the risk of patients experiencing severe symptoms and complications, there is an urgent need for a vaccine against this infection. Bacterial proteins involved in host cell attachment have previously been shown to be efficacious prophylactic vaccine antigens for other infections. We have shown that an O157 strain, NCTC12900 has 1.3-fold higher binding to HT29 cells than the commensal strain, HS ($p=0.0162$). We have used a proteomic approach to identify the bacterial proteins involved in attachment to two human gastrointestinal epithelial cell lines, HT29 and Caco-2. We have identified seven host cell attachment proteins in VTEC, strain NCTC12900, that are not found in commensal strain, HS. These antigens will be examined for their ability to protect mice from VTEC challenge.

Vaccines against bacterial pathogens
Presentations: Monday and Tuesday evening

A030

Potential glycoengineered anti-Burkholderia vaccines by exploiting the bacterial O-glycosylation machinery

Guan-bo Wang, Lena Glaser, Rebecca Ingram, Miguel Valvano

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

Abstract

Previously, we discovered a protein *O*-glycosylation (*ogc*) cluster conserved in all *Burkholderia* species, which glycosylates proteins with a trisaccharide glycan. Sera from *Burkholderia*-infected patients produce anti-glycan antibodies, suggesting that the *Burkholderia* protein glycosylation pathway can be exploited for potential vaccine development. Here, we successfully produced two prototypes of anti-*Burkholderia* vaccines: a recombinant glycoprotein-based vaccine and an *E. coli* LPS-display vaccine. To generate the former, we constructed a plasmid carrying a chimeric gene encoding three glycosylation sequons fused to the cholera toxin B subunit. The presence of the glycan was observed in recombinant proteins expressed in *B. cenocepacia* parental strain, but not in proteins expressed by the glycosyltransferase-deficient Δ*pglL* strain, as determined by SDS-PAGE and fluorescent lectin blots. For the development of an *E. coli* LPS-display vaccine, we constructed a plasmid expressing the *ogc* cluster, which was introduced into an *E. coli* strain unable to synthesize O-antigen but carrying the O-antigen ligase WaaL. Our results show that the LPS of this strain contained an additional moiety consistent with the *B. cenocepacia* trisaccharide glycan, as demonstrated by silver-stained LPS gels and lectin blot. This extra moiety was not detected in a Δ*waaL* mutant. These results suggest that the plasmid was able to provide the necessary functions for the synthesis and membrane translocation of the lipid-linked trisaccharide, which became a substrate for the WaaL ligase and incorporation into the *E. coli* LPS. Therefore, we demonstrate that the O-glycosylation pathway can be manipulated for the construction of potential anti-*Burkholderia* vaccines.

Microbial dark matter

Presentations: Monday and Tuesday evening

A031

Beyond the filter: filterable lotic microorganisms and their role in dissolved organic carbon (DOC) cycling

Lydia-Ann Ghuneim[1], Rafael Bargiela[1,2], Tatyana Chernikova[1,2], Stephan Toshchakov[3], Manuel Ferrer[4], Olga Golyshina[1,2], Peter Golyshin[1,2], David Jones[1]

[1]Bangor University, Bangor, United Kingdom. [2]Centre of Environmental Biotechnology, Bangor, United Kingdom. [3]Winogradsky Institute of Microbiology, Moscow, Russian Federation. [4]Institute of Catalysis, Madrid, Spain

Abstract

Microorganisms residing in lotic (river) systems are integral to the cycling of dissolved organic carbon (DOC). However, these communities are challenging to study due to the constant fluctuating conditions (weather patterns, anthropogenic activities, etc.) and our inability to culture their members *in vitro*. Recent evidence suggests that ultra-small and filterable species residing in the freshwater systems may utilize low molecular weight DOC. Therefore, the aim of this study was to (1) determine the taxonomic identity and community dynamics of filtered (0.22 µm pore size) microorganisms residing in water of the Conwy River (North Wales, UK), and (2) investigate their role in cycling of low molecular weight DOC (amino acids, sugars, and organic acids). We achieved this by comparing the communities of the unfiltered and 0.22 µm filtered fractions over a three-week period using a combination of radioisotope labeling, metagenomics, and metabolomics techniques. The results show that unfiltered and filtered communities are inherently different in their taxonomic makeup, and their ability to utilize low molecular weight DOC. This suggests that they may have different functional roles in freshwater ecosystems. Further work is required to elucidate the origin, abundance and functional significance of these ultra-small bacteria and archaea communities across a more diverse range of freshwaters.

Microbial dark matter

Presentations: Monday and Tuesday evening

A032

Phylogenetic and genomic characterisation of narnaviruses: a diverse group of non-encapsidated RNA viruses

Adam Dinan, Ingrida Olendraite, Katherine Brown, Andrew Firth

Division of Virology, University of Cambridge, Cambridge, United Kingdom

Abstract

Narnaviruses are a group of single-stranded, positive-sense RNA viruses, which are non-encapsidated and hence normally transmitted vertically. Narnaviral genomes encode a single protein: the RNA-dependent RNA polymerase (RdRp), which catalyses viral replication. Currently, there are just two recognised species within the genus *Narnavirus*, both of which are known to infect *Saccharomyces* yeast. Here, we systematically identify and characterise narnaviral genomes in public sequence databases, using a combination of *in silico* approaches.

We identify two major clades of narnaviruses, and propose the establishment of a taxonomic framework based upon their molecular characteristics. Codon usage bias across both clades was analysed and compared with those of potential host taxa from across the eukaryotic domain of life. In one clade, we demonstrate the widespread presence of a long reverse-strand open reading frame (rORF), which typically occupies > 90% of the full-length genomic RNA. Comparative analysis shows that the putative rORF-encoded proteins are highly divergent in amino acid composition, with a central region of increased conservation. These findings shed new light on one of the most divergent clades of eukaryote-infecting viruses.

Microbial dark matter

Presentations: Monday and Tuesday evening

A033

Comparative analysis of the composition and change of the microbiome of diabetic foot ulcers from patients on different therapies

Thomas Walker[1], Dr Janice Spencer[1], Dr Stephanie Dancer[2], Professor John Craft[1]

[1]Glasgow Caledonian University, Glasgow , United Kingdom. [2]Hairmyres Hospital , Hairmyres, United Kingdom

Abstract

Diabetic foot ulcers (DFUs) are slow healing wounds which arise from co-morbidities associated with diabetes. Often these ulcers become infected leading to gangrene, osteomyelitis and sepsis. Current treatment options include debridement and a topical irrigant which have limited success. Understanding the effects that these treatments have on microbiome of DFUs and on wound healing is poorly understood.

This study compared the efficacy of two irrigant solutions (Prontosan and Electrolysed water –E.W.) on their impact on the DFU microbiome, their role in DFU healing and their effect on biofilm viability. Sequential samples taken from 7 patients undergoing treatment with either one of the irrigants, over a 4 week period revealed commonly observed genera present included *Staphylococci* (96%), *Propionibacterium* (96%) and *Finegoldia* (89%). A unique composition and diversity was observed in the microbiome of each individual DFU. Increasing microbial diversity within the DFUs was correlated with an elevated percentage abundance of anaerobic and Gram negative genera whilst inversely correlated with facultative anaerobic and Gram positive genera. No significant reduction in diversity or species richness of the DFU microbiomes was observed after treatment with either irrigant.

Both Prontosan and E.W. had similar effects upon *S.aureus* biofilms reducing viability by 82.013% and 86.89% respectively however E.W. efficacy was strain specific. In addition, E.W. was ineffective at preventing biofilm formation in 6/8 (75%) *S.aureus* strains. Better understanding of the DFU microbiome and investigations into novel therapies is paramount to aid our ability to improve the quality of life for diabetic patients.

A034

Characterising the human intestinal mycobiome during healthy ageing

Steve James, Sarah Phillips, David Baker, Lindsay Hall, Simon Carding

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Although fungi are fundamental to the human microbiome, the diversity and dynamics of the mycobiome is poorly understood, particularly in considering their association with infectious disease, autoimmune disorders and atopy that affect immunocompromised individuals and infants. Characterising the human mycobiome faces several challenges relating to their low abundance and lack of standardized procedures for sample collection and isolation of viable cells and/or quality genetic material for culture-dependent and -independent taxonomic and functional characterisation.

To address these issues, we have developed a mycobiome analysis pipeline employing both culture-dependent and -independent methods to identify as well as isolate, where possible, the fungal taxa populating the human intestinal tract. In a proof-of-concept study this pipeline has been used to identify fungal populations in faecal samples obtained from a small cohort of young infants, aged 2 years or younger. All were born prematurely, and severely immunocompromised and at risk from invasive and potentially lethal microbial infections, including those caused by fungal overgrowth.

We have used this combined approach successfully to identify the fungi present in each individual infant, and to recover viable isolates. To date, *Candida albicans* and *C. parapsilosis* are the most frequently isolated fungi. While both are major opportunistic human fungal pathogens, *C. parapsilosis* is particularly problematic to preterm babies, due to its innate ability to form biofilms. Detailed characterisation of these isolates is currently underway.

Two large-scale longitudinal microbiome studies have started at the Quadram Institute, and our validated analysis pipeline will be incorporated to define the fungal component of each study participant.

Microbial dark matter

Presentations: Monday and Tuesday evening

A035

Extraction and identification of components of the biofilm matrix in *Pseudomonas* species biofilms

Laura Quinn, Caio Barros, Stefania Vitale, Eoin Casey

University College Dublin, Dublin, Ireland

Abstract

The surface associated communities of microorganism in biofilms are encased in a matrix of extracellular polymeric substances (EPS). The EPS is made up of mainly polysaccharides, proteins, nucleic acids and lipids and plays an important role in maintaining the integrity of the biofilm[1]. Although the general composition of the EPS is known, it can be highly variable among strains and among different growth conditions for the same strain. Due to the large variety of biopolymers in nature and the difficulty in their analysis, EPS has been called 'the dark matter of biofilms'[2].

In order to develop a comprehensive understanding of the matrix of the biofilm, EPS was extracted from four *Pseudomonas* spp., mCherry-expressing *Pseudomonas fluorescens*, GFP-expressing *Pseudomonas putida* and the wild types of *Pseudomonas fluorescens* and *Pseudomonas putida*. The extractions were carried out on biofilms grown on glass slides using the cation exchange resin (CER) method. Colorimetric methods were used to quantify the sugars and proteins present in the EPS. These colorimetric assays showed that there was a larger amount of proteins present compared to sugars. The proteins present in all four biofilms of *Pseudomonas* spp. were identified by LC-MS/MS while NMR and HPLC were used to identify the sugars present. The knowledge gained by these results have the potential to aid in the development of biofilm eradication methods through the targeting of specific components of the EPS.

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2. Flemming, H.-C. and J. Wingender (2010). *Nature Reviews Microbiology* **8**: 623.

A036

Diversity of DNA and RNA viruses in polar regions

Alberto Rastrojo[1], Carlos Fernandez-Linares[1], David Pearce[2], Antonio Alcami[1]

[1]Centro de Biologia Molecular Severo Ochoa, Madrid, Spain. [2]University of Northumbria, Newcastle upon Tyne, United Kingdom

Abstract

RNA viruses may be as abundant as DNA viruses but studies on RNA viruses are scarce. Metagenomic studies of RNA viruses in freshwater lakes in the Arctic (Svalbard) have shown that, as we described for Antarctic RNA viruses, most of them belong to the order Picornavirales. We have characterized their genetic variability (quasispecies), which may represent a mechanism of adaptation.

Polar regions harbour a great diversity of DNA viruses. We have carried out extensive bioinformatic analysis and assembled >9.000 new circular ssDNA viral genomes from polar regions that group in >100 clusters, largely increasing the existing database (~1.300 circular ssDNA viruses,<10 families). Additional analysis including ~32.000 circular ssDNA viral genomes, assembled from published viral metagenomic studies, group them within the >100 clusters we described. Interestingly, many clusters are composed exclusively of polar viruses, suggesting new viral families adapted to polar environments. Sequence variability, similar to RNA viral quasispecies, has been suggested in circular ssDNA viral genomes but this has not been explored. We have identified complex quasispecies (high sequence variability) in several polar circular ssDNA viruses.

These studies illustrate the power of our metagenomic approach to identify new viruses from polar regions. High genetic variability may represent a mechanism of rapid virus adaptation to changing conditions in natural ecosystems and facilitate virus colonization.

A037

Fundamental nanoparticle interactions with biofilms of *Pseudomonas* species

Henry Devlin, Caio Barros, Stephanie Fulaz, Dishon Hiebner, Laura Quinn, Stefania Vitale, Eoin Casey

University College Dublin, Dublin, Ireland

Abstract

The use of engineered nanoparticles (NPs) as a technique for antimicrobial delivery aimed at biofilm treatment is an emerging field of research. There have been numerous studies involving a wide range of NPs showing varying results regarding anti-bacterial effects [(1)]. However, research focusing on specific interactions between functionalized nanoparticles and the extracellular polymeric substances (EPS) of biofilms are limited. The complexity of the biofilm matrix may be hindering the understanding of the fundamentals which govern biofilm – nanoparticle interactions.

There are a wide range of physicochemical properties which influence the uptake and retention of nanoparticles within the matrix including NP size and charge properties, biofilm topography and porosity and EPS composition [(2)]. These aspects must be considered when studying biofilm – nanoparticle interactions.

In order to identify these specific interactions, a series of experiments were carried out using mCherry-expressing *Pseudomonas fluorescens* and GFP-expressing *Pseudomonas putida* biofilms. Using high throughput fluorescent intensity measurements and confocal microscopy, it was possible to investigate the uptake of surface functionalized silica NPs by the two biofilms and obtain valuable information regarding biofilm – nanoparticle interactions. The results suggest that specific NP surface functionalization has a major role in guiding the interaction and binding of EPS components, possibly due to electrostatic interactions between NPs and the EPS. The findings of this research will help with the future design of nanoparticles with specific modes of action towards components in the EPS.

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Microbial dark matter

Presentations: Monday and Tuesday evening

A038

Shining a light on microbial dark matter: a role for the forgotten B vitamins in marine algal communities?

Dominic Absolon[1], Alison Smith[1], Katherine Helliwell[2]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Marine Biological Associatoin, Plymouth, United Kingdom

Abstract

The ocean is home to a huge diversity of life that perform a wide range of functions key to earth systems. Marine algae communities contribute a significant proportion of net carbon fixation of the globe as well as forming the basis of the food webs for every trophic level above their own. Despite their importance, many of the taxa that make up these microalgal communities are relatively unknown and understudied, due to their recalcitrance to lab culturing. This concept is known as microbial dark matter and applies in particular to many non-photosynthetic lineages which have been long overlooked by the research community. To investigate whether dependencies of these taxa on certain B vitamins may play a role in this unculturability, data sets produced form the *Tara* oceans expedition will be analysed. The Stramenopile lineage will be analysed in greater detail for their metabolic potential to synthesise the B vitamins, and/or whether they may dependent on an external source. Both Single-Amplified Genomes (SAG) and metatrascriptomic data sets will be analysed to determine the complement of genes present in different Stramenopile lineages. This will then be correlated with the global distribution data attainable from analysing *Tara* data sets. By this means, we hope to understand more about the metabolic contribution of this taxonomic group to the community, how this supports the community as a whole and whether dependence on B vitamins is a contributing factor to unculturability.

A039

Small genomes and metabolic diversification of anaerobic methanotrophic archaea in a deep-sea cold seep

Yong Wang, Wen-Li Li

Institute of Deep-Sea Science and Engineering, CAS, Sanya, China

Abstract

Methane assimilation by anaerobic methanotrophic (ANME) archaea is an important way to regulating the effectiveness of the greenhouse in deep-sea cold seeps.Subsurface microbes have very low mutation rates, and their evolution is likely controlled by selection of different environmental settings.In this study, a total of 13 ANME genome bins representing five ANME types were obtained from the Jiaolong cold seep in the South China Sea. This includes the first report of ANME-1b, -2b and -2c genomes. Despite >50% genomic reduction of our ANME bins, compared with the reference genomes for the cultivated strains in bioreactors, the pathways of methane assimilation and carbon dioxide fixation were all identified in the bins. Our genomic bins lack the genes for TCA cycle and most of those for carbohydrate and inorganic ions transport and metabolism in the cultivated strains. We also identified the genes involved in utilization of alkanesulfonate in our ANME genomes perhaps for adaption to sulfur starvation. All the genes related to reduction of nitrogen and sulfur oxides were absent from the bins, indicating their syntrophic dependence on partner organisms. Acquisition and loss of the genes in the ANME genomes exhibited a momentous role in shaping genetic diversity and ecological divergence among sympatric microbes in the cold seep. Our study also detected *in situ*transcriptional activities of the ANME types in four subsurface layers, which provides further evidence for the critical role of the ANME in the carbon and nitrogen cycles.

Microbial dark matter

Presentations: Monday and Tuesday evening

A040

Investigation into the Physicochemical Interactions of Silica Nanoparticles and EPS Biomolecules within the Biofilm Matrix of *Pseudomonas spp.*

Dishon Hiebner, Caio Barros, Henry Devlin, Stephanie Fulaz, Laura Quinn, Stefania Vitale, Eoin Casey

School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland

Abstract

Difficulties in the removal of bacterial biofilms in the industrial and biomedical sectors have driven the development of new technologies. Although numerous studies have highlighted the use of nanoparticles (NPs) as antibiofilm agents, the fundamental physicochemical interactions between NPs and the biofilm matrix is still poorly understood[1]. The development of "smart nanoparticles" for biofilm removal requires an in-depth understanding of the complex interactions between NPs and biomolecules within the extracellular polymeric substances (EPS) of the biofilm matrix. These interactions are highly dependent on the physical and chemical properties of the NPs[2].

In order to identify and characterize the specificity of binding and the direct interaction between silica NPs (SiNPs) and EPS matrix components of *Pseudomonas spp.* biofilms, a range of experiments were carried out. Biofilms were exposed to SiNPs of different sizes, charges and surface functionalization while biomolecules such as proteins, polysaccharides, and eDNA were fluorescently labelled and their distribution, relative abundance and their colocalization with SiNPs within the biofilm was quantitatively assessed using CLSM microscopy.

Changes to the SiNPs size and surface-chemistry dramatically affected their interactions with biomolecules in the biofilm matrix. This includes the increased affinity (or interaction) of SiNPs to preferentially bind to proteins and beta-linked polysaccharides and also lead to changes in the degree to which aggregation of SiNPs occurs within and on the surface of the biofilm.

1. Ikuma K *et al.* (2015). *Front. Microbiol*. 6, 591

2. Bewersdorff*, et al. (2017). Int. J. Nanomed.* 12*,* 2001–2019

Microbial dark matter

Presentations: Monday and Tuesday evening

A041

Leveraging Structural Relationships as a Novel Mode of Viral Classification

Damian Magill[1], Timofey Skvortsov[1], Vincent O'Flaherty[2], John McGrath[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]National University of Ireland Galway, Galway, Ireland

Abstract

100 years have passed since the independent discovery of the humble bacteriophage (phage) by Frederick Twort and Felix d'Herelle in 1915 and 1917 respectively, and since then, it has become commonly accepted that phages represent the most abundant biological entities on Earth. Despite this fact, viral taxonomy lies in extremely treacherous waters, ever changing to accommodate the next series of phylogenetic mysteries.

The utilisation of genes such as the terminase large sub-unit can in some cases provide a robust taxonomic marker, but this is often found to fail at higher taxonomic levels. In addition, the rapid evolutionary dynamics and highly modular nature of phages provide yet more phylogenetic roadblocks, necessitating additional and multifaceted approaches as a means of resolution.

Here, we describe a novel approach towards the taxonomic classification of phage systems. Tools for accurately predicting the three dimensional structure of proteins are improving at an unprecedented rate due to the fact that the number of protein sequences far exceeds the number of experimentally determined structures. Our approach leverages these methods through a pipeline which compares models of phage marker genes in order to permit the inference of phylogenetic relationships based on cross model superimposition. We hope this method will supplement other approaches in providing a more holistic approach to viral classification.

Microbial dark matter

Presentations: Monday and Tuesday evening

A042

Characterisation of the Microbiome for two Hexactinellid Sponges and Purification of Associated Antimicrobial Agents from their Resident Microbes.

Matthew Koch, Poppy Best, Garry Farnham, Michele Kiernan, Alistair Bishop, Philip Warburton, Kerry Howell, Mathew Upton

University of Plymouth, Plymouth, United Kingdom

Abstract

The imminent threat of antimicrobial resistance has necessitated that the search for novel antimicrobials be widened to lesser-explored environments. Marine and freshwater sponges have emerged as the most prolific source of such compounds over the last decade, representing the most widely sampled phyla in the hunt for novel biologics over the last 45 years. Most of the work however has focused on sponges from shallow waters, with the deep-sea sponge microbiome highlighted as a major source of untapped antimicrobial potential.

Optimisation of bacterial recovery was carried out for two previously unstudied species of deep-sea Hexactinellid sponge species (*Pheronema carpenteri* and *Rhabdodictyum delicatum* recovered from the Rockall Trough, North Atlantic), using a variety of culture media, supplementation and environmental conditions. This optimisation was carried out in parallel with 16S rDNA metagenomic sequencing in order to determine community composition for both sponge species (IonTorrent, Life Technologies). All recovered isolates were assayed for antimicrobial activity, forming a panel of 'active' organisms. Two isolates (Ph16-28; A11) were selected for downstream purification and characterisation of the responsible antimicrobial agent via column chromatography. Isolate identities are currently being confirmed via draft whole-genome sequencing (MinION, Oxford Nanopore), and are suspected to be members of the *Bacillus* and *Streptomyces* genera.

Current data provides a working axiom for the cultivation of deep-sea sponge microbes and suggests the deep-sea sponge microbiome to be a promising source for novel antimicrobials.

Microbial dark matter

Presentations: Monday and Tuesday evening

A043

Uncovering the Dark Matter of the Metagenome One Read at a Time.

Nicholas Dimonaco[1], Chris Creevey[2], Robert Hoehndorf[3], Maxat Kulmanov[3], Wang Liuwei[3], Amanda Clare[1], Wayne Aubrey[1], Kim Kenobi[1]

[1]Aberystwyth University, Aberystwyth, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom. [3]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Abstract

Contemporary metagenomic annotation methods have proven insufficient in our attempts to better understand the complex environments around us. We call the yet to be annotated part of a metagenome it's 'dark matter'. The Gene Ontology (GO) is a hierarchical vocabulary used to describe gene product function and a large collection of curated genes with GO annotations already exists. DeepGO utilises deep learning to build models from these curated genes and gene products to predict GO categories for novel proteins.

One of the major problems with metagenomic studies today is the process of assembling the environmental DNA sequences into their original genomes. This is difficult, with chimeric metagenomically assembled genomes being common. To avoid this and the computational and time expense, we have modified DeepGO to perform protein function prediction directly from sequence reads with limited protein coding sequence prediction. Three independent models were trained as the following; The first 50 amino acids of a protein were used for training, The last 50 amino acids were used for training, A phasing window of 50 amino acids was used to train across the entirety of a protein sequence. These models were chosen to learn from the different parts of a protein sequence we are likely to capture from only the short unassembled sequence reads.

We compared the three models by producing a mock metagenomic community consisting of 6 model bacterial genomes. We evaluated the functions predicted from the unassembled sequence reads and the protein coding sequences predicted from the assembled metagenome.

A044

Effect of dietary olive oil and palm oil on rumen bacterial composition in dairy cows

Nathaly Cancino[1], Jaime Romero[2], Sharon Huws[3], Einar Vargas[1]

[1]Pontificia Universidad Catolica de Chile, Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Santiago, Chile. [2]Universidad de Chile, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Santiago, Chile. [3]Queen's University of Belfast, Institute for Global Food Security, School of Biological Sciences, Belfast, United Kingdom

Abstract

The rumen bacteria play a major role in lipid metabolism. Bacteria remove the double bonds of unsaturated fatty acids resulting in the production of saturated fatty acids, which are incorporated in milk. Crude olive oil (rich in unsaturated fatty acids) represents a potentially valuable feed source for dairy cows that might enhance the human-health beneficial composition of milk and dairy products.

This project studied the effect of supplementing dairy cow diets with olive oil (OO) and palm oil (HVO) on rumen microbiota. For 63 days the animals were fed a control diet (basal diet) with no added lipid and two fat-supplemented diets (30 g/kg DM). Rumen sampling were performed at the onset of the experiment and every 21 days for 63 days using a rumen scoop. Total microbial DNA was extracted from ruminal samples for high-throughput sequencing of the 16S rRNA gene through Illumina MiSeq platform.

Results revealed the dominance of phyla Firmicutes and Bacteroidetes. Firmicutes was the most prevalent phyla in diet control (75.2%), OO (71.1%) and HVO (75.2%). At genus level Succiniclasticum and Prevotella were the dominant genera, belonging to Firmicutes and Bacteroidetes respectively. Succiniclasticum decrease significantly their relative abundance during OO supplementation ($p < 0.0001$). Decrease in genera Succiniclasticum might imply propionate reduction and consequently increase milk fat percentage. In addition, milk composition analysis showed reduction in milk fat yield (14.6%) with OO supplementation however this diet also increased the total polyunsaturated fatty acids in milk ($P < 0.05$), which could be beneficial for the human health.

Microbial dark matter

Presentations: Monday and Tuesday evening

A045

The Human Gut Mycobiome in Inflammatory Bowel Disease

Chloe E. Huseyin[1,2], Jamie A. FitzGerald[1,2], Claire Shannon[1], Emilio J. Laserna-Mendieta[3], Fergus Shanahan[2,4], Pauline D. Scanlan[1,2], Marcus J Claesson[1,2]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]APC Microbiome Ireland, University College Cork, Cork, Ireland. [3]Research Unit, Department of Gastroenterology, Hospital General de Tomelloso,, Tomelloso, Spain. [4]College of Medicine, University College Cork, Cork, Ireland

Abstract

Introduction:

The human gastrointestinal (GI) tract is inhabited by a myriad of microorganisms including bacteria, viruses, archaea and fungi[1]. The microorganisms present in the GI tract are a popular target for investigation with respect to inflammatory bowel disease (IBD) aetiology[2]. Indeed, numerous studies have reported differences in the microbiome (bacterial population) between patients with IBD and controls[3] . However, studies of the mycobiome (fungal population) are less common[4, 5]. Moreover, studies investigating both the microbiome and mycobiome of the same cohort, even less so [6, 7].

Methods:

In this study, faecal samples were collected from patients with IBD [both ulcerative colitis (UC) and Crohn's disease (CD)] as well as healthy controls. The fungal internal transcribed spacer (ITS) 1 region was sequenced to characterise the mycobiome and V3-V4 16S region to characterise the microbiome of the same cohort. Two bioinformatic approaches were compared for the downstream analysis of the ITS sequencing data, followed by statistical analysis of the difference between the IBD groups and controls.

Results:

This pilot study investigated the inherent ecological interplay between the mycobiome and microbiome in IBD as well as provided data for power analysis to inform sample size for a larger cohort. We observed decreased alpha diversity in CD patients versus UC patients and differential abundance analysis highlighted the *Saccharomyces* genus as a biomarker for CD.

Future directions:

To further investigate differences in the mycobiome between the groups, as well as adding to the data showcasing the interactions between the mycobiome and microbiome.

Missing microbes and the hygiene hypothesis: New challenges and perspectives
Presentations: Monday and Tuesday evening

A046

Diversity of eukaryotic gut microbiota of northern Thai populations

Amara Yowang[1], Vasana Jinatham[1], Anastasios Tsaousis[2], Niwed Kullawong[1], Siam Popluechai[1], Eleni Gentekaki[1]

[1]Mae Fah Luang University, Chiang Rai, Thailand. [2]University of Kent, Canterbury, United Kingdom

Abstract

Gut eukaryome refers to the collection of fungi and protists in the gut. Until recently, all eukaryotic gut microbes were considered parasites and subject to elimination. Though this is true in some instances, critical evaluation of the literature reveals that most microbial eukaryotes are harmless often colonizing the human gut for long periods of time. Evidence is accumulating that the eukaryome plays important ecological roles in gut communities, as well as, in health and disease of the host. Nonetheless, systematic examination aiming to obtain baseline information of their prevalence and diversity in human populations is lagging. To address this knowledge gap, we collected fecal samples from a population of adults residing in north Thailand (n=211), who showed no gastrointestinal (GI) symptoms and had no history of GI diseases. We then examined the prevalence and diversity of two commonly found eukaryotic genera: the stramenopile *Blastocystis* (*Blastocystis* spp.) and the yeast *Candida* (*Candida tropicalis* and *Candida albicans*). Twenty three percent of individuals were positive for *Blastocystis*. Their sequences grouped in six of the nine clades that colonize humans. Twelve percent of the study population was positive for *Candida*, 4% for *C. albicans* and 8% for *C. tropicalis*, while concurrent colonization was also noted in some individuals. Eukaryotic bacterial interactions, as well as, interplay with diet and body mass index are also discussed. This is the first study providing data on the eukaryome of Thai populations and evidence that microeukaryotes traditionally considered as pathogenic asymptomatically colonize the gut of healthy humans.

A047

Electrochemical-Biosensor for the Detection of Coliform Presence in Drinking Water

Teri Bigham, James Davis

NIBEX, University of Ulster, Jordanstown, United Kingdom

Abstract

It is well established that drinking water contaminated with faecal matter poses a series risk of waterborne disease yet, it is estimated that almost 2 million people are reliant on such sources. The production of improved water sources for low- and middle-income countries has been a long-standing international commitment but ensuring that the drinking water is safe to drink remains a challenge – particularly in rural communities. The development of low-cost diagnostic systems capable of detecting coliform contamination is clearly required. The design of a disposable microfluidic based device that integrates the preconcentration, culture and detection of *E. coli* is presented as a possible solution. The device is based on a thin layer conductive carbon fibre filter modified with a riboflavin – ferrocyanide redox couple. The device utilises the fermentation characteristics of captured indicator bacteria to lower the local pH by the production of lactic acid and pyruvate after the introduction of lactose. The process can be monitored voltammetrically whereby ferrocyanide (internal reference) and riboflavin (pH probe) are readily detectable at the carbon fibre filter and allows the indirect measure of the change in pH as a function of time and bacterial concentration. The construction of the device and the electroanalytical responses are presented and the analytical efficacy, in terms of bacterial detection and in relation to present WHO criteria, is critically evaluated.

A048

Assessment of novel disinfection technologies, and bacterial contamination in the healthcare setting.

Jason Murray[1], Nigel Ternan[1], Chris Gill[1], Geoff McMullan[2], David Farren[3], Michael Scott[3]

[1]Ulster University, Coleraine, United Kingdom. [2]Queens Univeristy , Belfast, United Kingdom. [3]Antrim Area Hospital, Antrim, United Kingdom

Abstract

Worldwide, hundreds of thousands of healthcare acquired infections (HAIs) are reported each year. Contamination of hospitals is a source of, and allows dissemination of HAIs. In healthcare settings one of the major vectors of contamination is healthcare workers' uniforms. As surfaces become contaminated, bacteria can then be contacted by patients or staff who may indirectly spread bacteria to patients. Direct and indirect spread of bacteria could result in infection of patients and increased infection rates. Further consequences include increased levels of antibiotic use and costs.

A pilot study was conducted at Antrim Area Hospital, Northern Health and Social Care Trust. 100 pre-shift and 100 post-shift healthcare workers' uniforms were assessed for *Staphylococcus aureus* and *Enterococcus* spp. isolates. We found increased levels of antibiotic resistant *S. aureus* and *Enterococcus* spp. contamination on post-shift uniforms compared to zero to minimal contamination of pre-shift uniforms. A biobank of isolates was subsequently characterised for antibiotic sensitivity using European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines – 51% of *S. aureus* isolates were classed multi-drug resistant. Genomic diversity was assessed using Random Amplification of Polymorphic DNA (RAPD) – high levels of similarity was found amongst isolates. As one means of reducing uniform bioburden, we conducted analysis of a novel surface-active organisilane disinfectant named Goldshield (GS). GS was marketed as a long lasting antimicrobial prevent (re)contamination. GS technology displayed bactericidal, sporicidal and anti-biofilm properties in laboratory testing providing rationale for an intervention where GS could be incorporated into hospital laundry and assessed for potential use in infection control.

A049

Identification and characterisation of the key virulence determinants of asymptomatic pathogenic Escherichia coli

Jennifer Hallam

University of Glasgow, Glasgow, United Kingdom

Abstract

Diarrhoeal disease is a major public health concern, with over 500,000 childhood deaths recorded every year, (www.who.int). The leading cause of infantile diarrhoea is pathogenic *Escherichia coli* (EPEC), characterised by watery diarrhoea which varies in levels of severity. In recent studies a growing incidence of asymptomatic EPEC carriage has been recognised in Asia and the Middle East, suggesting the possibility that communities may act as a reservoir for this microorganism, (Kader, 2009; Alikhani, Mirsalehian and Aslani, 2018). Using a combination of molecular biology and bioinformatic analysis, we aim to characterise asymptomatic strains from these regions, determine their key virulence factors and find new ways of combatting this pathotype. At present we have developed a diagnostic multiplex PCR which incorporates known virulence genes of diarrhoeagenic *E. coli* pathotypes for quick and simple detection of EPEC. Additionally, we have optimised an infection assay using HeLa cells to phenotypically characterise typical and atypical colonisation and also identified bacteriocins capable of killing EPEC.

A050

Microbial community diversity within specialised diet types

Kirsty Davies, Omololu Fagunwa, Paul Humphreys, Simon Rout

University Of Huddersfield, Huddersfield, United Kingdom

Abstract

Dietary habit is likely to be a major driver of gut microbiome composition, in particular where certain dietary principles are held. In the vegan diet for example; no animal produce is consumed. In the raw food diet; in addition to the requirements of the vegan diet, food is not cooked. In the present study, the composition and diversity of faecal microbiota of individuals having western, vegan or raw diets is investigated. A culture-independent approach based on 16S (for bacteria) and 18S (for fungi) DNA gene amplicon sequencing is used to analyse microbial diversity of these individuals.

Preliminary results indicate taxa of the Phylum Proteobacteria constitute a greater proportion of the bacterial community (23%) within individuals partaking in a raw food diet compared to those with a vegan diet (1%). This was contrasted by the Phyla Tenericutes and Actinobacteria which were absent from the bacterial communities of the raw food diet individuals, but comprised 5% and 3% of the vegan participants respectively. A less marked variation between individuals was observed when comparing the communities obtained by 18S sequencing.

The high fibre nature of these two diets and differences in composition suggest that the microbiomes of these individuals may contain micro-organisms of interest; with respect to identifying novel bacteria involved in degradation of polysaccharides and other biological materials relative to their diet. Tailored media formulations have been developed to enrich for and identify such novel organisms from the faecal samples provided.

Missing microbes and the hygiene hypothesis: New challenges and perspectives
Presentations: Monday and Tuesday evening

A051

Human Peripheral Blood Interleukin-10 Expression Levels Increased Following *Helicobacter pylori* Eradication Therapy

Harry Jenkins[1,2], Akanksha Thakkar[1], Darren Letley[1,2], Kazuyo Kaneko[1,2], John Atherton[1,2], Karen Robinson[1,2]

[1]Centre for Biomolecular Sciences, School of Medicine, University of Nottingham, Nottingham, United Kingdom.
[2]Nottingham Digestive Diseases Biomedical Research Centre, Queen's Medical Centre, Nottingham University Hospitals, Nottingham, United Kingdom

Abstract

Helicobacter pylori (Hp) infection has been associated with reduced severity of extra-gastric immune-mediated diseases, including allergy and autoimmunity. We hypothesise that this is mediated through induction of suppressive regulatory T-cells (Tregs). It is important to understand whether eradication of the infection may worsen immune-mediated diseases in some individuals.

We analysed the expression of signature cytokines of $CD4^+$ T-helper (Th) subsets Th1, Th2 and Tregs; *IFNG*, *IL4*, and *IL10* respectively, in peripheral blood mononuclear cells (PBMCs), and serum anti-*Hp* IgG levels in 50 *H. pylori*-positive patients attending the Queen's Medical Centre, Nottingham, for antibiotic eradication of *H. pylori*. Blood samples were donated at month 0, 2, 6, 12 and 24 post-eradication, with informed patient consent and ethical approval. PBMCs were purified and cytokine mRNA expression quantified using reverse transcriptase quantitative PCR (RT-qPCR). Anti-*Hp* IgG levels were quantified by ELISA.

mRNA expression of *IL10* was significantly increased by a median of 2-fold ($p=0.005$) at 12 months, and 5-fold ($p=0.004$) at 24 months post eradication of the infection. No differences were observed in *IFNG* or *IL4*. Anti-*Hp* IgG responses were reduced by 3-fold at 12 months and 5-fold at 24 months ($p<0.05$).

Eradication of *H. pylori* resulted in markedly reduced serum antibody levels, however there were no significant changes in Th1 and Th2 cytokine expression. Surprisingly, expression of the suppressive cytokine *IL10* increased over time. In further work, this finding is being verified using flow cytometry to quantify Th1, Th2 and Treg cell frequencies.

Offence and defence

Presentations: Monday and Tuesday evening

A052

The role of copXL in community acquired methicillin resistant *Staphylococcus aureus* USA300 hyper-resistance to antibacterial copper toxicity

Inderpeet Kaur[1], Joanne Purves[1], Jamie Thomas[1], Gustavo Riboldi[2], Marta Zapotoczna[3], Emma Tarrant[4], Julian Ketley[5], Peter Andrew[1], Alejandra Londoño[6], Paul Planet[7,8], Joan Geoghegan[9], Kevin Walsdron[2], Julie Morrissey[1]

[1]University of Leicester, Leicester, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom. [3]Trinity College Dublin, Dublin, Ireland. [4]Newcastle University , Newcastle, United Kingdom. [5]University of Leicester, Leciester, United Kingdom. [6]Columbia University , New York, United Kingdom. [7]Columbia University, New York, USA. [8]Pennsylvania, Philadelphia, USA. [9]Trinity College Dublin, Ireland, Ireland

Abstract

Copper is an essential metal in both eukaryotes and prokaryotes, however excess levels are toxic. Bacteria have developed mechanisms, such as efflux and sequestration, to counteract these toxic effects. Significantly, copper has been shown to be important in host innate immunity as an antibacterial mechanism against invading pathogens, via active transport of copper into the phagosome.

Worryingly, there has been a global emergence of *S. aureus* strains with increased antibiotic resistance (e.g community-acquired methicillin resistant *S. aureus* (CA-MRSA)), which unlike typical *S. aureus,* can infect healthy humans with no previous exposure to healthcare situations. These isolates show increased resistance to innate immunity and reduced clearance from healthy airways compared to other clinical isolates.

Recently, we identified a novel horizontally transferred copper resistance locus, *copXL,* in CA-MRSA which is in addition to the core copper homeostasis operon (*copAZ)* found in all *S. aureus* and is not present in established *S. aureus* human lineages*. copX* encodes a copper efflux transporter and *copL* is predicted to encode a lipoprotein of unknown function. This operon confers resistance to extremely high concentrations of copper compared to other *S. aureus* and, notably, is important for survival within intracellular macrophages.

The recent evolution and success of USA300 may be due to possession of these additional copper resistance genes, enhancing bacterial fitness through increased resistance to copper-dependent bactericidal innate immunity. The function of CopL in *S. aureus* macrophage survival and copper hyper-resistance is currently being investigated to combat this highly effective copper resistance mechanism and spread of these highly virulent pathogens.

Offence and defence

Presentations: Monday and Tuesday evening

A053

Correlating prophage presence in Helicobacter pylori with Restriction-Modification systems.

Liam Crawford, Roxana Zamudio Zea, Andrew Millard, Sandra Beleza, Marco Oggioni

University of Leicester, Leicester, United Kingdom

Abstract

The human pathogen *Helicobacter pylori* colonises approximately half of the global population and infection can lead to a range of gastric diseases. The temperate bacteriophages in *H. pylori* have been poorly characterised, most likely due to a very high number and strain-to-strain variability of restriction modification (RM) systems, which can be easily more than 20 in any strain. This work aims to study the prevalence of bacteriophages and RM systems in over 460 strains of *H. pylori* isolated from 184 gastric samples from asymptomatic subjects.

H. pylori prophages were identified using the PHASTER tool. The gold standard RM systems were downloaded from Rebase and the RM genes were identified in the 460 genomes using Blast+.

The analysis for phage genomes showed 57 intact bacteriophages, ranging from 12 – 30 Kb in length, in 25 subjects (14%). Approximately half of the bacteriophages were shown to have integrated in the Lipid A biosynthesis gene *lpxD*. Using 107 RM system genes, we built a presence/absence matrix of the genes in all our *H. pylori* genomes, which was ordered according to a maximum likelihood gene presence/absence tree generated by FastTree. This clustering revealed the presence of multiple pairs of strains, one strain carrying an intact prophage whereas the other is lacking a prophage but containing the same RM systems, potentially allowing for a prophage donor and recipient pair. The availability of this panel of donor and recipient strain pairs is an ideal starting point to study the molecular biology of bacteriophages in *H. pylori*.

Offence and defence

Presentations: Monday and Tuesday evening

A054

Bacterial growth conditions affect the production of virulence factors associated with *Pseudomonas aeruginosa*.

Sophie Glossop, Paul Humphreys, Andrew Collett

University of Huddersfield, Huddersfield, United Kingdom

Abstract

Pseudomonas aeruginosa is a common cause of nosocomial infections and a frequent coloniser of chronic wounds often forming persistent biofilms which are difficult to treat. *Pseudomonas* can also express a variety of virulence factors causing localised inflammation and impaired wound healing. This study investigates how the bacterial microenvironment can influence production of virulence factors associated with *Pseudomonas* and investigate how extracellular secretions effect an in vitro model of wound healing.

Pseudomonas strains PS3 (isolated from a chronic wound bed) and fluorescens (a laboratory strain) were grown in the presence of either ethanol or glucose in simulated wound fluid for 24 or 80 hours. Protease, haemolysin, pyocyanin and biofilm production were quantified in each of the different culture conditions. Corresponding supernatants from the cultures were used to challenge an in vitro keratinocyte scratch assay.

PS3 80 hour cultures showed an increased production of all virulence factors compared to 24 hours cultures. The cultures grown in ethanol produced a greater concentration of haemolysin and pyocyanin. In addition, supernatant from cultures grown with ethanol for 80 hours showed increased toxicity resulting in greater keratinocyte death and longer healing times in the scratch assay. These studies will further our understanding of the effect of the microenvironment on bacterial virulence and potential pathogenicity.

Offence and defence

Presentations: Monday and Tuesday evening

A055

Poster withdrawn

A056

Harnessing the *Klebsiella*- macrophage arms race

Brenda Morris, Joana Sá Pessoa, José Bengoechea

Queen's University, Belfast, United Kingdom

Abstract

Klebsiella pneumoniae is a Gram-negative, multi-drug resistant human pathogen causing urinary tract infections, pneumonia and septicaemia. *K. pneumoniae* subverts phagolysosomal maturation and survives in the *Klebsiella* containing vacuole (KCV). In this work, we seek to identify the carbon sources and metabolic pathways used by intracellular *K. pneumoniae*. *In vitro*, *K. pneumoniae* is capable of carrying out glycolysis/ gluconeogenesis, the pentose phosphate pathway, the citric acid cycle (TCA) and the glyoxylate pathway. To dissect intracellular *Klebsiella* metabolism, we have followed a genetic approach generating mutants in key enzymes of each pathway. These mutants were assessed for *in vitro* growth kinetics using different carbon sources, adhesion, phagocytosis and intracellular survival in macrophages, and the ability to trigger inflammation. The virulence was assessed by infecting *Galleria mellonella*. Our results demonstrate that neither bacterial morphology nor growth kinetics in enriched media or minimal media supplemented with glucose are affected by mutations in central carbon metabolism. Loss of the glycolytic/ gluconeogenic enzyme fructose bisphosphate aldolase results in very poor growth kinetics when supplied only with acetate. Intracellular survival analysis have demonstrated that the enzymes of the glyoxylate pathway are not important for intracellular survival, however interruption of glycolysis/ gluconeogenesis and TCA pathways result in increased macrophage clearance. We have further shown that loss of the enzymes malate synthase (glyoxylate pathway) and malate dehydrogenase (TCA cycle), reduce virulence of *K. pneumoniae* in *G. mellonella* infection. Deciphering the metabolism of *K. pneumoniae* within the KCV may open new avenues of investigation for therapeutic targets to control these infections.

Offence and defence

Presentations: Monday and Tuesday evening

A057

An analysis of the role of Statins in reducing the virulence of *Candida albicans.*

AHMAD AJDIDI, Kevin Kavanagh

Maynooth university, biology department, Maynooth, Co. Kildare, Ireland

Abstract

The yeast *Candida albicans* has the ability to induce several systematic and superficial diseases in the immunocompromised or immunosuppressed host. Statins are widely used drugs for the control of cholesterol biosynthesis in the body and are therefore used in treatment of hypercholesterolemia. There is increasing evidence for the potential use of statins in preventing and treating fungal infections. The aim of this study was to assess the effect of statins on *C. albicans* and to characterize the proteomic alterations occurring in *C. albicans* in response to statin. Pre-growth of *C. albicans* in the presence of statin lead to lower levels of ergosterol, reduced adherence to buccal epithelial cells and decreased virulence in *Galleria mellonella* larvae. Cells also showed increased permeability as measured by elevated amino acid and protein leakage. Proteomic analysis of *C. albicans* exposed to statin revealed differential abundance of proteins related to ergosterol biosynthesis such as squalene monooxygenase (4.52 fold increase), and lanosterol synthase (2.84 fold increase). Proteins involved in oxidative stress response such as small heat shock protein 21 (6.33 fold decrease) and glutathione peroxidase (2.05 fold decrease) and adherence related proteins such as yeast-form wall protein 1 (6.26 fold decrease) and Ecm33p protein (2.06 fold decrease) were also altered in abundance. These results indicate that statins have the ability to reduce the growth of *C. albicans* and induce an oxidative stress response. Statins may have potential to control fungal infections *in vivo* if used alone or in combination with conventional antifungal agents.

A058

The Cathelicidin antimicrobial peptide (LL-37) stimulates the growth and pathogenicity of the pulmonary lung pathogen *Aspergillus fumigatus*

Gerard Sheehan[1], Gudmundur Bergsson[2], Noel G. McElvaney[2], Emer P. Reeves[2], Kevin Kavanagh[1]

[1]Medical Mycology Laboratory, Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland.
[2]Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Co. Dublin, Ireland

Abstract

The pulmonary mucus of Cystic Fibrosis (CF) patients displays elevated levels of the Cathelicidin antimicrobial peptide LL-37. The interactions between the pulmonary antimicrobial peptide arsenal and *Aspergillus fumigatus,* a common pathogen of CF patients, have been neglected in the literature.

Exposure of *A. fumigatus* to LL-37 and its derived fragment RK-31 (1.95 µg/ml) for 24 h, has a stimulatory effect on growth (199.94 ± 6.17%, $p < 0.05$) and (218.20 ± 4.63%, $p < 0.05$) respectively, whereas scrambled (SCR)-LL-37 did not. Exposure of mycelium to 5 µg/ml LL-37 for 48 h increased hyphal wet weight (4.37 ± 0.23 g, $p < 0.001$) compared to the SCR-LL-37 treatments. The levels of Immunosuppressive metabolite gliotoxin was significantly increased at 24 h from LL-37 exposed hyphae (169.1 ± 6.36 ng/mg hyphae, $p < 0.05$) compared to the SCR-LL-37 treatments. The whole cell proteomic response *A. fumigatus* to LL-37 revealed an increase in proteins associated with growth (eIF-5A), tissue degradation (aspartic endopeptidase) and allergic reactions (Asp F13). By 48 h there was an increase in proteins indicative of cellular stress, growth and virulence.

A. fumigatus conidia incubated in LL-37 and RK-31 displayed increased pathogenicity and fungal burden in the *Galleria mellonella* larvae model of aspergillosis. These results find that endogenous LL-37 paradoxically stimulates *A. fumigatus* growth and this may result in increased fungal growth and secretion of toxins in the lungs of CF patients.

A059

Elucidating the Contribution of the Pseudomonas aeruginosa CPX System in Biofilm Formation

ANA PAIVA, Morgan Alexander, Paul Williams

The University of Nottingham, Nottingham, United Kingdom

Abstract

An investigation of *Pseudomonas aeruginosa*attachment to a diverse range of polymers in a high-throughput microarray format resulted in the discovery of novel biofilm-resistant and biofilm-stimulating materials. These findings raised questions about the nature of the surface interactions involved and in particular the sensory mechanisms use by *P. aeruginosa*to distinguish between different surface chemistries. To further investigate this, Tn5 mutants of the *P. aeruginosa*PAO1 Washington sub-line were tested for biofilm formation on the polymer microarrays. This revealed that a Tn5::*cpxR* mutanthad a significant difference in biofilm formation when compared with PAO1-W.

In *Escherichia colicpxR* forms an operon with *cpxA* and *cpxP*. Together they form the CPX two-component signalling system controlling biofilm formation in response to cell envelope stress. To further characterise the *P. aeruginosa cpx*system, Δ*cpxR,*Δ*cpxP*and Δ*cpxA*deletion mutants were constructed. No significant differences were observed in their growth or in the swimming, swarming or twitch motility phenotypes normally required for surface colonization. Unlike *E.coli* the PAO1-W *ΔcpxA*mutant was able to invade lung epithelial cells and did not display increased sensitivity to antibiotics. However, the Δ*cpxR*mutant showed increased biofilm formation on glass and eDNA secretion in both biofilm and liquid modes of growth.

This work highlights the relationship between biofilm formation and the CPX system in *P. aeruginosa.* However, further assays need to be conducted in order to understand the sensory mechanism(s) involved in surface sensing via the CPX system.

Offence and defence

Presentations: Monday and Tuesday evening

A060

Characterisation of the proteomic response of *Pseudomonas aeruginosa* to *Aspergillus fumigatus* in a nutrient poor environment

Anatte Margalit, James C. Carolan, Kevin Kavanagh

Maynooth University, Maynooth, Ireland

Abstract

Aspergillus fumigatus and *Pseudomonas aeruginosa* are the most prevalent fungal and bacterial pathogens, associated with cystic fibrosis (CF)-related infections, respectively. Co-colonization is rare, and although it is well established that *P. aeruginosa* predominates as the primary pathogen, less is known as to the reason why this is the case.

In this study, we demonstrate that *P. aeruginosa* PAO1 proliferates more rapidly when grown in a nutrient-poor medium conditioned with the supernatants from a co-culture of *A. fumigatus* and *P. aeruginosa*, than when grown in a medium conditioned with either *A. fumigatus* or *P. aeruginosa* supernatants. Label free quantitative proteomics was used to determine the possible causes of this observation. Large changes in the proteomic profile of *P. aeruginosa* were observed when cultured under these conditions. Between the groups, more than 550 proteins were identified as being statistically significant ($p<0.05$) and differentially abundant (fold change of +/- two). Comparative analysis identified changes in a number of central metabolic pathways including nitrogen, nucleic acid and amino acid metabolism. Large differences in the abundance of proteins associated with DNA replication, ribonucleotide binding, cell wall formation, the periplasmic space and ABC transporters were observed. 124 proteins were identified exclusively in *P. aeruginosa* grown in both the co-cultured and *A. fumigatus* supernatants. These included transcriptional and post-transcriptional regulators and anaerobic-response proteins. The proteomic profile as described here may help to explain why *P. aeruginosa* is so successful at out-competing *A. fumigatus* under nutrient limiting conditions such as those found in the CF lung.

A061

Regulation of Rab32 and its role in mediating *Salmonella* killing in macrophages

Kieran Sefton, Stefania Spanò

University of Aberdeen, Aberdeen, United Kingdom

Abstract

The GTPase Rab32 is involved in killing the human-adapted pathogen *Salmonella enterica* serovar Typhi (*S*. Typhi) in macrophages. Recruitment of Rab32 to the *Salmonella* Containing Vacuole (SCV) appears critical for killing of the bacterium, yet the underpinning mechanism remains poorly understood. The broad-host *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), which is present in a vacuole devoid of Rab32, survives and replicates in macrophages. This bacterium delivers the bacterial effectors GtgE and SopD2, which target Rab32 and prevent its recruitment to the SCV. The absence of these effectors in *S*. Typhi or in a *S.* Typhimurium strain deficient for *gtgE* and *sopD2,* results in bacterial killing in macrophages and their inability to cause infection in mice. We aim to understand the role of Rab32 in regulating this killing mechanism. Rab GTPases are inactivated by Rab GTPase activating proteins (GAPs). Remarkably, *S.* Typhimurium inactivates Rab32 through the delivery of the GAP SopD2, however the endogenous GAPs targeting Rab32 in macrophages remain unknown. To identify these GAPs, we investigated GAPs showing activity towards Rab32 or closely related Rabs *in vitro*. We observed that altering the expression of a candidate Rab32 GAP influences localisation of Rab32 to SCVs and affects the *Salmonella* killing capacity of macrophages. Therefore, we propose that this GAP is a negative regulator of Rab32 that converts Rab32 to an inactive state. Identification of this GAP as an inhibitor of *Salmonella* killing will contribute to understanding of Rab32 trafficking and its role in macrophage bacterial killing.

A062

The impact of the colonic milieu on enterohaemorrhagic *E. coli* outer membrane vesicle production.

Daniel Yara[1,2], Regis Stentz[2], Tom Wileman[1], Stephanie Schüller[1,2]

[1]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [2]Quadram Institute Bioscience, Norwich Research Park, Norwich, United Kingdom

Abstract

Haemolytic uraemic syndrome (HUS) is a complication which may arise upon enterohaemorrhagic *E. coli* (EHEC) colonisation of the colon. The development of HUS is associated to Shiga toxins (Stxs) release leading to organ damage. It has been found that Stx release can occur within outer membrane vesicles (OMVs). Hence, the effect of different intestinal environment cues on EHEC OMV production was examined. OMVs were purified following EHEC growth in Luria broth (LB), simulated colonic environmental medium (SCEM) with or without bile salts, and in the presence or absence of human cell lines. Yield was quantified through SDS-PAGE and densitometric analysis of outer membrane proteins F/C and A. Furthermore, OMV uptake by HEp-2 and T84 cells was analysed by incubating such cells with OMVs labelled fluorescent lipophilic dye and fluorescence microscopy. Different OMV yields were attained under different conditions, with human cell growth medium and SCEM producing significantly more than cultures grown in LB, with further increases in the presence of bile salts. The presence of HEp-2 cells did not affect OMV yield, yet a lower yield was attained in the presence of T84 cells. These results suggest that colonic environmental factors may influence EHEC OMV production *in vivo*. OMVs were also shown to be up-taken by both cell types. Such observations with T84 cells suggest that the human colonic epithelium can uptake OMVs. Coupled with Stx, this data may offer a paradigm on how OMVs contribute to Stx translocation across the gut barrier, subsequently leading to HUS.

A063

Identification of host-factors restricting *Salmonella* Typhi

Virtu Solano Collado, Massimiliano Baldassarre, Stefania Spanò

The Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

Abstract

Salmonella enterica serovar Typhi (*S.* Typhi) is a human adapted pathogen that causes typhoid fever, a systemic infection responsible for more than 200,000 annual deaths. With a high incidence in the developing countries, the risk of infection is increasing worldwide as a consequence of the spreading of antimicrobial resistance and the current vaccines do not providing full protection. Thus, it is essential to identify new targets for therapy. Spanò and Galán discovered recently a Rab32-dependent antimicrobial pathway that is required to prevent the growth of *S.* Typhi in macrophages derived from mouse. Despite this finding, the exact mechanisms used by macrophages to kill *S*. Typhi as well as the molecular basis of these mechanisms in the adaptation to the human host are unknown.

To identify host genes required to kill *S.* Typhi we recently optimized the conditions to perform silencing screenings in primary mouse macrophages. We used pooled short-hairpin RNAs (shRNAs) to knockdown gene expression in macrophages combined with fluorescence microscopy and flow-cytometry to identify and isolate macrophages containing different numbers of bacteria. Macrophages containing larger amounts of intracellular bacteria were sorted and targeted genes identified by next-generation sequencing. A small-scale screen allowed us to identify Rab GTPases required to control *S*. Typhi survival in mouse macrophages and to optimize the screening conditions to perform a genome-wide screen in primary macrophages. This will identify the genes that macrophages use to control *S*. Typhi infection and will extend our knowledge of the immunity mechanisms controlling the growth of intracellular pathogens.

Offence and defence

Presentations: Monday and Tuesday evening

A064

Mechanisms underlying Enterohaemorrhagic *Escherichia coli* O157: H7 manipulation of the bovine cellular immune response

Amany Hassan, David Gally, Tim Connelley, Alexander Corbishley

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

Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 can cause haemorrhagic diarrhoea and potentially fatal renal failure in humans. Ruminants are considered the primary reservoir for human infection. Studies investigating the response of cattle to colonization generally focus on humoral immunity, leaving the role of cellular immunity unclear. These bacteria colonise their host by tight attachment to the epithelium, using a type three secretion system to inject a cocktail of effectors into the host cell. Injected effectors manipulate the innate response in several ways to promote bacterial persistence. Transcriptional profiling of responses at the terminal rectum, the primary site of colonisation in cattle, reveals a bias towards a T-helper type 1 response, indicating that cellular immunity may be involved in bacterial clearance. Mathematical modelling also indicates that injected effectors have a reduced human MHC-I epitope density, whilst structural bacterial proteins do not. This implies that host cellular immune responses target injected effectors and have exerted selective pressure on their evolution. My on-going research is examining the expression of MHC-I on the surface of cultured bovine epithelial cells following infection with *E. coli* O157. Initial results demonstrate a decrease in MHC-I surface expression during EHEC O157 colonisation after three hours. The basis of this reduction is being investigated using defined mutants in type three secretion genes. The longer-term objective is to use peptide elution and mass spectrometry to examine the presentation of effector protein peptides and determine whether *E. coli* O157 has evolved to interfere with this process.

Offence and defence

Presentations: Monday and Tuesday evening

A065

A potential novel metal-sensing multikinase network in the opportunistic pathogen, *Burkholderia cenocepacia*.

Jack Plume[1], Matt Robinson[2], Steve Porter[1], Alan Brown[1]

[1]University of Exeter, Exeter, United Kingdom. [2]Laos-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Mahosot Hospital, Vientiane, Lao, People's Democratic Republic

Abstract

Burkholderia cenocepacia is an opportunistic pathogen of particular threat to the cystic fibrosis community, and is able to thrive in a variety of environmental niches, such as industrial waste-contaminated soils and aquatic environments. Bioinformatic predictions suggest that four previously uncharacterised putative metal-sensing two-component systems (TCS) in *B. cenocepacia* may cross-regulate one another, forming a novel metal-sensing multikinase network, allowing the integration of several environmental stimuli to form a coordinated cellular response. This study aims to characterise the extent of interaction between these TCSs, ultimately revealing the complexity behind the metal response in *B. cenocepacia*.

Deletion mutants constructed in *B. cenocepacia* K56-2 have implicated two of these TCSs in the response to metal stress, with BCAM0714/5 conferring cadmium and zinc resistance and BCAM0442/3 conferring copper resistance. Additionally, deletion of BCAM0442/3 significantly reduces the ability to form biofilms, suggesting a link between the copper response and biofilm formation. Phosphorylation studies using [γ-^{32}P] ATP demonstrate phosphotransfer between histidine kinases and non-cognate response regulators, reinforcing the idea of a multikinase network that can integrate several stimuli from separate TCSs.

Work thus far indicates that a novel metal-sensing multikinase network may indeed exist within *B. cenocepacia*, which may be underpinning its ability to grow in metal-containing environments and counteract metal toxicity strategies employed by macrophages. Future work revolves around elucidating the regulon of each TCS, as well as investigating the potential role of each TCS in virulence and intracellular survival.

Offence and defence

Presentations: Monday and Tuesday evening

A066

Citrobacter rodentium triggers the rapid onset of conserved infection signatures in a susceptible mouse strain

Danielle Carson, Rachael E Barry, Gad M Frankel

Imperial College London, London, United Kingdom

Abstract

Citrobacter rodentium murine infection is the 'gold standard' model for investigating host response to human diarrhoeal pathogens, Enteropathogenic and Enterohaemorrhagic *Escherichia coli*. Mouse strains have varying susceptibility to *C. rodentium* with some developing self-resolving mild diarrhoea whereas others develop severe diarrhoea and dehydration resulting in lethal disease. One of the primary defences against these invading pathogens are intestinal epithelial cells (IECs) which line the gastrointestinal tract forming a protective barrier. Previously, we have characterised changes in the metabolic landscape of *C. rodentium* infected IECs from resistant C57Bl/6 mice, which develop self-limiting colitis upon infection. Here, we examine the global proteomic response of infected IECs from a lethally susceptible host, C3H/HeNCrl. We highlight conserved infection signatures between resistant and susceptible mice, including the upregulation of cell cycle and DNA replication processes at the peak of infection. Temporal analysis of the IEC landscape revealed reduced expression of the differentiated Reg4+ and Slc26a3 containing cells three days post inoculation (DPI), earlier than reported for C57Bl/6. Further investigation revealed the onset of other infection-induced host responses also occurring by three DPI. Bioluminescent imaging showed *C. rodentium* to colonise a larger proportion of the colon and microbiome analysis revealed the absence of the *C. rodentium* competing phyla, Bacteroides from the host-pathogen interface. Our results suggest that the absence Bacteroides from the uninfected C3H microbiome may enable *C. rodentium* to extensively colonise the colon accelerating the onset of host protective responses which can ultimately be of detriment and lead to increased disease severity in C3H/HeNCrl.

Offence and defence

Presentations: Monday and Tuesday evening

A067

CpxR Modulates Type VI Secretion System Activity in a Clinical Isolate of *Serratia marcescens*

Connor Bowen[1], Alex V Predeus[2], Blanca M Perez-Sepulveda[2], Jay C D Hinton[2], Sarah J Coulthurst[1]

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

Abstract

Serratia marcescens is found in a number of environments, including soil, water courses and in clinical settings, where it can cause opportunistic infections. To flourish in these different niches, *S. marcescens* strains have developed a number of strategies to compete with other microorganisms. These include the production of diffusible antimicrobial molecules and the actions of contact-dependent antibacterial systems, such as the Type 6 Secretion System (T6SS). The T6SS is a proteinaceous nanoweapon that can be deployed by *S. marcescens* and many other species to deliver effectors that inhibit or kill neighbouring cells. Much of the work on the T6SS in *S. marcescens* has been performed using the model insect pathogen, strain Db10, with a focus on the mechanism of the machinery and identification of effector proteins. Less is known about the role of the T6SS in clinical isolates of *S. marcescens*. Here we use a multi-drug resistant clinical isolate of *S. marcescens,* SM39, to study the action and regulation of the T6SS in a pathogenic strain. We show that SM39 encodes a fully functional T6SS that displays antibacterial action at environmental temperatures. We further observed that a single amino acid change in the regulator CpxR drastically changes the T6SS profile of the isolate. Subsequently, we have adopted an RNAseq based approach to analyse the impact of this mutation. Our findings suggest that the regulator CpxR plays a major role in controlling the action of the T6SS in a clinical isolate of *S. marcescens*.

Offence and defence

Presentations: Monday and Tuesday evening

A068

A conserved transcription factor regulates virulence in distinct pathogenic *Escherichia coli*

Nicky O'Boyle, James Connolly, Andrew Roe

University of Glasgow, Glasgow, United Kingdom

Abstract

The sensing of environmental signals and subsequent co-ordination of virulence factor expression is a key determinant of the success of bacterial pathogens. We recently discovered a highly conserved LysR-type transcriptional regulator (YhaJ) which is part of the core-genome of *Escherichia coli*. We showed that this regulator controls the expression of the type three secretion system (T3SS) of enterohaemorrhagic *E. coli* (EHEC), which would allow for the restriction of colonisation within a favourable niche. In this study, we investigated the global role of YhaJ in EHEC and uropathogenic *E. coli* (UPEC). In spite of the high sequence conservation of YhaJ in these two pathotypes (99% at AA-level), RNA-seq revealed no overlap between the transcriptional responses of EHEC and UPEC to deletion of *yhaJ*. Deletion of *yhaJ* in UPEC affected the expression of a distinct group of colonisation factors, including the *fim* and *foc* type I fimbriae. ChIP-seq revealed pathotype-specific binding sites for YhaJ-FLAG, with an essential virulence determinant *nleA* (a T3SS effector) being detected for EHEC. Interestingly, a binding site for *fimA* was detected in EHEC but not UPEC. EHEC possess a non-functional *fim* locus due to the invertible *fimS* switch element being locked in the off orientation. Indeed, DNA-binding assays revealed that YhaJ bound *fimS* from both strains exclusively in the "off" orientation and in UPEC this binding increased the rate of switching to the "on" state. These data provide important insight into the effects of intraspecies transcriptional rewiring for the control of virulence factor expression.

Non-human pathogens

Presentations: Monday and Tuesday evening

A069

Characterization of Keratinophilic Fungal Species and Other Non-Dermatophytes in Hair and Nails Samples in Riyadh, Saudi Arabia

Suaad AlWAKEEL

college of Science , Biology Department,Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, RIYADH, Saudi Arabia

Abstract

The presence of fungal species on the surface skin and hair is a known finding in many mammalian species and humans are no exception. Superficial fungal infections are sometimes a chronic and recurring condition that affects approximately 10-20% of the world's population. However, most species that are isolated from human tend to occur as co-existing flora. This study was conducted to determine the diversity of fungal species isolated from the hair and nails of workers in the central region of Saudi Arabia where there are not many observational studies on the mycological species. Male workers from Riyadh, Saudi Arabia were recruited for this study and samples were obtained from their nails and hair for mycological analysis which was done using Saboraud's agar and sterile wet soil. Fungal isolates were examined microscopically. Twenty four hair samples yielded a total of 26 species from 19 fungal genera. *Chaetomium globosum* was the most commonly isolated fungal species followed by *Emericella nidulans*, *Cochliobolus neergaardii* and *Penicillium oxalicum*. Three fungal species were isolated from nail samples, namely, *Alternaria alternata*, *Aureobasidium pullulans,* and *Penicillium chrysogenum*. Most of the isolated fungal species (17 of the 26 or 65.38% of the isolated fungal species). Most of our isolated fungal species have not been thoroughly characterised nor morphologically classified.

Non-human pathogens
Presentations: Monday and Tuesday evening

A070

Stage-specific gene regulation in *Perkinsus olseni* parasites

Jana Zielinski, Elin Einarsson, Imen Lassadi, Ross Waller

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

Abstract

Perkinsus species are important marine parasites of molluscs including mussels and oysters with substantial commercial and environmental impact. *Perkinsus* forms a sister lineage to the apicomplexan parasites (e.g. malaria agent, *Plasmodium*) and share similar invasion-related structures with these better studied pathogens. However, much less is known of the transmission and invasion biology of *Perkinsus* spp. We are developing genetic tools to study these parasites, and *Perkinsus* is emerging as an experimental model for marine parasites. The life cycle of *Perkinsus* species start with motile zoospores that are ingested by the host via filtration of sea water. After infection of the mollusc, these cells develop into a replicating non-motile vegetative form of the parasite, the trophozoite. Upon host death or morbidity, the parasites are released back into the water column where they differentiate into zoosporangia that release up to 100 motile zoospores. *In vitro*, *Perkinsus* species can be maintained as trophozoites in an axenic sea water-based media, and this is the best studied form of the parasite. However, *Perkinsus olseni*, can be triggered to sporulate *in vitro* using Ray's fluid thioglycollate media, and we seek to better study this phase of the lifecycle that is essential for disease spread. To do this, we are determining stage-specific gene expression, during the transformation from trophozoites to zoospores, by RNA-Seq. This will both identify genes that are specific to this growth stage, and also identify zoospore-specific promoters that can be used in experimental manipulation and study of this poorly known cell stage.

Non-human pathogens

Presentations: Monday and Tuesday evening

A071

Genetic studies of European foulbrood: virulence, persistence and antibiotic resistance

Nicola Burns[1,2], Giles Budge[3], Ville Friman[1], Thorunn Helgason[1]

[1]University of York, York, United Kingdom. [2]Fera Science, Sand Hutton, York, United Kingdom. [3]Newcastle University, Newcastle, United Kingdom

Abstract

Honey bees (*Apis mellifera*) are important because of their significant contribution to worldwide pollination (Gallai, *et al.*, 2009). Populations are now under threat from multiple major pathogens including fungi, viruses, parasites, and bacteria. European foulbrood (EFB), is a bacterial pathogen of the honey bee, and is detected in hives globally. The causative agent *Melissococcus plutonius* is a gram-positive bacterium that infects the gut of the larva, resulting in death when the bee larva is between 4-5 days old. Previously, isolates have been differentiated into strain types (STs) and clonal complexes (CC) which vary in virulence and disease persistence. The genetic basis of these variations in EFB disease is unknown.

Whole genome sequencing of more than 50 *M. plutonius* UK isolates has speculatively identified potential genes related to virulence features such as biofilm formation, toxin production, antibiotic resistance and mobile genetic elements. These theoretical genetic differences will be tested in *in vivo* using future larval experiments. *In vitro* antibiotic experiments will also test resistance of strains *M. plutonius* to oxytetracycline, the antibiotic most commonly used in the UK for EFB. If the presence or absence of specific bacterial genes can reliably predict disease severity, then screening and management of EFB in the UK may be improved in the future.

Non-human pathogens

Presentations: Monday and Tuesday evening

A072

The study of rotavirus phylogenetic diversity, re-assortment and Interspecies transmission and associated virome in Northern Irish livestock and wildlife

Paula Lagan Tregaskis[1,2], Mark Mooney [2], Ken Lemon[1]

[1]Agri-food and Biosciences Institute , Belfast , United Kingdom. [2]Queens University Belfast, Belfast , United Kingdom

Abstract

Background

Rotavirus (RV) is a highly infectious pathogen of livestock causing diarrhoea and dehydration, and substantial economic loss. RV is an RNA virus with a segmented genome that has evolved significantly creating diverse strains. RV is believed to be endemic within livestock and the genotyping of positive isolates will allow the diversity of the rotavirus to be phylogenetically mapped for evidence of re-assortment and interspecies transmission. Rotavirus positive samples and rotavirus negative samples were examined using a metagenomics approach through next generation sequencing (NGS) to study the virome of symptomatic animals.

Methods

RV in symptomatic livestock, and a small subset of wildlife and exotic animal faeces samples (*n=326)*, originating from Northern Ireland, were screened by RT PCR for the RV VP6 gene. NGS libraries using a *de novo* metagenomics approach were run on the MiSeq reagent V3 600 cycle as pairend reads. Data was quality checked by Fast QC, assembled in SPAdes, processed through NCBI blast (n) and then MEGAN to display the taxonomical content.

Results

The prevalence of RV VP6 gene was n=108 (33%). Initial Sanger sequencing results showed porcine G3, G4 and G5 and bovine P7 and P13 strains. Preliminary metagenomic results indicates re-assortment and interspecies transmission with a positive bovine sample showing RV acquired re-assortment from human and equine RV strains. Analysis shows there is a large viral community present in both RV positive and RV negative animals. The metagenomic profiling through NGS data set will give a better understanding of the symptomatic virome in livestock.

Non-human pathogens

Presentations: Monday and Tuesday evening

A073

Biofilm production and other virulence factors in Streptococcus spp. isolated from clinical cases of bovine mastitis in Poland

Edyta Kaczorek-Łukowska, Joanna Małaczewska, Roma Wójcik, Andrzej Siwicki

Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Abstract

Mastitis is a common disease in dairy cattle. An important aetiological agent of this disease is bacteria of the genus Streptococcus; hence, exploring the mechanisms of virulence in these bacteria is an extremely important step for the development of effective prevention programmes. The purpose of our study was to determine the ability to produce biofilm and the occurrence of selected invasiveness factors among Streptococcus isolated from cattle with the clinical form of mastitis in northeastern Poland. Most of the isolates analyzed demonstrated an ability to produce biofilm (over 70%). Virulence genes were searched for in S. agalactiae, S. uberis and S. dysgalactiae. For S. agalactiae, only four genes were confirmed: rib (33%), cylE (78%), bca (37%), and cfb (100%). The genes pavA, scpB, bac and lmb were not present in any of the tested strains. The dominant serotypes were Ia (n = 8) and II (n = 8), in addition to some strains that were not classified in any of the groups (n = 6). Out of the eight selected genes for S. uberis only one was not found (lbp). Finally, two genes were chosen for S. dysgalactiae (eno and napr), and their presence was confirmed in 76% and 86% of the strains, respectively. The experiment showed that strains of Streptococcus spp. isolated from dairy cattle with clinical cases of mastitis in the northeastern part of Poland possess several invasiveness factors that can substantially affect the course of the disease, and this should be considered when developing targeted prevention programmes.

Non-human pathogens

Presentations: Monday and Tuesday evening

A074

Characterizing Avian Pathogenic Escherichia coli from Diagnostic Cases in Georgia, USA – Comparison of Gene Profiles with Tissue of Isolation

Catherine Logue, Darby Newman, Lisa Nolan, Nicolle Barbieri

University of Georgia, Athens, USA

Abstract

Colibacillosis caused by Avian Pathogenic *Escherichia coli* (APEC) is a significant cause of morbidity, mortality and carcass condemnation to the poultry industry worldwide resulting in significant economic losses. We assessed a multiplex PCR for classifying diagnostic APEC and characterized these isolates using gene profile analysis.

48 *E. coli* isolates collected between August and October 2018 through the Poultry Disease Research Center (PDRC) Diagnostic Laboratory were analyzed. Isolates were screened using multiplex PCR targeting genes associated with APEC chromosomal and plasmid virulence. Isolates were assessed for relationship between gene profile and host tissue of origin.

Overall, isolates met the criteria for definition as well-developed pathogens with more than 90% of isolates positive for the genes *iroN*, *ompTp*, and *hlyF*; 78% were positive for *aerJ* and 67% for *iss*. A significantly lower prevalence was observed for *cvaC*, *etsB*, *ireA* and *papC* (range 5-36%). When overall gene prevalence was examined for tissue of isolation, we found that APEC from the ovary, bone marrow, pericardium and lung had higher average numbers of genes compared to isolates recovered from skin and yolk sac. Genes associated with the ColV virulence plasmid (*iss*, *iroN*, *hlyF* and *ompTp*) were detected in 43 of 48 isolates (89.5%) further confirming the ColV plasmid is the defining trait of the APEC subpathotype.

The use of a multiplex panel to screen for APEC has shown good correlation with pathogenesis, and tissue source and correlates well with invasive strains. Path panel diagnostics is available through PDRC, providing significant value to APEC screening.

A075

A longitudinal survey of the gut virome of commercial broiler chickens from prehatch to slaughter

James Trudgett, David Brennan, Veronica Casement, Paul McAdam, Victoria Smyth

Agri-Food & Biosciences Institute, Belfast, United Kingdom

Abstract

Uneven flock growth (UFG) occurs when a broiler flock exhibits a wide range of bodyweights at slaughter. Automated chicken processing plants operate on an average bodyweight for the flock being processed so UFG can cause disruption to the processing flow that requires manual remediation. It is known that enteric virus infections of young broiler flocks lead to varying degrees of growth restriction that range from runts at hatch and severe early stunting, which are generally culled, to poor flock performance and UFG.

In order to investigate the make-up of communities of enteric viruses (virome) associated with poor growth and also their infection timings and persistence in broiler flocks, a longitudinal survey of 7 commercial broiler flocks of varying performance over 3 successive broiler crops was undertaken with gut and faecal samples collected from 50 birds at each of 12 timepoints from pre-hatch to slaughter. The samples were pooled for each timepoint, then processed to enrich for viruses by removing host cells and bacteria. The RNA was extracted from each timepoint sample, amplified by whole transcriptome amplification followed by whole genome library preparation for the Illumina MiSeq platform and libraries multiplexed. Bioinformatics analysis used ViromeScan which facilitates metagenomic studies of quality-trimmed sequencing reads. The presence of diverse viral families was seen including the known major enteric viruses of the astrovirus, picornavirus, reovirus and parvovirus families. A greater diversity of viruses was observed in the flocks of poorer performance than in those flocks of good performance.

Non-human pathogens

Presentations: Monday and Tuesday evening

A076

Studying a mycovirus from *Dothistroma septosporum*, causative agent of pine needle blight

John Daudu[1], James Snowden[2], Katherine Tubby[2], Robert Coutts[1], Ioly Kotta-Loizou[3]

[1]University of Hertfordshire, Hatfield, United Kingdom. [2]Forest Research , Farnham, United Kingdom. [3]Imperial College London, London, United Kingdom

Abstract

Dothistroma needle blight caused by *D. septosporum* has emerged in the British Isles as a major threat to Corsican pine, lodgepole pine and Scots pine. There is increasing evidence that mycoviruses can reduce the growth and pathogenicity of fungal plant pathogens. The aim of the present study is to characterise a double-stranded RNA virus found in *D. septosporum* and investigate for putative hypovirulence, a common feature noted for mycoviruses, which might be used for biological control to invasion by more aggressive strains of the fungus. To this end the viral genome was cloned and sequenced revealing four genomic segments, each one containing a single open reading frame (ORF) flanked by 5' and 3' untranslated regions. The ORFs encode the RNA-dependent RNA polymerase, the capsid protein, a protein of unknown function and a putative protease, respectively. Phylogenetic analysis of the sequences obtained revealed their similarity to members of the established family *Chrysoviridae*, genus *Alphachrysovirus*, which are encapsidated in isometric particles and are known to elicit hypovirulence in their hosts. Subsequently, virus-free and virus-infected isogenic lines were generated to determine any effects of the mycovirus on fungal fitness and pathogenicity. More specifically, the virus-infected isolate is currently being assessed in comparison to the virus-free one in terms of radial growth in solid culture, biomass in liquid culture, pathogenicity in pine trees and production of the mycotoxin dothistromin. In conclusion, this study reports the first mycovirus ever found in *D. septosporum*.

A077

Non-sporulating variants of *C. difficile* from animal faecal samples

Natalie Vardy[1], John McGrath[1], Derek Fairley[2,1], Geoff McMullan[1], Mairead Connor[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Belfast Health and Social Care Trust, Belfast, United Kingdom

Abstract

Clostridioides difficile, formerly known as *Clostridium difficile*, is a pathogen of increasing agricultural and clinical significance. This Gram-positive, anaerobic, toxigenic bacterium produces extremely robust spores which are highly resistant to environmental pressures, allowing this organism to persist in the environment for extended periods.

*C. difficile*is capable of infecting both animals and humans. As a commensal organism it does not typically pose a threat to its host unless antibiotic treatment disrupts the normal gut flora. However following a decline in microbial diversity, *C. difficile*opportunistically colonises the host gut and proliferates, thereby causing *C. difficile*infection (CDI). CDI is transmitted via the faecal-oral route with spores surviving transit through the gastrointestinal tract to the gut. Symptoms of CDI range from mild diarrhoea to pseudomembranous colitis (PMC), toxic megacolon and death. *C. difficile*ribotypes present in farm animals have been found in workers tending to them, suggesting a potential zoonotic transmission.

Sporulation is intrinsic to *C. difficile*'s transmissibility and the spores are highly resilient. Previous joint work at both the Queen's University Belfast and Royal Victoria Hospital (Belfast) identified a non-sporulating variant of ribotype 078, the most prevalent ribotype infecting humans in Northern Ireland. Investigations into the prevalence of the non-sporulating variant in a range of animal and clinical derived ribotype 078 isolates are currently ongoing. Additionally, the presence of the non-sporulating variant in other toxigenic clades of *C. difficile*is undergoing assessment.

Non-human pathogens

Presentations: Monday and Tuesday evening

A078

Brucella Sequence Type 27 Isolated from Dwarf Sperm Whale (*Kogia sima*) Stranded in the Costa Rican Pacific Coast

Marcela Suárez-Esquivel[1], Nazareth Ruiz-Villalobos[1], Gabriela Hernández-Mora[2], Rocío González-Barrientos[2], Jose David Palacios-Alfaro[3], Elías Barquero-Calvo[1,4], Esteban Chaves-Olarte[4], Nicholas Thomson[5], Edgardo Moreno[1,4], Caterina Guzman-Verri[1,4]

[1]Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica. [2]Servicio Nacional de Salud Animal, Ministerio de Agricultura y Ganadería, Heredia, Costa Rica. [3]Fundación Keto, San José, Costa Rica. [4]Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica. [5]Welcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

Abstract

Brucella organisms are Gram-negative, intracellular facultative extracellular bacteria that infect a variety of animals. On March 2018, a pregnant dwarf sperm whale (*Kogia sima*), stranded and aborted on Herradura beach at the Pacific coast of Costa Rica. *K. sima* is a criptic species; very little is known of its biology and worldwide distribution, however it is still hunted in Asia. *Brucella* sp. was recovered from multiple tissues of the female and the calf, and examined by biochemical tests, MLVA-16, brucellader and HRM real time. The isolates were used for whole genome sequencing and the reads were aligned to *Brucella abortus* 9-941 as the reference, alltogether with other *Brucella* species. A total of 27,365 variable sites were extracted and a phylogenetic reconstruction by maximum likelihood was produced. The phylogenetic tree revealed that the *K. sima* isolates are related to *Brucella* sp. F5/99, a singular strain recovered on 1992 from a bottlenose dolphin captive in California, classified as sequence type (ST) 27 by multi-locus sequence type. This ST27 was described in *Brucella* isolates with zoonotic capacity and therefore transmission to humans from Peru and New Zealand. This is the first report of *Brucella* ST27 recovered from a host of the Eastern Tropical Pacific and of *Brucella* infection in a dwarf sperm whale. Our results shows that the range of marine mammals infected by *Brucella* sp. is wider than our current knowledge, and that biosafety measures should be increased when handling the stranded mammals, as the zoonotic transmission is of major risk.

A079

DEVELOPMENT OF ANTIMICROBIALS AGAINST ACANTHAMOEBA

Alemao G. Carpinteyro Sanchez[1], Fraser J. Scott[2], Colin J. Suckling[1], Craig W. Roberts[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Huddersfield, Queensgate, United Kingdom

Abstract

Acanthamoeba is a free-living amoeba widely distributed in the environment which exists as two stages in their life cycle: a motile and trophic replicating trophozoite and a resistant cyst stage. In recent years, the incidence of infections due to *Acanthamoeba* spp. has shown a remarkable increase. This parasite is the causative agent of a sight-threatening infection of the cornea known as *Acanthamoeba* keratitis (AK) and a fatal disease of the central nervous system known as Granulomatous Amebic Encephalitis (GAE) mainly in immunocompromised patients. Although the trophozoite form is much more readily eliminated, at present there are not harmless effective treatments or a single drug that can eliminate both cystic and trophozoite forms. A particular set of compounds known as Minor Grove Binders (MGBs) have the characteristic of binding specifically to minor groove region of double-stranded DNA. The main effects of these MGBs are their ability to interfere with biological functions of DNA such as transcription machinery, also induction of apoptosis, hence cell death. These molecules have received great attention since they can be empirically screened and iteratively refined via chemical synthesis to target various entities such as tumors, bacteria, viruses and parasites. MGBs have been tested *in vitro* using a colorimetric alamarBlue viability cell assay against *Acanthamoeba castellanii* Neff strain. To date, 2 hit compounds were able to inhibit trophozoites producing IC50s of 1.56 μM and 12.5 μM.

A080

Chimeric genes in chimeric genomes

Andrew Keith Watson

Sorbonne Université, Paris, France

Abstract

It is now broadly accepted that genomes are chimeric entities. Prokaryotic genomes include genes acquired by lateral gene transfer from other cellular genomes and from mobile genetic elements. While the extent of lateral gene transfer is reduced in eukaryotic genomes, the entire group is underlined by an endosymbiosis between archaea and bacteria, meaning that any eukaryotic genome has a chimeric origin. Further, secondary endosymbioses in eukaryotes, such as the acquisition of the plastid, mean that photosynthetic eukaryotic lineages are even more chimeric, including genes from additional sources. Genes or gene families in genomes can also have chimeric origins. The fusion and fissions of genes from different gene families contributes extensively to the formation of new genes and gene families. Thus, in chimeric genomes, fusions and fissions may combine components of different phylogenetic origins, or combine components from an external origin with respect to the host lineage in new ways. Our recent work has identified hundreds of candidate chimeric genes associated with different evolutionary transitions, including the origin of eukaryotes, the origins of photosynthetic eukaryotes, and the origin of Haloarchaea. Here, we explore the commonalities in what kind of components make up putative chimeric genes across these different transitions.

A081

Characterisation of Vibrio cholerae chromosome II segregation proteins

Sat Chodha

University of Sheffield, Sheffield, United Kingdom

Abstract

Background: *Vibrio cholerae* chromosome II uses the *parABSII* system to segregate from the midcell to quarter cell positions. ParB2 binds to parS2 sites, proximal to ori2, to form foci. The ATPase, ParA2, was observed to oscillate from pole-to-pole. However, little is known about how chromosome II is segregated using the Par system. Here, we use biochemical assays to characterise ParA2 and ParB2 activities.

Methods: Electrophoretic Mobility Shift Assay (EMSA),

Thin layer chromatography (TLC),

Size exclusion chromatography/multi-angle light scattering (SECMALS),

Circular dichroism (CD),

Fluorescence spectrophotometry.

Results: EMSA analysis produces ParA2-DNA binding curves from EMSA analyses to show high affinity of ParA2 to DNA in the presence of ATP or ATPγS. There is weak affinity to DNA in presence of ADP or no nucleotide, while full binding is not achieved. TLC analysis shows weak ParA2 ATPase activity that is stimulated by ParB2 and DNA, 4-fold and 2-fold, respectively. EMSA analysis also shows ParB2 binds *parS2* with high affinity to form specific binding species, and binds weakly to non-specific DNA.

Conclusions: these data demonstrate that specific ParB2-parS2 nucleoprotein complexes are formed, and stimulates ParA2-ATP hydrolysis to mediate chromosome segregation.

A082

Accessory genome drives virulence and resistance of the ocular isolate of *P. aeruginosa*

Dinesh Subedi[1], Gurjeet Singh Kohli[2], Ajay Kumar Vijay[1], Scott Rice [2], Mark Willcox[1]

[1]The University of New South Wales , Kensington, Australia. [2]Nanyang Technological University , Singapore, Singapore

Abstract

Accessory genome leads to rapid evolution of genetic materials, allowing traits such as antibiotic resistance and virulence to spread through the bacterial community. Although extensive research has been carried out to understand the accessory genome of *P. aeruginosa*, no single study exists which examine the complete accessory genome of ocular *P. aeruginosa.* We obtained the complete genome an ocular isolate *of P. aeruginosa* strains PA34 utilising hybrid genome assembly of whole genome sequence reads from Illumina and Oxford Nanopore Technology followed by PCR. In-depth genomic analysis was done using various bioinformatics tools. The complete genome of PA34 includes a chromosome of 6.8 Mbp and two plasmids of 95.4 Kbp (pMKPA34-1) and 26.8 Kbp (pMKPA34-2). PA34 had a larger accessory genome (1,213 genes) than three reference genomes and had 543 unique genes. These exclusive genes encode features related to metal and antibiotic resistance, phage integrase and transposons. At least 24 GIs were predicated in the complete chromosome, of which, two were integrated into the novel sites. Eleven GIs were found to be associated with either carrying or replacing pathogenic genes. A bacteriophage carried aminoglycoside resistance gene (also called resistance island). Two different plasmids carried other six antibiotic resistance genes. The large accessory genome of the ocular isolate PA34, which are different than other strains remarkably shaped the virulence and antibiotic resistance. This provides insights that further complete genome study in a larger sample requires to unveil complexity of accessory genome of ocular *P. aeruginosa*.

A083

Interaction of the bacterial master initiator protein DnaA with the DnaA-trio replication origin element

Daniel Stevens, Heath Murray

Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

The highly conserved master bacterial initiator protein, DnaA performs several activities at the origin of replication (*oriC*) to start new rounds of DNA synthesis. These activities are guided by essential sequence information encoded by the origin. Firstly DnaA monomers are recruited to the origin by binding specific double-stranded sequences termed DnaA-boxes. Specificity for these has long been understood. DnaA then assembles into a filament that interacts and stretches a single DNA strand inducing unwinding. A new element within the origin, the DnaA-trio, has been identified to provide specificity for single-strand binding by the filament. The sequence and function of the DnaA-trio is known but the mechanism for specific recognition remains unidentified.

To address how DnaA specifically binds DnaA-trios we began investigating the initiator specific motif (ISM) of DnaA *in vivo* and *in vitro*. The ISM is an insertion in the AAA+ motif (ATPasaes associated with various cellular activities) unique to replication initiator proteins containing residues implicated in filament formation and single-strand DNA binding. Utilising an inducible heterologous replication initiation system we performed an alanine scan of the ISM identifying residues essential for DnaA function *in vivo*. Several residues were identified as being essential for DnaA activity. Alanine substitution variants were purified and investigated *in vitro* for their ability to form filaments non-specifically in solution and specifically on DnaA-trios, as well as to unwind duplex DNA.

Taken together the results provide new insights into the structure and function of the DnaA ISM and provide clues towards identifying protein specificity for DnaA-trios.

Genetics and genomics forum
Presentations: Monday and Tuesday evening

A084

Characterization of prophages in *Salmonella* Typhimurium definitive type 8.

Salina Thapa, Manal Mohammed

University of Westminster, Westminster, United Kingdom

Abstract

Background

Salmonella Typhimurium DT8 has been associated with human outbreaks. Anderson phage typing scheme has been used for more than four decades for subtyping of *Salmonella* Typhimurium, but it has shown some limitations. Here, we characterize prophages among *Salmonella* Typhimurium DT8 strains to test the potential use of prophage sequence profiles for subtyping.

Method

A total of 54 isolates of *Salmonella* Typhimurium DT8 were selected for this study which was part of an outbreak study by Ashton et. al (2015) [*]. Twenty-six isolates were associated with an outbreak in the States of Jersey in 2013 and 28 isolates were non-outbreak-associated isolates. Comparative genomics using a range of bioinformatic tools was carried out including SPAdes for *de novo* assembly of WGS data. PHASTER was used for characterisation of prophages (http://phaster.ca/).

Result

All DT8 strains are lysogenic for three prophages (RE-2010, ST64B and Gifsy-2). Moreover, the three prophages showed identical sequence among outbreak and non-outbreak isolates.

Interestingly, prophage SSU5 was detected in one non-outbreak isolate. Prophage SSU5 is closely related to cryptic plasmid *pHMC2* that infects only rough strains of *Salmonella* Typhimurium. This study is the first to report the presence of prophage SSU5 in *Salmonella* Typhimurium DT8.

Conclusion

It is crucial to use accurate, reliable and highly discriminative subtyping methods for epidemiological characterisation and outbreak investigation of *Salmonella* Typhimurium.

Prophage profiling might be unsuitable subtyping method. The emerging genetic analysis should be combined with the conventional method for epidemiological surveillance until WGS-based analysis can be improved and standardized.

Genetics and genomics forum
Presentations: Monday and Tuesday evening

*****https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4336196/

A085

Genome-guided screening of bacterial isolates to identify potential antibiotic producers.

Opeyemi Awolope, Noelle O'Driscoll, Andrew Lamb

Robert Gordon University, Aberdeen, United Kingdom

Abstract

The urgent need for new antibiotics cannot be overemphasized. Bacterial secondary metabolites remain a relatively untapped source of new therapies. The ability to produce these bioactive compounds is however not universal to all bacterial species. Two key indicators are bacterial genome size (>3Mb), and the presence of antibiotic-encoding biosynthetic gene cluster (BGCs) within the genomes. BGC distribution is largely determined by phylogeny. Another attribute of some antibiotic producers is the ability to withstand nutritional stress. We exploited these attributes to isolate and identify potential antibiotic producers. A minimal substrate medium was used to isolate nutritionally versatile bacterial strains from topsoil collected from the rhizosphere. The genera of isolates were identified by 16S rRNA gene sequence comparison as *Pseudomonas*, *Hafnia* and *Obesumbacterium*. The typical genome size of species in these genera are 6.2Mb, 4.7Mb and 5.0Mb respectively. The antiSMASH database was browsed by phylogeny to determine the distribution pattern of BGCs in these genera. *Pseudomonas* strains have an average of 7 BGCs within their genomes that may encode antibiotics, whilst *Hafnia* and *Obesumbacterium* strains have 2 and 0 respectively. Therefore, the isolated *Pseudomonas* strain has the greatest potential to biosynthesis antibiotics. However, the biosynthetic potential of other isolates may be understated given the typical genome size of species in their genera, and their ecological origin. Consequently, all isolates are prime candidates for the next stage of the project which involves genome mining for cryptic or silent genes that may encode novel compounds with antibiotic properties. More isolates are also being recovered.

A086

Characterisation of Extended Spectrum Beta-Lactamases (ESBL) resistance in multi-drug resistant S*almonella Concord*

Winnie Lee[1,2], David Greig[2], Martin Day[2], Marie Chattaway[2], Satheesh Nair[2]

[1]University of Hertfordshire , Hatfield, United Kingdom. [2]Public Health England, London, United Kingdom

Abstract

Salmonellosis in children from Ethiopia is caused mainly by *Salmonella* Concord, which are highly invasive, multi-drug resistant and extended-spectrum β-lactamase (ESBL) producers. *S.* Concord infections have been observed in children adopted from Ethiopia, now living in Europe and United States. *S.* Concord infections are present in parents of these adopted children, posing a significant dilemma for treatment. Data from *Salmonella* isolates are stored in Public Health England's in-house Gastro Data Warehouse (GDW) database. Resistance profiles for 37 pure *S.* Concord isolates were determined using agar incorporation methods using EUCAST guidelines. The minimum inhibitory concentration (MIC) was determined for 18 antimicrobial agents and ESBL resistance was confirmed using Double Disk Synergy and ESBL E-test methods. ESBL resistance was present in 8 isolates with resistance most commonly seen against penicillins and cephalosporins. Isolates 408 and 537, and isolate 527 displayed resistance to 78% and 56% of the antibiotics respectively. Drug resistant regions in isolates 408, 527 and 537 that have been previously sequenced by an Illumina HiSeq 2500 were characterised. ESBL genes $bla_{CTXM-15}$ was present in isolate 527 and 408 and bla_{SHV-12} was found in isolate 537. Replicons from plasmids, InCI2, InCFIB, InCF2 and IncH2 were located in the assembled sequences of the three previously sequenced isolates. In conclusion, the possible mechanisms causing spread of ESBL resistance in *S.* Concord is most likely due to acquisition of plasmids through horizontal gene transfer from various *Enterobacteriaceae,* however further research must be conducted to confirm this to advance antimicrobial research in this area.

A087

What Factors Contribute to the Long-term Persistence of *Shigella* species as pathogens?

Rebecca Bennett

University of Liverpool, Liverpool, United Kingdom

Abstract

The genus *Shigella* is a globally important pathogen responsible for the infectious disease shigellosis which causes an estimated 125 million cases worldwide and up to 100,000 deaths. *Shigella* species are now recognised as a priority organism by the World Health Organisation due to the increasing antimicrobial resistance (AMR) observed. For adults, treatment of *Shigella* infections commonly consists of ciprofloxacin, however, recently there have been confirmed cases of ciprofloxacin-resistant strains. Most *Shigella* is already resistant to ampicillin and trimethoprim so added resistance to ciprofloxacin is a worrying development. Also of note is the role played by toxin-antitoxin systems where they are critical for maintaining large plasmids that typically contain genes of interest such as AMR genes and virulence factors. The Murray collection comprises of several hundred bacterial strains from the pre-antibiotic era (1917 - 1954) from a range of geographic locations. Within this collection there are many *Shigella* isolates which are now publicly available for analysis. There are also available datasets for modern collections which have been sequenced for past studies. We will use these data sets to perform bioinformatic analysis and characterise the dynamic changes in the genetic composition across time. The Murray Collection isolates provide a prospective from the pre-antibiotic era and so from the comparisons with modern isolates we will observe the effect of routine antibiotic use has on the evolution of antimicrobial resistance. Through this investigation the hope is to provide unprecedented insight into how AMR and toxin-anti-toxin systems contribute to the long-term persistence and success of *Shigella*.

Genetics and genomics forum

Presentations: Monday and Tuesday evening

A088

Phase variation of Opa proteins in hypervirulent serogroup W meningococcal isolates.

Neelam Dave[1], Luke Green[1], Jay Lucidarme[2], Neil Oldfield[3], Christopher Bayliss[1]

[1]University of Leicester, Leicester , United Kingdom. [2]2Meningococcal Reference Unit, Manchester, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom

Abstract

Serotype W, sequence type 11 *Neisseria meningitidis* are an increasing cause of morbidity and mortality. The mechanisms underpinning the success of this clone remain unclear. Meningococci express up to four Opa proteins, which mediate adhesion to/invasion of host cells. Opa expression undergoes phase variation– a high frequency ON/OFF switch in gene expression due to insertion/deletions of pentameric repeats (5'-CTCTT-3') in the coding region. NadA functions as an adhesion, and is phase-variable through tetrameric repeats in a regulatory. WGS and PCR-based fragment size analysis of 121 invasive and 51 carriage MenW:ST11 strains was conducted to determine gene expression patterns between invasive and carriage. Inactivating mutations were constructed in *opa*, *pilE* and *nadA* genes of two representative MenW isolates. These mutants were tested in *in vitro* infection assays for adhesion and invasion of A549 cells. We observe that four *opa* loci are present in all MenW:cc11 isolates and that OpaB and OpaD share 100% sequence similarity. Repeat number variability was detected between the 'original UK' strain and the novel '2013-strain' of the hypervirulent MenW:cc11 South American sublineage. No significant differences in the patterns of Opa expression were observed between invasive and carriage isolates. *In vitro* assays with *pilE* and *nadA* deletion suggest that NadA has an effect on invasion, while the double mutant shows a reduction in both adhesion and invasion. Our findings indicate that the Opa proteins do not contribute to the invasiveness of MenW:cc11 strains, but may give a niche specific advantage by enhancing phase variation-mediated immune evasion.

A089

Characterisation of *rpoS* alleles in UPEC strain CFT073

Naoise McGarry, Stephen Smith

Trinity College, Dublin, Ireland

Abstract

Escherichia coli is a major cause of urinary tract infections, bacteraemia and sepsis. CFT073 is a well-studied, prototypic urosepsis isolate. This laboratory has previously shown that strain CFT073 is serum-resistant, with resistance being imparted by factors including capsule and extracellular polysaccharides *inter alia*. It has become apparent that not all CFT073 strains are identical, some harbour a 5 bp insertion in the *rpoS* gene which results in a truncated, non-functional RpoS. In this study, we compare CFT073 wild-type and an *rpoS* mutant. The gene sequence of *rpoS* in each isolate was verified. Next, the sensitivity of the bacteria to hydrogen peroxide was determined. Finally, wild-type and mutant strains were rendered bioluminescent. Bioluminescence permits the real-time detection of viability. The contribution of RpoS to serum resistance was examined by bioluminescence.

A090

Fitness costs of methicillin resistance in Staphylococcus aureus

Jacqueline Gill

University of Oxford, Oxford, United Kingdom

Abstract

Staphylococcus aureus is a Gram-positive bacteria, and a common human pathogen. It is a leading cause of healthcare-associated infections worldwide, causing potentially fatal bacteraemia. Infections can be treated using antibiotics, but many strains have quickly developed resistance to the most common antibiotics.

Methicillin resistance in *Staphylococcus* is acquired through the uptake of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*). SCC*mec* is a large mobile genetic element, which can integrate into the *Staphylococcus* genome. It carries the *mecA* gene, which confers resistance to broad-spectrum β-lactam antibiotics, including methicillin.

Several different SCC*mec* elements have been described, some of which can carry additional antibiotic resistance genes, alongside *mecA*. These can include resistance to common antibiotics or resistance to heavy metals. Smaller SCC*mec* types do not encode any additional antibiotic resistance genes. These have been shown to confer no fitness cost to the bacteria. Because of this, smaller SCC*mec* types are becoming increasingly more common, particularly in community-associated MRSA infections.

A091

Fitting linear mixed models to a highly-structured dataset effectively controls for population structure in bacterial genome-wide association studies

Samuel Kidman[1], Emem-Fong Ukor[2], Andres Floto[2], Julian Parkhill[1]

[1]Wellcome Trust Sanger Institute, Hinxton, CB10 1SA, United Kingdom. [2]Cambridge Centre for Lung Infection, Royal Papworth Hospital, Cambridge, CB23 3RE, United Kingdom

Abstract

Cystic fibrosis is an inherited, autosomal recessive disease that causes an accumulation of viscous mucus within the lung, leading to chronic bacterial infections. *Pseudomonas aeruginosa* is a Gram-negative, opportunistic pathogen that can invade the cystic fibrosis lung, and is the most common bacteria isolated from cystic fibrosis patients.

In the largest study of its kind, ~4,200 *P. aeruginosa* isolates were collected from nine cystic fibrosis patients. The isolates were whole-genome sequenced and screened for 14 virulence-related phenotypes. We aim to associate phenotype with genotype by fitting both linear models and linear mixed models association tests. During this study, linear mixed models were found to effectively control for strong population structure signals, allowing relevant associations to be uncovered. When sub-sampling the dataset into unstructured, single-patient groups, fitting linear mixed models were found to associate genotype with phenotype as effectively as fitting linear models. We also found that aggregating non-synonymous SNPs in the same gene to a single knockout mutation increases the power of the association tests to identify relevant associations.

The future direction of this study will involve filtering the results to identify any phenotypically-relevant associations. These putative associations will then be experimentally verified.

A092

Identification of Staphylococcus Aureus and Methicillin Resistant S. Aureus in Jail Populations

Ryann Whealy[1,2], Daryn Erickson[1,2], Jill Cocking[3,1], Crystal Hepp[3,1], Viacheslav Fofanov[3,1]

[1]Pathogen and Microbiome Institute, Flagstaff, USA. [2]Northern Arizona University, Flagstaff, USA. [3]School of Informatics, Computing & Cyber Systems, NAU, Flagstaff, USA

Abstract

Staphylococcus aureus is the leading cause of soft tissue and skin infection, but also causes pneumonia, endocarditis, and bone infection. The same infections can be caused by methicillin resistant *S. aureus* (MRSA), but are much more difficult to treat because MRSA is highly resistant to antibiotics.

In ethnic and socioeconomic minority groups, methicillin resistance in *S. aureus* is 30% higher than the general population. As a result, methicillin resistance is likely to have higher prevalence in jails and prisons where the demographic is disproportionately represented by ethnic minorities, individuals with lower average socioeconomic status, and less access to healthcare. Jail inmates are an understudied but very important population because diseases acquired in jails can be easily introduced into the general population and vice versa because cycling into and out of jail is very common. The presence of *S. aureus* is an important determinant of the development of soft tissue and other infections, making the detection of this bacteria and the identification of antibiotic resistance essential in assessing risk of disease.

In this study, we identified S. aureus in samples from a United States county jail through bacterial plating and DNA extraction. Methicillin resistance was identified in *S. aureus* positive samples through PCR and sequencing. Our analysis has found elevated rates of S. aureus in the jail population being studied. This project will determine if rates of *S. aureus* and methicillin resistance in *S. aureus* isolated from this demographic are disproportionate to the general population.

A093

Causative Agents of Early Childhood Caries: Challenges and Solutions in Culturing and Sequencing

Daryn Erickson[1,2], Ryann Whealy[1,2], Jill Cocking[3,1], Crystal Hepp[3,1], Viacheslav Fofanov[3,1]

[1]Pathogen and Microbiome Institute, Flagstaff, USA. [2]Northern Arizona University, Flagstaff, USA. [3]School of Informatics and Cyber Systems, NAU, Flagstaff, USA

Abstract

Dental caries, also known as cavities or dental decay, effects children at a rate five times higher than asthma in the United States. This disease is highly preventable but causes extreme pain and costs millions of dollars to treat every year. The presence of Streptococcus mutans and Streptococcus sobrinus plays a major role in the development and progression of tooth decay; therefore, it is important to establish colonization of these bacteria to assess the risk of developing the disease.

Streptococcus bacteria are difficult to grow, extract, and sequence due to strict necessary growth conditions. In this study, we evaluated the performance of different storage and culturing protocols and developed strategies for reducing interference by unwanted bacterial and fungal genomes when sequencing extracted samples. We compared storage conditions of samples at various temperatures, and with and without glycerol. We decided the best storage method was at -80°C in a specialized solution known as Aimes. When sequencing cultures, we encountered various unwanted bacterial and fungal genomes. To reduce this, we modified our culturing methods by including growth in anaerobic conditions and using serial isolation streaking. These modifications have limited the growth of aerobic specimens and increased culture purity before extraction. With this study, we will be able to better understand the oral microbiome and aim to identify virulence factors in S. mutans and S. sobrinus that contribute to the high rates of dental caries in children.

A094

Evolvability of orthologous genes

Hind Abdalaal[1], Joakim Näsvall [2]

[1]Uppsala University, Uppsala, Sweden. [2]Uppsala, Uppsala, Sweden

Abstract

The project aims to generate a library of laboratory orthologous genes for studying evolvability. In this study, we have taken one of histidine biosynthetic genes *hisA* from *Salmonella enterica* through alternating rounds of weak selection (simulated by random mutagenesis through error-prone PCR and screens for partial loss of *hisA* function) followed by strong purifying selection (simulated by additional rounds of random mutagenesis and screens for restored *hisA* function). We have started from the wild -type *hisA* gene expressed from relatively weak arabinose inducible promoter *ParaBAD.* Twenty different single nucleotide polymorphisms (SNPs) that cause slow growth in the absence of histidine were isolated. In twelve of these deleterious mutants the activity was restored to wild-type levels by additional SNP after another round of random mutagenesis.

In this study, in some of the compensated *hisA* variants it was difficult to isolate further deleterious mutations, suggesting that these compensating mutations can mask the effects of additional deleterious mutations. By combining the compensating mutations with known deleterious mutations, complete or partial compensation was seen.

Orthologs from one of the diverging lineages were also compared for their evolvability toward TrpF activity (ability to synthesize tryptophan) using fluctuation test. Two orthologs in this lineage showed higher evolvability compared to the wild type *hisA.*

Genetics and genomics forum

Presentations: Monday and Tuesday evening

A095

Genetic regulation of compost and plant degradation in *Agaricus bisporus.*

Keith Dunne[1,2], Martin O`Donoghue[2], Helen Grogan[2], Kerry Burton[3], Mary Heneghan[1]

[1]Institute of Technology Sligo, Sligo, Ireland. [2]Teagasc, Dublin, Ireland. [3]Independent Consulant, Warwickshire, United Kingdom

Abstract

Agaricus bisporus, the common button mushroom, is a major contributor to the global carbon-nitrogen cycle through its role as a secondary decomposer of plant and leaf litter. It is also an economically significant mushroom with a domestic farm gate value in excess of €120 million. This research aims to elucidate the molecular mechanisms involved in compost and plant matter degradation by *A. bisporus*. An examination of the entire genome using microarray analysis was carried out on *A. bisporus* at 48-hour intervals over the course of its commercial lifecycle. This data revealed several genes that appear to be involved in the genetic regulation of mushroom production. From this data, genes with potentially critical roles in compost utilisation and plant matter degradation were identified. Five genes of significant interest were selected for further study. These include three carbohydrate degrading genes and an unknown gene which appears to be unique to *A. bisporus*, all of which had an expression profile that matched the commercial growth pattern. The fifth gene was selected based on its significant down-regulation during peak mushroom growth. To further compliment this data a bioinformatic analysis of known transcription factor binding sites present within each promoter of *A. bisporus* is also being carried out. The data generated from this study will provide crucial insight into the mechanisms behind the regulation of *A. bisporus* growth and its ability to degrade plant matter and compost degradation.

A096

AphA is a master regulator of natural competence in *Vibrio cholerae*

James Haycocks, Gemma Warren, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

Vibrio cholerae is the etiological agent of cholera, a disease affecting 4.3 million people each year (WHO, 2015). The symptoms of cholera include profuse watery diarrhoea and vomiting, leading to coma and death in serious cases. The El Tor biotype of *V. cholerae* is globally predominant, and the success of this biotype to cause disease is thought to be due, in part, to the ability of the organism to successfully colonise two different environments; i) marine and brackish coastal waters where *V. cholerae* forms biofilms on the chitinous surfaces of shellfish, and ii) the human intestine. When bound to chitinous surfaces *V. cholerae* expresses genes required for biofilm formation, chitin utilisation and natural competence. Conversely, within the human host, *V. cholerae* switches on the expression of virulence genes.

The transcription factor AphA was discovered as a regulator of genes encoding the toxin co-regulated pilus (TCP) of *V. cholerae*. It is also known that AphA is active when *V. cholerae* populations have a low cell density. Hence, AphA is generally considered a regulator of virulence and quorum sensing. We have mapped DNA binding by AphA across the entire *V. cholerae* genome. The majority of AphA target genes encode cell surface and cell envelope proteins. Unexpectedly, we show that AphA also targets key gene clusters within the natural competence regulon of *V. cholerae*. Using biochemistry and genetics we show that AphA is a master repressor of natural competence and acts to antagonise transcription activation by the cyclic-AMP receptor protein.

Genetics and genomics forum
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A097

Whole Genome Analysis of the Common Pathogens Associated with Microbial Keratitis.

Michael Glenn[1], Stephen Kaye[2], Timothy Neal [2], Paul Roberts [2], Craig Winstanley [2], Derek Fairley[3], David Simpson[1]

[1]Centre for Experimental Medicine, Queens University, Belfast, United Kingdom. [2]Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool , United Kingdom. [3]Belfast Health and Social Care Trust, Belfast, United Kingdom

Abstract

Background

Microbial Keratitis is a serious infection of the cornea and a major cause of corneal opacity and vision loss worldwide. Whole-genome sequencing (WGS) of keratitis bacterial isolates has the ability to provide a wealth of clinically-valuable information. The potential of metagenomics has also been evaluated as current techniques for pathogen identification are laborious, time-consuming and associated with poor culture rates, making metagenomics an exciting future prospect.

Methods/Results

Samples from keratitis patients were collected and isolates grown in agar before DNA was extracted from pure-cultures and WGS data was obtained from a total of 30 isolates, including common causative agents *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Libraries were prepared using a miniaturised Nextera XT protocol and sequencing was carried out on Illumina's NextSeq platform. Genomic analysis was then based around MLST-typing, comparative genomics, and the identification of key genes inferring antibiotic resistance and virulence. Our pathogens exhibited diverse evolutionary lineages and a range of virulence-associated genes, providing insight into pathogenicity. The ability to obtain sufficient DNA quantities for metagenomics sequencing was also assessed using various host depletion and microbiome enrichment techniques.

Discussion/Conclusion

The clinical role of next-generation sequencing is currently limited due to associated costs and required computational resources, however in the future this method could replace current techniques for pathogen identification. WGS provides important insights into the genomes of the common keratitis infectious agents, improving understanding of the aetiology of corneal infections and metagenomics shows future potential to be the centre of culture-independent pathogen identification.

Genetics and genomics forum
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A098

The genome wide binding properties of the regulator of multiple antibiotic resistance (MarR) in the Escherichiacoli genome.

Alistair Middlemiss, James Haycocks

The University of Birmingham, Birmingham, United Kingdom

Abstract

The ability of bacteria to adapt and respond to environmental stresses has catalysed the formation of complex genetic systems. One system, the multiple antibiotic resistance (mar) operon, is negatively regulated by MarR. Upon detection of an antibiotic stress (or other environmental stress) MarR dissociates from the *marRAB*promoter, allowing the activator of multiple antibiotic resistance (MarA) to be transcribed. In order to identify the complete regulon of MarR we generated a set of tools to tag the protein with a 3x FLAG or 8x Myc tag at either the N- or C-terminus. Following tagging and a chromatin immunoprecipitation and DNA sequencing (ChIP-Seq) experiment we have shown that MarR has a single binding target in the *Escherichia coli*genome.

A099

Exploring the Role of VpsT in *Vibrio cholerae* Lifestyle Switching

Tom Guest[1], James Haycocks[2], Gemma Warren[1], David Grainger[1]

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

Abstract

Biofilm formation is an important stage of the *Vibrio cholerae* lifecycle. The transcription factor VpsT is a master regulator of biofilm formation that activates the expression of biofilm matrix components in response to elevated intracellular c-di-GMP. VpsT has also been shown to repress the expression of *rpoS* and genes involved in motility. This suggests VpsT may have a wider role in the transition from a motile to sessile lifestyle.

To better understand the role of VpsT in *V. cholerae* lifestyle switching ChIP-seq (chromatin immunoprecipitation and DNA sequencing) was used to map VpsT binding across the genome. Our data reveals many additional targets, defines the DNA binding motif for VpsT, and expands the VpsT regulon to include genes involved in c-di-GMP metabolism, motility and virulence. The VpsT interaction at target promoters is c-di-GMP dependent and can repress or activate gene expression.

A100

Phylogenetic Analysis of Avian Orthoreovirus strains circulating in the UK and Ireland.

Pauline Sheridan[1], Victoria Smyth[1], Nikki Marks[2]

[1]AFBI, Belfast, United Kingdom. [2]QUB, Belfast, United Kingdom

Abstract

Avian orthoreoviruses (ARV) are double stranded RNA viruses with segmented genomes that cause economically significant disease in commercial domestic poultry despite widespread use of vaccination. While 80% of ARV isolates are not associated with clinical disease, some ARVs are associated with poor growth in young chicks and tenosynovitis (lameness) in older birds. Many clinical ARV isolates of circulating strains have been classified as belonging to at least 6 genogroups which are very different to previously described genogroups.

The aim of this study is to investigate possible genomic factors which may influence ARV pathogenicity. Whole genomes of multiple ARV isolates associated with tenosynovitis or growth retardation were sequenced and a further set of isolates obtained from clinically unaffected flocks was also sequenced. Viral RNAs were extracted and converted into complementary DNAs, which were prepared for whole genome shotgun sequencing on a MiSeq instrument. Each genomic segment of the viral isolates was analysed phylogenetically and attributed to genetically similar clusters, with comparison to published genomes of ARVs from Asia, America and continental Europe and then individual isolates were categorised on the basis of segment assortment. The UK sequences clustered in distinct clades, which in four of the ten segments were divergent from other global strains. In the remaining segments the UK strains formed several clades with close phylogenetic relationship to subsets of Asian and American strains. The most variable regions were found in segment S1 which encodes the cell attachment protein sigma C, with other segments showing more conservation of sequence.

A101

Genotypic and phenotypic diversity in *Candida tropicalis*

Caoimhe O'Brien, João Oliveira-Pacheco, Geraldine Butler

Conway Institute, UCD, Dublin, Ireland

Abstract

Candida tropicalis is a human pathogen with significant mortality rates, which is particularly prevalent in tropical regions. The first genome sequence of *C. tropicalis* was published in 2009. In this study, we sequenced 75 *C. tropicalis* isolates with the aim of studying the intra-species diversity of this pathogen at a phenotypic and genetic level. These isolates were collected from both clinical and environmental sources, and sequenced using Illumina technology.

Phylogenetic analysis of the sequenced isolates using SNP trees and PCA analysis reveals several multi-isolate clusters, which are unrelated to geographical origin. Overall, the genomes of the isolates were stable, with only 4 out of 75 isolates demonstrating any aneuploidy. However, the genomes are highly heterozygous, with one variant, on average, every 136 bases. Variant analysis revealed three highly divergent isolates, with one variant, on average, every 7 bases. Further examination of these isolates revealed that they are the products of hybridisation between two parents; one which is almost identical to the reference strain (>99.9%), and a second, unidentified parent, which is approximately 4.5% different in its sequence to the reference strain. Differences in sequence indicate that these hybrids arose from separate hybridisation events.

Phenotypic differences are also observed between isolates under certain conditions, particularly in the presence of cell wall stressors, metal stressors and antifungal drugs. Cosine similarity was used to identify correlations between genetic variants and phenotypic variation in all isolates, identifying variants that may be responsible for particular phenotypes. Work is ongoing to confirm these observed correlations.

A102

Influence of hybrid genes on flavor profiles in lager yeasts

Isa Sawad

Moyne Institute, Trinity college, Dublin, Ireland

Abstract

Abstract

Saccharomyces pastorianus is an inter species hybrid of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus* and is used in the fermentation of lager beers. At least two distinct types (Group I and II) have been identified based on chromosome content and structure. Different recombination events between the parental chromosomes has led to the production of a set of unique hybrid genes containing parts from *S. cerevisiae* and *S. eubayanus*. One such hybrid gene is *XRN1* which plays a major role in mRNA degradation, and influences the steady state levels of cellular mRNAs. Group I strains have two hybrid *XRN1* genes, *HYB1* and *HYB2* while Group II strains contain copies of *HYB1* as well as *S. cerevisiae*-like *XRN1* alleles.

We have investigated the role of the different lager yeasts alleles of *XRN1* on the steady state pool of mRNAs by RNAseq and RT-PCR. We show that the different alleles of *XRN1* have different influences on RNA stabilization, and specifically appear to influence the pool of transcription factors. We also aimed to understand how those different alleles influence the amount of mRNA stabilization when co-expressed in the lager yeasts and find that the steady state pools of transcription factors differ in Group I and II strains.

Furthermore, we want to understand if and how the number of copies of *XRN1* influence the steady state levels of mRNA in lager yeasts. For that we designed strains with a unique di- or polyploid combination of Group I and Group II genes, using CRISPR-Cas9 with the aim of understanding the role of different alleles and allele combinations of hybrid genes on cellular and molecular processes in particular those involved in flavor production during fermentation.

A103

Molecular Characterization Of The Activity And Requirements Of A Novel And Promiscuous Bacteriophage Integrase

Mohammed Mohaisen[1], Heather Allison[2], Alan McCarthy[2]

[1]Anbar University, Anabar, Iraq. [2]Institute of integrative biology, University of Liverpool, Liverpool, United Kingdom

Abstract

Stx bacteriophages are responsible for the dissemination and production of Shiga toxin genes (stx) across the Shigatoxigenic *E. coli* (STEC). These toxigenic bacteriophage hosts can cause severe, life-threatening illness, and Shiga toxin (Stx) is responsible for the severe nature of EHEC infection, a subset of pathogenic STEC. At the point of Stx phage infection, the injected phage DNA can direct its integration into the bacterial chromosome becoming a prophage; the host cell is then known as a lysogen. Unusually, our model Stx phage, Φ24B, can integrate into at least four distinct sites within the *E. coli* genome that shared no easily identifiable recognition sequence pattern. The identification of what are actually required for phage and bacterial DNAs recombination has been tested using both *in vitro* and *in situ* recombination assays. These assays enabled the simple manipulation of bacterial attachment site (*attB*) and phage attachment site (*attP*) sequences.

The aim of the study is to fully characterize the requirements of this promiscuous integrase, carried by the Stx phage Φ24B (IntΦ24B), to drive integration. These assays enabled us to identify the minimal necessary flanking sequences for *attB* site identified (21 bp and 49 bp from the right and left the cross over region, respectively) and the *attP* site (200 bp each side). Furthermore, we identified that the Φ24B integrase does not need Integration Host Factor (IHF) to drive integration.

Fi

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A104

Investigating The Influence Of Host Genetics On The Metagenome:The Intestinal Microbiome Of Two Arid Mammalian Species

Peter Osborne[1], Ella Pasternak[2], Noga Kronfield-Schor[2], Lindsay Hall[3], Shabhonam Caim[3], David Thybert[1]

[1]Earlham Institute, Norwich, United Kingdom. [2]Department of Zoology, University of Tel Aviv, Tel Aviv, Israel. [3]Quadram Institute, Norwich, United Kingdom

Abstract

Numerous factors have been shown to influence microbiome composition, including host genetics, diet and environmental variables. Recent studies have explored how host genetics influence human gut microbial communities, and those of pre-clinical models e.g. mice. However to date no studies have determined the influence of host genetics and extreme environments on wild animal microbiomes. Furthermore, when 'wild' species have been utilised this has typically involved working on captive individuals, restricted to a single species or limited to single sampling without replicates. Here we present work which takes advantage of a unique opportunity to directly investigate host organism genetic influences and environment on the resident microbiome.

Our dataset comprised samples from individuals of two closely related sympatric, independently adapted arid mouse species, subject to the same stresses and diet; Acomys cahirinus and Acomys russatus. These desert dwellers are very highly adapted to the extreme environment they live in, and therefore represent an exciting opportunity to probe key microbiome-environment-host genetic questions. Samples are replicates from wild individuals, separated by a period of four months.

Wild individuals were captured on two occasions and faecal samples collected. DNA extracted and subjected to shotgun metagenomic sequencing, generating NGS data. Preliminary analysis allowed us to probe community composition and relative abundance within individuals, and between members of the same species. Utilising Kraken, Centrifuge and Metaphlan we have found that abundant taxa include Lactobacillus and Roseburia. Ongoing analysis will enable us to establish the relative influence of host genetics on the metagenome composition of the two species.

A105

Accelerated Evolution of Lager Yeast Strains for improved flavour profiles

Roberto de la Cerda, Ursula Bond

The Moyne Institute. Trinity College Dublin (TDC), Dublin, Ireland

Abstract

Fermented food and beverages have accompanied humans throughout history. Several species of microorganisms can transform raw materials into products with different and improved characteristics, for example alcoholic beverages (wine and beer) or dairy products, such as yogurt. In a world of constant change and competitiveness, brewers have to modulate and create new products to satisfy consumers. Therefore, the aim of my project is to generate lager yeast capable of producing molecules that improve the organoleptic profile of alcoholic beverages and understand the genetic and biochemical changes that increases the production of these aromatic molecules. The initial work has focused on the Ehrlich Pathway and the ester production. To produce changes in the genome, two approaches have been followed: one chemical way, using Radicicol, and one physical way, Heat Shock Thermal Stress (HSTS). Both ways inhibit Heat Shock proteins. Furthermore, this inhibition produces DNA damage, introducing Single sStrand Breaks (SSBs) and Double Strand Breaks (DSBs). The result of this inhbition is damage in the DNA and, furthermore, mutations in the DNA (aneuplidies and INDELS). After the Accelerated evolution, the evolved strains were plated on media containing amino acid analogues. These amino acid analogues select for cells with decreased feed-back inhibition of amino acid biosynthesis. Different mutant strains were generated with these two ways. These strains will be tested by different approaches, as growth curves, genome ploidy using cCGH and CHEF gel and qPCR analysis to measure the flux throughout the fermentation.

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A106

National Collection of Type Cultures: The Bacteriophage and Plasmid Collections and Repositories

Mohammed-Abbas Fazal, Juandem Agendia, Kazutomo Yokoya, Matthew Hannah, Julie Russell, Sarah Alexander

Public Health England , London, United Kingdom

Abstract

The National Collection of Type Cultures (NCTC) is the world's oldest bacterial collection that was specifically established to provide strains globally to support scientific research. In addition to the general catalogue NCTC has a fully curated bacteriophage and plasmid archive that to date has not recently been made available to the wider scientific community. The NCTC bacteriophage collection consists of over 100 bacteriophage and their corresponding bacterial hosts which were originally deposited primarily for their value in bacterial typing. The collection consists of bacteriophage from the following hosts: *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli* and Campylobacter. The NCTC Datta plasmid collection is a curated collection of over 500 unique plasmids which were originally curated to examine the biology of plasmid transfer between and within bacterial strains.

The NCTC bacteriophage collection is currently being fully characterised using a range of modern methods including genomic sequencing and electron microscopy. The plasmid collection is also being characterised using genomic sequencing and restriction digest profiles. It is intended that once characterised and rebanked both the plasmid and bacteriophage collections will be made available in 2019 to scientists to support research and development. The NCTC bacteriophage and plasmid collections will both be dynamic, representing an active repositories into which microbiologists can deposit phages and plasmids to support accessibility and reproducibility in science.

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A107

Characterization of the mobilome of invasive non-typhoidal *Salmonella* Dublin

Manal Mohammed[1], Sabrina Cadel-Six[2,3], Marie-Leone Vignaud[2,3], Myron M. Levine[4]

[1]School of Life Sciences, University of Westminster, London, United Kingdom. [2]Université Paris-Est, Marne-la-Vallée, France. [3]ANSES, Laboratory for Food Safety, Salmonella and Listeria Unit, Maisons-Alfort, France. [4]Center for Vaccine Development, School of Medicine, University of Maryland, Maryland, USA

Abstract

Background:

Human infection by non-typhoidal *Salmonella* Dublin is mainly characterised by self-limiting gastrointestinal illness. However, a proportion of cases are associated with invasive illness (bacteraemia, septicaemia and meningitis). The genetic basis of invasiveness of *S.* Dublin in humans is not well characterised. Acquisition of mobile genetic elements (MGEs) might contribute to bacterial virulence and pathogenesis. The aim of this study is to characterize the mobilome of *S.* Dublin.

Method:

A set of *S.* Dublin isolates from human invasive and non-invasive (gastroenteritis) cases and from veterinary cases were selected for whole genome sequencing (WGS). Isolates included 12 human invasive isolates from blood of children admitted to l'Hôpital Gabriel Touré in Mali with fever and severe clinical illness. For comparison, we included 10 clinical non-invasive isolates and 8 veterinary isolates from raw milk and raw milk cheeses. WGS of multiplexed libraries was carried out on Illumina HiSeq using 250bp PE protocol. *De novo* assembly was carried out using SPAdes and draft genomes were annotated using Prokka.

Result:

S. Dublin strains harbour several MGEs including the *S.* Dublin virulence plasmid, *Salmonella* pathogenicity islands; SPI-6 and SPI-19 harbouring T6SSs and the novel pathogenicity island ST313-GI. Interestingly, Vi antigen was present in two veterinary strains but was absent from other strains indicating that Vi antigen is not the main virulence determinant in *S.* Dublin.

Conclusion:

WGS revealed several MGEs forming the mobilome of *S.* Dublin that may contribute to bacterial virulence. Comparative transcriptomics will be carried out to identify differentially expressed genes in invasive strains compared with non-invasive and veterinary strains.

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A108

Genome Dynamics of *Streptomyces clavuligerus*

Lis Algora, Paul Herron

University of Strathclyde, Glasgow, United Kingdom

Abstract

Streptomyces clavuligerus is the producer of clavulanic acid; a β-lactamase inhibitor that is used in combination with the antibiotic amoxicillin. Genome sequencing has established that *S. clavuligerus* contains a linear chromosome and four linear plasmids: pSCL1, pSCL2, pSCL3 and pSCL4. Although pSCL4 carries 20% of the *S. clavuligerus* coding sequences, none are thought to be essential with the exception of *tap* and *tpg* that encode terminal proteins necessary for priming of the lagging strand at the telomeres. In order to confirm plasmid essentiality and the genomic architecture of *S. clavuligerus*, we first corroborated the available genome sequences by physically mapping the genome using Pulsed-field Gel Electrophoresis, this confirmed the presence of the replicons pSCL2, pSCL3 and pSCL4 with sizes of 150, 450 and 1,800 kilobases respectively. In addition, bioinformatics and physical analyses of the *S. clavuligerus* genome allowed the identification of similar non-archetypal telomeres in the chromosome and pSCL4 at one end of each replicon. Despite this, the other telomere remains unidentified, which suggests there is a dynamic chromosome-plasmid relationship in *S. clavuligerus.* Furthermore, in order to study the essentiality of pSCL4 we first introduced a copy of *tap-tpg* onto the chromosome prior to plasmid curing and/or *tap-tpg* deletion. Consequently, targeted genome sequence and further physical analyses, especially of the telomeres, will permit us understand the complex dynamic relationship between the megaplasmid and the chromosome in this important industrial microorganism.

A109

Phylogenetic analysis of integrases of *Acinetobacter baumannii* genomic islands

Zaaima AL-Jabri[1,2], Roxana Zamudio [2], Eva Horvath-Papp [2], Joseph D. Ralph [2], Zakariya AL-Muharrami [1], Marco Oggioni[2], Kumar Rajakumar[2]

[1]sultan Qaboos University, muscat, Oman. [2]University of Leciester, Leicester, United Kingdom

Abstract

Acinetobacter baumannii among other pathogens is capable of acquiring new virulence determinants via mobile genetic elements (MGE). Analysis of the distribution of genomic islands (GIs) has shown in many species that most MGEs cluster tightly with specific lineages on a phylogenetic core genome tree.

This study aims to address the questions on the association between GIs distribution among certain clonal lineages and to test the contribution of G08 and G62 in *A. baumannii* to metal susceptibility phenotypes.

The whole genome phylogenetic single nucleotide polymorphism (SNP) tree was build using the feature frequency profile. As input, the 101 complete *A. baumannii* genomes deposited in GenBank were used. The matrix was generated using command line BLAST and the R platform to generate the output. Susceptibility testing of heavy metals was performed on wild type and GI-deletion mutants using eight different heavy metals.

Whole genome phylogenetic SNP tree constructed on all complete deposited *A. baumannii* genomes showed that the integrase of the metal resistance G08 and G62 are present only in single or very few sequence types (STs). To test the hypothesis that these GIs even if present only in defined lineage are still mobile, G08 and G62 were selected respectively in strains AB0057 and ATCC17978. Phylogenetic analysis of the respective GI integrases confirmed that the G08*int* and G62*int* belong to a separate clade within the *Acinetobacter* tyrosine recombinases. Susceptibility testing in *A. baumannii* strain ATCC 17978, AB0057, and their respective ΔG62 and ΔG08 mutants confirmed the contribution of the GI-encoded efflux transporters to heavy metal decreased susceptibility.

A110

Use of genomic data from an open access curated non-redundant reference sequence database to identify a new species of Yersinia isolated from the pork chain in the Republic of Ireland

Scott Nguyen, Dechamma Muthappa, Evonne McCabe, Daniel Hurley, João Anes, Séamus Fanning

University College Dublin - Centre for Food Safety, Dublin, Ireland

Abstract

In the field of microbiology, the taxonomic discrimination of bacteria at the species and the subspecies level has been traditionally rooted in biochemical and phenotypic typing methods. These traditional microbiological identification methods are often used to identify pathogenic organisms. *Yersinia enterocolitica* is an important foodborne pathogen and the etiological agent of yersiniosis, a self-limiting gastrointestinal infection which is characterized by diarrhea and abdominal pain. A *Yersinia* isolate originally isolated from an Irish pig environment in a farm-to-fork surveillance study was identified putatively as Y. *enterocolitica* via traditional microbiological typing methods. However whole genome sequencing (WGS) revealed this isolate was not closely related to known *Y. enterocolitica* strains. Upon querying the curated Reference Sequence (RefSeq) database, two other closely related *Yersinia* isolates were identified. These closely related *Yersinia* isolates had been sequenced by the US Food and Drug Administration Center for Food Safety and Applied Nutrition (CFSAN) and the Chinese Academy of Sciences (CAS) and the isolates were identified as *Y. kristensenii* and *Y. enterocolitica* respectively. Through the use of WGS technologies and bioinformatics, these three strains are tentatively classified as a new *Yersinia* species. This study highlights the importance of using WGS to resolve new isolates and how traditional microbiology identification methods has led to the misclassification of three isolates in separate unrelated locations.

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A111

Dissemination of a phage-encoded virulence factor in a pandemic *S*. Typhimurium

Eleonora Tassinari[1,2], Matt Bawn[1,3], Geraldine Duffy[2], Robert A. Kingsley[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Ashtown Food Research Centre, Dublin, Ireland. [3]Earlham Institute, Norwich, United Kingdom

Abstract

Salmonella Typhimurium is the second most common cause of foodborne salmonellosis. Phage-mediated horizontal gene transfer contributes to the virulence of *S*. Typhimurium. An example of a phage-encoded virulence gene is *sopE*, a T3SS effector, found rarely in Typhimurium and associated with epidemics. The current pandemic and multi-drug resistant monophasic variant of *S*. Typhimurium (*S*. 4,[5],12:i:-) acquired *sopE* in multiple events following the lysogeny of a previously undescribed bacteriophage, mTmV. The current study aimed to further investigate the association of the virulence gene with *S*. 4,[5],12:i:- and assess its epidemiological impact. To this end, a large collection of clinical S. Typhimurium isolates from the UK have been analysed for the *sopE* gene and mTmV presence using a phylogenomic approach. While a large proportion of *S*. 4,[5],12:i:- (41%) carried the *sopE* gene, few isolates outside the epidemic clade harboured it. Notably, the mTmV bacteriophage was identified only in *S*. 4,[5],12:i:-, although laboratory experiments demonstrated that the phage host range is not restricted to it. Nonetheless, we identified the phage in other *S*. *enterica* serovars circulating in the same ecological niche of *S*. 4,[5],12:i:-. In addition, a genomic characterisation of mTmV was performed revealing an unexpected level of phage variation. Finally, we identified a novel phage-like element harbouring the gene. The study revealed the large dissemination and selection of the virulence gene in the current epidemic, which is mobilised by multiple and distinct mobile genetic elements.

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A112

Comparison of temperate bacteriophages of *Pseudomonas aeruginosa* from the lungs of chronically infected non-cystic fibrosis bronchiectasis patients over a period 10 years.

Libby Duignan[1], Adnan Tairq[1], Jeremie Hamel[2], Jean-Guillaume Emond Rheault[2], Anthony de Soyza[3], Audrey Perry[4], Roger Levesque[2], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]University of Laval, Quebec City, Canada. [3]University of Newcastle, Newcastle upon Tyne, United Kingdom. [4]Freeman Hospital, Newcastle upon Tyne, United Kingdom

Abstract

The accessory genome of *Pseudomonas aeruginosa* (PA), is frequently perceived insignificant compared to the core genome, however typically contains temperate bacteriophage (phage) genomes that effect the bacterial host. PA presence in chronically infected lungs correlates with loss of lung function particularly in cystic fibrosis (CF) and non-CF bronchiectasis (nCFBR). This study focuses on isolates from chronically infected nCFBR patients isolated over a decade, shedding light on how temperate phages change over the course of a chronic infection with antibiotic treatment. As temperate phages insert themselves into the bacterial genome they also have the ability to cause genetic diversity to their host's genome, which drives evolution at an increased rate. By analysing the PA genomes isolated from the chronically infected lungs of patients over a period of 10 years. It was possible to predict the temperate phages in the genomes as well as induce the phages from the bacteria and sequence them. This then identifies which phages are rooted within the genome and which are inducible and therefore may transfer, granting horizontal gene transfer between strains in the lungs. The aim of this study is to ascertain whether by comparing the phages longitudinally and horizontally (when multiple strains were seen within a sample) it is possible to determine if these phage have a role in the PA infection within the lungs becoming chronic. This may also give an idea to why these PA infections are chronic and are so hard to clear from the lung, which is yet unknown.

A113

Investigating Virus Diversity in Humans and Non-Human Animals in Vietnam

Jordan Ashworth[1], Lu Lu[1], Mark Woolhouse[1], on behalf of the VIZIONS Consortium[2]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Oxford University Clinical Research Unit (OUCRU), Ho Chi Minh City, Vietnam

Abstract

Despite ~60% of human pathogens being of zoonotic origin, the diversity of viruses in non-human hosts remains understudied. To investigate the viral diversity present in Vietnam, the Vietnam Initiative on Zoonotic Infections (VIZIONS) collected 2,100 rectal swabs and faecal samples from several non-human hosts (including pigs, rats and bats) as well enteric hospital patients and individuals with high animal contact. Samples underwent metagenomic sequencing (Illumina HiSeq), assembly (MetaSPAdes) and screening for viral sequences (Blastn and DIAMOND Blastp). The majority (87.5%) of viral sequences identified in humans shared high nucleotide identity (>95%) to previously described viral species, with the exception of sequences assigned to Anelloviridae and Picobirnaviridae. Approximately half (46.5%) of viral sequences identified in non-human hosts shared between 60 to 80% nucleotide identity to its closest match in GenBank. For all hosts other from rats the family with the highest frequency of low identity hits was Picobirnaviridae. Sources of diversity at a viral family level were mostly host specific, with pigs having diverse astrovirus and smacovirus sequences, rats for rotavirus and adenovirus and bats for coronavirus and parvovirus. These results highlight the historic bias of sequencing viruses from human hosts and the need to shift focus to non-human hosts. Pig, bats and rats in Vietnam harbor a large diversity of potentially novel viruses that may be threats to animal and human health.

A114

Large-scale generation of mutant strains in *Candida parapsilosis* using CRISPR-Cas9

Lisa Lombardi, Letal I. Salzberg, Eoin Ó Cinnéide, Caoimhe O'Brien, Geraldine Butler

UCD, Conway Institute, Dublin, Ireland

Abstract

In order to streamline the study of the pathogenic yeast *Candida parapsilosis*, we developed a plasmid-based CRISPR-Cas9 system (pRIBO) for gene editing in this species. However, pRIBO was not feasible for large-scale generation of mutant strains. We recently addressed this bottleneck by generating pCP-tRNA, an improved version of pRIBO in which the guide sequence can be easily cloned into the *Sap*I-digested plasmid, and the release of the mature sgRNA is mediated by endogenous RNase cleavage of the *C. parapsilosis* $tRNA^{Ala}$, and by self-cleavage of the HDV ribozyme. We are currently using pCP-tRNA for the systematic generation of mutant strains. Suitable guides were computationally designed to induce Cas9 cleavage within the first 25% of each *ORF* in the genome, which is then repaired by recombination with a repair template (RT) containing 30 bp homology arms, 11 bp to introduce a stop codon in the functional reading frame, and a unique tag. In 4 months, 288 plasmids targeting genes encoding transcription factors, phosphokinases, or unknown functions, were transformed into *C. parapsilosis* with the corresponding RTs. The system resulted in gene editing of 62% of the 288 genes at high efficiency (80-100% of the colonies tested were positive); 16% of the genes in the panel may be essential based on homology with related species, and we believe that the remaining 22% may be successfully edited by selecting a different guide. In conclusion, we demonstrate that pCP-tRNA is a valuable tool for high throughput generation of mutants in *C. parapsilosis*.

A115

Oligonucleotide Transcription Factor Decoys as tools to control bacterial transcription

Louis Laurence[1], Michael McArthur[1,2], Nichola Wong[1]

[1]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [2]Procarta Biosystems, Norwich, United Kingdom

Abstract

Transcription Factor Decoys (TFDs) are short synthetic oligonucleotides that contain the binding site for specific bacterial transcription factors. When translocated into bacterial cytoplasm they can rapidly kill cells when targeted against essential bacterial pathways. Translocation is currently achieved by combination of the TFD with a proprietary lipidic delivery agent, CM2, to form nanoparticles. These interact with highly conserved anionic phospholipids, such as Cardiolipin, to effect delivery to both Gram-positive and Gram-negative bacteria. Simplifying translocation would be an advance that would allow serial screening of large libraries of TFDs to delineate genetic regulatory networks in numerous types of bacteria, including emerging strains. To achieve this we have combined key chemical moieties of the CM2 delivery molecule to the oligonucleotide conjugate by Click chemistry and show that these discrete conjugates are capable of translocation. Confocal laser scanning microscopy was used to monitor the uptake of the TFD-conjugates to *E. coli* and in parallel their effect on the targeted genetic pathways was confirmed with reporter strains and plating under selective conditions. Hence, it was confirmed that these conjugates can be used as tools to efficiently and specifically modify gene expression by inhibition of selected transcription factors.

A116

Do Uropathogenic *E.coli* require changes before it can spread into the bloodstream ?

Cosmika Goswami, Stephen Fox, Alistair Leanord, Thomas Evans

University of Glasgow, Glasgow, United Kingdom

Abstract

Background: Uropathogenic *Eschericha coli* are the leading cause of urinary tract infections (UTIs). The microbe can spread from bladder to kidney and finally to blood. We wished to determine whether genetic changes accompany the passage of these infections from urine to blood.

Material/methods: 12 paired urine and blood samples were collected from patients in Greater Glasgow and Clyde; the interval between sample collection time between pairs was less than 48 hours. Whole genomic sequencing of these paired samples was performed using the Illumina MiSeq platform. *De novo*assembly of reads was carried out using Shovill assembler; whereas SNPs were identified using SMALT, VarScan and Gubbins tools.

Results: Urine and blood samples in each pair had the same MLST type. Surprisingly, however, there were multiple differences in the presence of plasmid genes, phage elements and insertion sequences within pairs, as well as numerous SNPs. For example, the mercury reductase *merA* gene and mercuric transport protein *merP* have been acquired in a plasmid of a blood isolate compared to the contemporaneous urine sample. We also identified missense mutations in genes involved in several metabolic pathways in bloodstream isolates. Several of the observed gene deletions/insertions and SNPs were found in more than one of the paired blood and urine isolates.

Conclusions: The observed sequence differences between contemporaneous blood and urine isolates suggests that genomic differences accumulate within the urinary bladder prior to blood stream invasion. The observed blood stream variants may thus possess a selective advantage in invasion and/or survival within blood

A117

Molecular approaches to understand the effect of acetic acid in uropathogenic E. coli

Fatemah ALatar[1], Francesca Bushell[1], Thippesh Sannasiddappa[1], John Herbert[2], Francesco Falciani[2], Peter Lund[1]

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

Abstract

Acetic acid has long been known for its antibacterial activity. We are using TraDIS to investigate the molecular mechanisms by which acetic acid acts as an antibacterial agent.

To do this, we grew a high-density transposon library in uropathogenic *E. coli* EO499 serotype 131 in M9 media at pH 7 and pH 5.5 with acetic acid concentrations of 40mM and 4mM, respectively, or without added acetic acid. Sequencing libraries were generated from total bacterial populations after growth, and sequenced using a transposon-specific primer to generate positions and frequencies for each transposon. By comparing numbers of reads before and after the stress, we identified candidate genes where transposon inserts led to a decrease of fitness under acetic acid stress. Eight of these were chosen for further study: *nuoM, nuoG, sucA, sthA, pitA, apaH, rssB* and *ytfP.*

Because of the difficulties of constructing gene deletions in the uropathogenic strain for validating the TraDIS results, we tested the relative fitness of the corresponding gene deletion mutants from the Keio library (in strain BW25113), with the growth conditions used for EO499. Interestingly, only a few knockouts showed a reduction in relative fitness in time course competitions at pH 5.5 with acetic acid. This may due to the differences between strains used in TraDIS and competition. To overcome this issue, we have also isolated transposon mutants from *E. coli* EO499 transposon library for the determination of relative fitness. The results will be presented.

A118

Investigation on MFS Family in C. parapsilosis with Crispr

Fang Zhao, Geraldine Butler

University College Dublin, Dublin, Ireland

Abstract

*C. parapsilosis*causes candidiasis especially among newborn babies. The function of specific transporters is considered to be one key feature underlying drug resistance in *Candida* species. Drug transporters fall into two main classes - ATP-binding cassette (ABC) transporters, and the major facilitator superfamily (MFS). Particularly, Some members of members of the drug/H (+) antiporter family (DHA1) of the MFS superfamily function as multidrug transporters. We find that the DHA1 family in *Candida* species can be divided into several clades. These include MDR1/FLR1, associated with multidrug resistance in *C. albicans* (1 member in *C. parapsilosis*); TPO4, associated with polyamine transport (1 member in *C. parapsilosis*); NAG3/4, associated with transport of N-acetyl glucosamine (2 members in *C. parapsilosis*); TPO2/3, associated with polyamine transport (1 member in *C. parapsilosis*); YHR048w, with no known function (no members in *C. parapsilosis*); and TPO1/FLU1, possibly associated with fluconazole resistance (8 members in *C. parapsilosis*). We propose to use CRISPR-based gene editing to explore the function of all 13 members of the DHA1 family in *C. parapsilosis.* To date we have individually edited 10 members of the family by introducing stop codons near the start site of translation (ATG). We are currently editing the remaining 3 genes, and we are attempting to combine at least two edited genes in the same background. We will then test the phenotype of each edited strain in the presence of various drugs.

A119

The impact of natural genetic variation on protein aggregation in *Saccharomyces* species

Laura Petch, Mick Tuite, Nadia Koloteva-levine, Irene Hung

University of Kent, Kent, United Kingdom

Abstract

Phenotypic heterogeneity in the yeast *Saccharomyces cerevisiae* has input from both genetic and epigenetic determinants. The epigenetic factors are largely based on inherited changes in protein structure resulting in the aggregation of the alternative conformational form of a protein. Such epigenetic factors include prions, protein-based epigenetic determinants that undergo self-perpetuating, heritable changes in their conformation. To date prions have been almost exclusively studied in laboratory-bred strains of *S. cerevisiae* and we are broadening their study to wild strains i.e. non-laboratory strains of *S. cerevisiae* and three related *Saccharomyces* species. In addition to searching for prion-related phenotypes in these yeast species we are also examining the ability of these genetically-related species to facilitate and perpetuate the formation of other amyloid-forming proteins, specifically the Alzheimer's disease associated protein Abeta 42 and Huntingtin (Htt)-associated polyglutamine (polyQ) and to define their impact on the microbial host. Our results to date indicate that each of these species can propagate the amyloid forms of Abeta 42 and polyQ, but these amyloid states are differentially impacted upon by the endogenous prion state of the host yeast species.

A120

Shiga toxin prophage analysis of clinically relevant enterohaemorrhagic *E. coli* isolates using Nanopore Sequencing

Daniel Yara[1,2], David Greig[3], Tim Dallman[3], Claire Jenkins[3]

[1]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [2]Quadram Institute Bioscience, Norwich Research Park, Norwich, United Kingdom. [3]National Infection Service, Public Health England, London, United Kingdom

Abstract

Enterohaemorrhagic *E. coli* O157 produces different Shiga toxin (Stx) subtypes which can contribute to the development of disease. The advancement of severe disease such as haemolytic uraemic syndrome is significantly associated to Stx2a subtype carriage. Three distinct EHEC lineages exist, where in the UK, stx2a has found in three STEC O157 sub-lineages: Ic, I/II and IIb. Stx encoding phages from the three sub-lineages where examined to determine whether the bacteriophage which conferred the stx2a synthesis are the same or different. Relevant representative EHEC O157 strains were sequenced using the MinION Platform. The sequences were assembled and annotated, allowing Stx encoding prophage identification. Such prophages and constituent genes were then aligned for comparison along with various reference strains. Results reveal that outbreak EHEC strains carry Stx2c encoding prophages and that such prophages were conserved, supporting past studies which suggest a single integration event and clonal expansion of Stx2c bacteriophages. Analysis of Stx genes revealed that some Stx2c phages carry Stx2a encoding genes, suggesting recombination between different Stx encoding phages. Variability was observed for Stx2a encoding phages, suggesting that there are various Stx2a encoding phages circulating in the UK.

A121

A genome wide study to understand triclosan resistance mechanisms

Muhammad Yasir, Keith Turner, Andrea Telatin, Andrew Page, Mark Webber, Ian Charles

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Triclosan is an antibacterial and antifungal agent added to cleaning products used widely in healthcare and households. A recent study has reported that triclosan resistance involves generic mechanisms of antibiotic resistance and as such triclosan exposure may select for mutants resistant to antibiotics of clinical importance. Triclosan is known to be bacteriostatic at low concentrations and bactericidal at high concentrations although the molecular basis for this is not understood. Identifying the repertoire of resistance mechanisms will be helpful in assessing how the universal use and release of triclosan into the environment from a range of products may impact resistance to antibiotics of clinical importance. Here we used K-12 *E coli* strain BW25113 as a model organism for a genome wide approach to assay all the genes simultaneously for their role in resistance to triclosan and we made about 1 million transposon mutants. A modified sequencing method to identify transposon insertions found more than half a million unique insertion sites. We identified more than 300 genes involved in survival of *E. coli* in the presence of triclosan as compared to untreated controls. The list included some novel mechanisms as well as most of the previously reported genes involved in triclosan resistance, such as *fabI, acrA, gcvP, fadB, tktA, gltA, wzzB* and *fadL*. We conclude that the transposon directed insertion site sequencing technique used, is a robust tool to screen for resistance mechanisms, and that triclosan has multiple resistance mechanisms, some of which were not seen until now.

A122

Investigation of molecular mechanisms of polymerase I (Pol-l) inhibitor PMR-116 using Isothermal Titration Calorimetry(ITC).

Konstantin Panov, Sameerh Alsahafi

Queen University, Belfast, United Kingdom

Abstract

Cancer has been identified as a group of diseases characterized by abnormal cell growth. In eukaryotic cells, the nucleolus is the region of the nucleus that constructs ribosomal subunits. polymerase 1 transcription site has been used as the marker to particular aggressive tumours seen when the nucleoli increases in size and number. The nucleolar size correlates with the level of rRNA synthesis, which is transcribed by RNA polymerase 1 (RNAP1) during the initial stage of ribosome biogenesis. In recent years the rRNA transcription has emerged as novel target for anti-cancer therapy and specific RNAP1 transcription inhibitor are currently undergoing clinical trials for anti-cancer therapy. Thus, the proposed therapeutic strategies for solid tumour growth or inhibition of cancer cell proliferation is the selective inhibitor RNAP1 transcription. The currently available inhibitors are characterised by a different mechanism and different levels of genotoxicity. Two drugs, (CX-5461 and PMR-116) are small molecules and selective inhibitor of RNAP1 transcription which have moderate effect on transcription by other nucleolar polymerases and protein translation. CX-5461 inhibits transcription by displacing essential promoter recognition factor SL1 thus preventing an initiation of transcription. PMR-116 demonstrated a great potential as RNAP1 inhibitor, is characterized by low cytotoxity and very high anti-cancer effect. However, the exact molecular mechanisms of RNAP1 inhibition is unknown. Therefore, this experiment aims to identify the stage of the transcription cycle affected by PMR-116 by using a combination in vitro and vivo based assays. Also, to determine the drug target into the molecular mechanism of PMR-116 by using biochemical methods including Isothermal Titration Calorimetry (ITC).

A123

High throughput approaches to study laboratory-based evolution of E. coli for enhanced growth at low pH

Mathew Milner, Hrishiraj Sen, Pete 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 have evolved six populations of E. coli MG1655 by iterative growth and dilution at pH 4.5 over five months, keeping a frozen fossil record of intermediate populations during this process.

Whole genome sequencing of the evolved strains 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 different parameters on evolutionary trajectories, but as evolution experiments take a long time, we are currently investigating the potential of traDIS to 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.

A124

Characterising the gut virome by cross comparison of sequence platforms and isolating *Bacteroides* bacteriophages from sewage water

Mohammad Tariq[1], Shen-Yuan Hsieh[1,2], Fiona Newberry[1,2], Tom Wileman[1,2], Simon Carding[1,2]

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

Abstract

The human gut microbiota includes viruses, termed the 'virome'. Outnumbering the bacterial abundance on average 10:1. Recent studies suggested that changes in intestinal virome may lead to chronic gastrointestinal (GI) inflammation and bacterial dysbiosis. Thereby causing diseases such as inflammatory bowel disease (IBD), type I diabetes (T1D) and myalgic encephalomyelitis (ME), also known as chronic fatigue syndrome (CFS). Conversely, bacterial dysbiosis caused by increases in aerobic bacteria and decreases in anaerobic *Bacteroides* spp. have been described in some inflammatory mediated diseases and in considering that *Bacteroides* spp. are prominent members of the normal gut flora, their associated bacteriophages merit investigation. In this study we characterise *Bacteroides* related bacteriophages and their genomes isolated from sewage waste water environment. As part of this study we have made direct comparisons of Illumina HiSeq PCR-free and Oxford nanopore MinION PCR-free sequencing for viral metagenomics in addition to determining the extent of PCR related biases sequence generated viromes.

A125

Using the CRISPRi/dCas9 interference system in *Mycobacterium tuberculosis* and *Mycobacterium bovis* to identify novel therapeutic targets.

Valwynne Faulkner[1], Amanda J Gibson[1], Shan Goh[2], Sharon L Kendall[1]

[1]Royal Veterinary College, London, United Kingdom. [2]University of Hertfordshire, Hertfordshire, United Kingdom

Abstract

Mycobacterium tuberculosis (*Mtb*) remains one of the most important infectious diseases in public health worldwide. It has been estimated by the WHO that up to 1.7 million deaths occurred as a result of human tuberculosis in 2017. It also estimated that there were approximately 147,000 new cases of *Mycobacterium bovis* in the same period, a zoonotic pathogenic mycobacterium which can cause human pulmonary tuberculosis and is 99% similar to *Mtb* at the genetic level. Long, expensive treatment regimens which can lead to poor adherence and the emergence of multi-drug resistance have added to the public health crisis. The development of effective new vaccines or treatments is crucial. A quarter of the genome of *Mtb* remains uncharacterized, with genes defined as conserved hypothetical proteins or just hypothetical proteins. A number of them are predicted as essential for *in-vitro* growth, some of which could be potential drug targets. Characterizing essential genes in mycobacteria is challenging and efforts have been made using a number of methods.The recent development of the Clustered Regularly Interspaced Short Palindromic Repeat interference dCas9 system (CRISPRi/dCas9) in *Mtb* gives us the opportunity to specifically target essential genes and turn down gene expression using an inducible promoter to verify essentiality and further study their function. We have constructed *Mtb* and *M. bovis* CRISPRi/dCas9 strains using the two-plasmid system by Singh et al., 2016 and single guide RNAs designed to target a number of common predicted essential genes. Growth rates of the strains are monitored following induction of the system.

A126

mfBiclust: An R Package for Biological Bicluster Analysis in the transcriptomics dataset

Quan Gu, Jonathan Lim

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

Abstract

Biclustering algorithms are unsupervised machine learning algorithms that find paired subsets of samples and variables exhibiting co-dependence in a transcriptomics dataset. While matrix-factorization-based biclustering is especially suited to revealing enrichment patterns in metabolomic datasets, a full matrix-factorization-based biclustering pipeline does not exist. Here we present mfBiclust, an R package with a Shiny-based GUI that enables users to apply recently developed biclustering pipelines to the transcriptomics datasets. In our general matrix-factorization pipeline, a data matrix is approximated as the product of two factors. The optimal number of biclusters for a dataset can be estimated by bi-cross-validating truncated singular value decompositions. Biclustering results can be visualized and exported, facilitating functional characterization of the observed biclusters. mfBiclust is thus potentially useful for analyzing any genomics and transcriptomics assay.

A127

Genomic analysis reveals the re-emergence of a nosocomial outbreak caused by multidrug resistant *Klebsiella pneumoniae*

To Nguyen Thi Nguyen, Stephen Baker

Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam

Abstract

Multidrug resistant (MDR) *K. pneumoniae* is listed among the most urgent public health threats due to its virulence and insusceptibility to a wide range of antimicrobials. Infection with this pathogen frequently leads to fatal outcomes, especially in low-income hospital settings. Patan Hospital is a 450-bed government hospital located within the Kathmandu Valley, Nepal. The hospital has previously witnessed multiple outbreaks caused by MDR *K. pneumoniae* in the neonatal intensive care unit (NICU). Particularly, a carbapenemase producing sequence type (ST) 15 *K. pneumoniae* clone was responsible for an outbreak with mortality rate up to 75% in 2012. Recently in 2015, this same NICU suffered again from an MDR *K. pneumoniae* outbreak. In this study, using whole genome sequencing (WGS) and state-of-the-art analytic approaches, we aimed to define the nature of this recent outbreak. We found that the 2015 outbreak in Patan Hospital was caused by the same MDR ST15 *K. pneumoniae* reported in 2012. Albeit genetically similar, these recent strains were susceptible to carbapenems due to deletion of the *bla*NDM-1 cassette. Using Bayesian phylogenetic inference, we determined the outbreak strain was introduced to Patan Hospital in late 2010 and subsequently caused major outbreaks in NICU in 2012 and again in 2015. This clone acquired four different plasmids encoding resistance to numerous therapeutic antimicrobials, and this may underlie its successful propagation and associated high mortality. Insights provided through this study are invaluable in tailoring infection control strategies as well as raising public awareness

A128

Properties and Directionality of Intragenic Promoters in *E. coli*

Emily Warman, Shivani Singh, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

The histone-like nucleoid structuring (H-NS) protein targets and binds AT-rich DNA. Oligomerisation of H-NS along AT-rich genes represses transcription from spurious intragenic promoters within the coding sequences of AT-rich genes.

In this work, we sought to better understand why promoters occur so frequently within AT-rich DNA. To do this, we compared the properties of i) canonical promoters ii) promoters within H-NS bound genes and iii) promoters generated by random combinations of nucleotides.

We have identified and analysed many active intragenic promoters distributed within AT-rich genes in the *E.coli* genome. We show that these promoters, and promoters from randomly generated AT-rich DNA, differ from canonical promoters in several ways. In particular, spurious promoters are often dependent on AT-tracts upstream of the promoter -10 element. These AT-tracts play a key role by altering DNA curvature and facilitating interactions between the promoter and RNA polymerase sigma factor. Promoter regions inside genes are also often bidirectional.

A129

Establishing classical and quantitative genetic tools for *Kluyveromyces marxianus*

Franziska Huff[1,2], Arun S. Rajkumar[1], Edward J. Louis[3], John Morrissey[1]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]University of Leicester, Leicester, United Kingdom. [3]Centre of Genetic Architecture of Complex Traits, University of Leicester, Leicester, United Kingdom

Abstract

Kluyveromyces marxianus is a non-conventional yeast that has several advantages over baker's yeast in economical fermentation processes. It is highly thermotolerant, fast-growing, resistant to multiple industrially relevant stresses and able to utilize of a broad variety of carbon and nitrogen sources due to its metabolic diversity. However, there still is a lack of knowledge about this yeast's genetics, its physiology, industrial performance, population structure and strain variation. Recent population genetics studies distinguished two *K. marxianus* haplotypes depending on the environment they were isolated from (haplotypes "A" and "B"). Ploidy differences linked to these different haplotypes were found, but a genomic basis remains elusive.

Preliminary data suggests substantial intraspecies differences in the mating type loci and related genes of *K. marxianus* strains. Therefore, the architecture of the mating type loci was further analysed. For a more fundamental understanding of the link between ploidy, mating types and the genetic differences between the A and B haplotype, we generated mutants to control mating-type switching and construct stable haploids for both haplotypes. We established mating and sporulation protocols as well as a reliable, fast mating type assay via multiplex-PCR. Mating of strains with industrially interesting traits forms the basis for quantitative genetics analysis, which have proven to be a powerful analytic tool to study complex traits. Our research will provide a firm base of fundamental knowledge about *K. marxianus*' population structure and the genetic basis of the underlying traits that are of extraordinary interest for industrial applications.

A130

Coinfection Promotes Plasmid Stability

Laura Carrilero Aguado[1], James P. J. Hall[2], Michael Brockhurst[1]

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

Abstract

Bacteria exist in complex communities that include not only many other bacterial species but also diverse mobile genetic elements which accelerate bacterial evolution via horizontal gene transfer. How multiple mobile genetic elements interact in bacterial populations and how this affects plasmid dynamics remains poorly understood. We experimentally evolved populations of *Pseudomonas fluorescens* SBW25 either singly-infected or co-infected with two conjugative mercury resistance plasmids either with or without positive selection (*i.e*. addition of Hg(II)). We show that coinfection led to higher levels of mercury resistance in the bacterial population in the absence of positive selection. Consistent with this, in the absence of positive selection the plasmids could stably coexist within bacterial cells resulting in the maintenance coinfection. By contrast, with positive selection, plasmid coexistence was destabilised, leading to the dominance of a single plasmid in several replicate bacterial populations. Plasmid co-infection appears to alter the trajectory of compensatory evolution to ameliorate the cost of plasmid carriage and may have selected for alternative mechanisms compared to singly-infected populations. Stable plasmid co-infection without positive selection for plasmid-encoded traits suggests that environments where plasmids are useless may be hot-spots for genomic innovation via plasmid-plasmid recombination.

A131

The Environmental Microbiome Toolkit for Urban Designers

Harry Watkins[1,2], Jake Robinson[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]Landscape Institute Plant Health and Biosecurity Group, N/A, United Kingdom

Abstract

Environmental microbiome research shows that microbial communities help to shape complex ecological processes that influence human and environmental health. Researchers are currently investigating the potential health-inducing interactions between humans and the environmental microbiome, particularly in urban areas. However, not only are there inherent technical issues to overcome in implementing the findings of this research, but there are also complex social, political and economic factors that affect the design, construction and management phases. Drawing on a recent paper discussing opportunities for Microbiome-Inspired Green Infrastructure (MIGI) (Robinson et al 2018), we set out criteria for an 'Environmental Microbiome Toolkit for Urban Designers' that could be used by planners, architects, landscape architects and civil engineers. We provide a worked example of this toolkit to design a public space in Sheffield, demonstrating practical design techniques to consider the environmental microbiome and its role in human and ecosystem health.

The Landscape Institute (LI) is the professional body that regulates and represents landscape architects, providing guidance across all spheres of the profession. One of the key functions of the LI is to develop and maintain the LI Plan of Work, regulating the work that landscape architects undertake at each stage of a project, from landscape assessment through to conceptual and detailed design, contract administration and landscape management. We apply these industry-standards to show how the environmental microbiome should be considered in landscape assessment, design and management, bridging the gap between research and practice and providing a common reference point for future policy development and industry regulation.

A132

Assessing the Presence and Genetic Basis of ESBL-producing Bacteria in Pre-boil Wort, Dairy Cattle Manure and Dog Faeces

Andrew Nally

University of Portsmouth, Portsmouth, United Kingdom

Abstract

Antimicrobial resistance (AMR) is becoming the biggest problem facing human and animal health care, with a predicted 10 million deaths per year due to antibiotic resistance by 2050 (Review on Antimicrobial resistance, 2014). The focus of many AMR studies is on problems inside clinical settings, such as hospitals and veterinary practices, yet, as this study shows, often the most diverse sources of resistance genes are found in relatively unexplored areas of the environment; in particular dog faeces and dairy cattle manure.

Chromogenic media analysis showed dog faeces had the greatest number and diversity of ESBL-producing bacteria. Multiplex PCR showed inconclusive evidence of what the resistance genes were in both dairy cattle manure and dog faeces. Phylogenetic analysis of the 16S rRNA showed *Flavobacteriaceae*-like bacteria in dairy cattle manure but the presence of *Enterobacteriaceae*-like bacteria, most similar to *Escherichia coli*, in dog faeces.

The major conclusion of this study is that ESBL-producing bacteria are present in high abundance in both dog faeces and dairy cattle manure and that ESBL-producing bacterial species in dog faeces showed higher diversity than first expected while pre-boil wort had no ESBL-producing bacteria.

The results of this study have crucial significance for future studies and the wider community. The statistical, microbiological and phylogenetic data collected provide strong evidence of the role of the environment in the spread of antibiotic resistance. Secondly, the study utilises a holistic visual model to map the spread of resistance and inform the wider community of the issue of AMR in the environment.

A133

Molecular Identification of fungi isolated from coastal regions of Red Sea, Jeddah, Saudi Arabia

Suaad ALwakeel

Princess Nourah bint Abdulrahman University, RIYADH, Saudi Arabia

Abstract

To isolate fungal communities from coastal areas of the Red Sea in Saudi Arabia and identify and classify them by molecular techniques. Samples were collected from the seaside of the Red Sea in Jeddah, Saudi Arabia in March 2012 and stored in sterile screw cap bottles for further analysis. Phenotypic and genotypic characterization of fungal isolates was done using standard techniques. Eight fungal genera including *Aspergillus*, *Penicillium*, *Thielavia*, *Fusarium*, *Emericella*, *Cladosporium*, *Scytalidium*, and *Alternaria.* Most isolated fungi showed significant growth on petroleum media and were thus considered capable of biodegradation of crude oil-based substances. The fungal genera isolated from the Red Sea had 97% - 100% similarity with the related fungi recorded in the GenBank in which they were deposited. The morphological and molecular structure of these marine fungal isolates closely resembles their terrestrial counterparts in the Genbank. The capabilities of these fungal species to utilize petroleum as a source of carbon speaks to future applications in which marine fungi may be utilized in the breakdown of petroleum-based waste in an ecologically efficient manner.

A134

Bioinformatics technology in clinical and public health microbiology applying computational methods

Hyunjo Kim

Gachon University, Seungnam, Korea, Republic of

Abstract

The role of clinical genomics in infectious disease diagnostics and public health microbiology is the topic of discussion during a recent decade. Although much of this work is aimed at describing the structure of outbreak communities, the methodology works equally well to identify pathogens in clinical samples. Clinical genomics is the exploitation of genome sequence data for diagnostic, therapeutic, and public health purposes. Central to this field is the high-throughput DNA sequencing of genomes and metagenomes. The key concept in using clinical genomics methodology is that detection of microbes is independent of culture and is not limited to targets used for in-depth PCR assays. Rather, it is a process of generating large-scale sequence data sets that adequately sample a specimen for microbial content and then of applying computational methods to resolve the sequences into individual species, genes, pathways, or other features.

Keywords: clinical microbiology, infectious diseases surveillance, public health, clinico genomics, whole-genome sequencing, bioinformatics pipeline

A135

Effect of fractions of toxic cyanobacterium, Nodularia spumingena KAC 66 and its purified nodularin and nodulopeptin 901 on Inhibition of colorimetric protein phosphatase 1 assay and lethality against two species of Daphnia

Shaista Hameed[1,2], Linda Lawton[3], Christine Edwards[2]

[1]Sardar Bahadur Khan Women's University, Quetta, Pakistan. [2]The Robert Gordon University, Aberdeen, United Kingdom. [3]The Robert Gordon University, Aberdeen, United Kingdom

Abstract

The strains of *Nodularia* are known to produce potent hepatotoxic nodularins (NOD) and other bioactive metabolites i.e. nodulopeptins and spumigins. Contemporary, three new nodulopeptins were also isolated from *N. spumigena* KAC 66 collected from the Baltic Sea.

The wide distribution and toxicological effects of *N. spumigena* on food chain, has caused significant attention towards the effects of the species. In the present investigation the toxicity of NOD and nodulopeptin 901, produced by *N. spumigena*, was fractionated by Reversed Phase Flash Chromatography (RPFC) and their toxicity was determined by their lethality to water fleas, *Daphnia pulex* and *D. magna* along with inhibition of protein phosphatase 1 assay (PP1; **eukaryotic threonine phosphatase/protein serine).** All fractions showed lethality to Daphnids and inhibitory activity against PP1, the toxicity was due to additional compounds as NOD and nodulopeptin 901 were only detected in 7 fractions. The pure NOD was lethal to *D. pulex* and *D. magna* LC_{50}= 8.4 µg · mL^{-1} and 5.0 µg · mL^{-1}, respectively. The newly characterized nodulopeptin 901 was also tested against *D. magna* (LC_{50}=>100 µg · mL^{-1}). NOD and nodulopeptin 901 inhibited PP1 with weak IC_{50} 0.038 µg · mL^{-1} and 25 µg · mL^{-1}, respectively. The anabaenopeptin A (ANA) and anabaenopeptin B (ANAB) were used as reference peptides as they have similarity in structure with newly characterized nodulopeptin 901. The ANA, ANB and linear nodularin (LNOD) inhibited PP1 with IC_{50} 70 µg · mL^{-1}, 100 µg · mL^{-1} and 20 µg · mL^{-1}, correspondingly.

This is the first study that indicates the toxic and inhibitory activities of nodulopeptin 901 and fractions of *N. spumigena* KAC 66 against daphnids and PP1.

A136

Effect of Different Biocoagulants on the Amino acid Content of Soft Cheese (wara) Produced From Sheep Milk

Ayodele Ogunlade[1], Victor Oyetayo[2]

[1]Federal Polytechnic Ado Ekiti, Ado Ekiti, Nigeria. [2]Federal University of Technology Akure, Akure, Nigeria

Abstract

Soft cheese (wara) is an unripened cheese consumed in several parts of West Africa due to its various nutritional qualities. Soft cheese, a coagulated product of raw milk is usually produced from cow milk using *Calotropis procera*. This study therefore sought to assess the effect of the different coagulants such as *Calotropis procera, Carica papaya*, lemon juice and steep water from cereals (maize, millet and sorghum) on the amino acid content of soft cheese produced from sheep milk. Raw milk sample was collected from sheep and processed into soft cheese by these coagulants and the amino acid composition of the sample was carried out using standard methods. The result revealed that *Calotropis procera* coagulated soft cheese has the highest essential amino acid content Leucine (10.21g/100g), while steep water from millet coagulated soft cheese has the lowest essential amino acid content methionine (0.72g/100g). However, lemon juice coagulated soft cheese has the highest non essential amino acid glutamic acid (16.27g/100g) in all the cheese samples. In conclusion, this study revealed that highly nutritious soft cheese can also be gotten from sheep milk other than the commonly used cow milk and other coagulants such as lemon juice can compete favorably well with *Calotropis procera* in production of highly nutritious soft cheese. It is therefore recommended that soft cheese produced from sheep milk coagulated by lemon juice should be incorporated into daily diet due to its highly nutritional content.

A137

Optimization of process parameters for enhanced CMCase production by *Aspergillus flavus* (RTM 3) NFCCI-4154 isolated from textile waste dumping site of Central India

Priya Sutaoney[1], Rachana Choudhary[2], Ashwini Kumar Gupta[1]

[1]School of Studies in Life Science, Pt.Ravishankar Shukla University, Raipur, India. [2]Department of Microbiology, Shri Shankaracharya Mahavidyalaya, Durg, India

Abstract

Fungal cellulases are industrially important, multi component extracellular enzymes having wide range of applications, and are categorized as endo-glucanase, exo-glucanase and β-glucosidases. These enzymes are non-aggregating, less complexed and require complete synergism for degradation of cellulose molecule into soluble sugars. However, endo-glucanase also known as CMCase has proved a vital role in textile industries for biopolishing and bio-finishing of fabric as well recycling of textile waste generated during manufacturing. This study aimed for enhanced production of CMCase by fungal isolate from textile waste dumping site of Raipur (21.2514° N, 81.6296° E) under submerged fermentation. The fungal isolate was identified as *Aspergillus flavus* (RTM 3) NFCCI-4154 based on morphotaxonomy and ITS gene sequencing analysis and deposited to National fungal culture collection India. The sequence was submitted to NCBI Gene bank database (accession number MK036350).The process parameters optimized for maximization of CMCase production were recorded at an incubation period of eight days at 28^{o}C and pH 5.50 with inoculum size of five mycelial discs 10 mm in diameter and agitation rate of 100 rpm. Analysis of nutritional parameter revealed ammonium chloride (0.3%) as best nitrogen source. 1.5% carboxy methyl cellulose as primary substrate with supplementary 2% sigma cell 20 (avicel) as carbon source and sodium deoxycholate (0.04%) were best additive for hyper production of CMCase. This optimization study increased the CMCase production by 2.88 fold as compared from control. The finding suggests *Aspergillus flavus* NFCCI-4154 as potent CMCase producer which can be further exploited in textile industries and waste management.

A138

Molecular Identification of *Bacillus spp* as Starter Culture for the Production of Ugba (*Pentraclethra macrophylla*), a Nigerian Fermented Condiment

Kenneth Nwanekwu

Imo State University, Owerri, Nigeria

Abstract

The isolation, physiological and molecular identification of *Bacillus* spp for the development of starter culture in Ugba production were carried out. Sixty two bacteria were isolated and 55 Phynotypically characterized as Gram positive, rod shaped, motile, spore-forming and catalase positive typical characteristics of *Bacillus* spp. Seven isolates were suspected to be *Micrococcus* and *Staphylococcus* spp. They were Gram negative, cocci and catalase negative. The genotypic identification of the suspected Bacillus spp was done using amplification and partial sequencing of the 16S rRNA gene. The sequence analysis of 16S rRNA gene fragment analyzed from isolates confirmed the organisms as *Bacillus cereus* (30)*, Bacillus subtilis*(17) and *Bacillus licheniformis* (8). *Bacillus subtilis* was the dominant species in Ugba fermentation as it had the highest recorded number of isolates. This study indicated that molecular characterization of organisms as a good tool for the identification of organisms for the development of starter culture for use in food fermentation.

A139

Freshwater invertebrates as vectors of horizontal gene transfer in enterococci

Temilola Olanrewaju, Mary McCarron, James Dooley, Joerg Arnscheidt

Ulster University, Coleraine, United Kingdom

Abstract

Aquatic environments remain potential hotspots of antimicrobial resistance genes which may be transferred from environmental bacteria to waterborne pathogens. Filter feeding invertebrates occupy central positions in freshwater ecosystems and can facilitate horizontal gene transfer through the aggregation of antibiotic-resistant bacteria in their guts. However, the rate at which these transfer events may occur has not been established. This study investigated the potential of *Daphnia magna* to facilitate conjugal gene transfer in *Enterococcus faecalis*. In microcosm experiments, adult *Daphnia* was fed in *Daphnia* media containing an *E. faecalis* donor strain carrying a *vanA* gene-bearing conjugative plasmid and a rifampicin-resistant recipient strain in a 4 h feeding period. This was followed by a 24 h gut clearance period in bacteria-free *Daphnia* media, after which the daphnids were homogenised. Transconjugants were detected on antibiotic selection tryptone soy agar plates. Results revealed that *Daphnia* disseminated transconjugants within the initial feeding period. Transconjugants were also obtained from homogenised *Daphnia* after gut clearance. Conjugation in bacteria requires the aggregation of bacteria cells on a suitable substrate which, in this case, was facilitated by filtration and passage through the narrow *Daphnia* gut. The recovery of vancomycin-resistant transconjugants from homogenised *Daphnia* is evidence that its gut could harbour *Enterococcus* long enough for conjugation to occur before being excreted. Our study has shown that *Daphnia* facilitated conjugal gene transfer in *Enterococcus faecalis*. This finding holds ecological significance as grazing on enterococci could potentially lead to the acquisition of new resistance genes within the *Daphnia* gut in faecally contaminated waters.

A140

Characterization and identification of corrosive bacterium isolated from petroleum product transporting pipelines

Arezoo Pedramfar, Keivan Beheshti Maal, Sayed Hossein Mirdamadian

Islamic Azad University Flavarjan Branch, Isfahan, Flavarjan, Iran, Islamic Republic of

Abstract

Corrosion is the main feature of the oil pipelines destruction. Biocorrosion has been detected in various industries, especially in the oil industry.
The rusted pipes samples were obtained from the Gandomkar petroleum pipeline station, Iran. For screening the corrosion producing bacteria, the samples were cultured in the selective culture medium, manganese agar and Iron oxidizing agar incubated at 30°C for 18 hours. The purified individual colonies were subjected to macroscopic, microscopic and molecular examinations, respectively.
The cultivation of corrosion based material on manganese agar isolated cream-coloured colonies, convex with the surrounding smooth. The microscopic examinations showed Gram-negative coccobacilli. Based on macroscopic, microscopic and molecular examinations the bacterial isolate was identified as *Stenotrophomonas maltophilia* strains PBM-IAUF- 4 with the accession number of KU145280.1 in Gene Bank. The cultivation of corrosion based material on Iron oxidizing agar isolated cream-coloured colonies cream-coloured colonies that had swarming, convex. The results showed Gram-negative bacilli. Based on macroscopic, microscopic and molecular examinations the bacterial isolate was identified as *Kluyvera intermedia* strains PBM-IAUF- 1 with the accession number of KU145277.1 in Gene Bank.
This is the first report of isolation and identification of corrosion-producing bacteria from, Gandomkar, Iran. The first isolated bacterium was identified as *Stenotrophomonas maltophilia*. The second isolated bacterium was identified *Kluyvera intermedia*. Both bacteria were isolated for the first time in the world from pipeline corrosion samples. This study confirmed the role of bacteria in the corrosion of oil pipelines.

Keywords: Industrial Microbiology, *Kluyvera intermedia,* Microbial Corrosion, Petroleum, Pipeline, *Stenotrophomonas maltophilia*

A141

Characterization of root-nodulating bacteria isolated from Genista cinerea (Fabaceae) growing in North African drylands using Bayesian Markov chain Monte Carlo (MCMC) methods and 16S rRNA sequencing

Dekak Ahmed[1], Menasria Taha[1], Benhizia Yacine[2], Chenchouni Haroun[1]

[1]Tebessa University, Tebessa, Algeria. [2]University of Constantine 1, Constantine, Algeria

Abstract

This study aims to phenotypically characterize endosymbiotic bacteria isolated from root-nodules of a wild legume (*Genista cinerea*: Fabaceae) growing in arid and semiarid region of northeast Algeria.

A phenotypic characterization was conducted using physiological tests and nutritional experiments .Data were analyzed using Markov Chain Monte Carlo sampler for multivariate generalized linear mixed models (MCMCglmm) to detect growth differences between isolates and RS. Similarities between isolates and RS were assessed using agglomerative hierarchical clustering (AHC), whereas multiple factor analysis (MFA) was performed to understand factors influencing each group of isolates/RS. In addition a molecular identification using 16S rRNA sequencing was done to identify the genera of the isolated bacteria

Symbiotic and cultural characteristics revealed the existence of a large physiological diversity among tested isolates, which showed a broad capability to assimilate different carbonaceous and nitrogenous substances, with consistent and large tolerances to pH [4–10], temperature [4–55°C], and salinity [NaCl=2–10%]. Although, the endosymbiont isolates have broad metabolic diversity, they formed two distinct groups with high level of similarity with RS. Group 1 included fast-growing and salt-tolerant isolates characterized by tolerance to acidity with high growth in alkaline conditions. Group 2 covered slow-growing acid-sensitive isolates that high salinity negatively affected their growth. Results of AHC and MFA evidenced that bacterial diversity of endosymbiont isolates showed high level of similarity with RS, a proof that they are rhizobial strains.

Our findings indicate that both fast- and slow-growing rhizobia nodulated *Genista cinerea* growing in semiarid North African regions. These rhizobia are poly-extremophiles adapted to diverse environmental stresses and belonging to *Betaproteobacteria and Gammaproteobacteria* Class.

A142

Antimicrobial peptides derived from phage display for use in poultry

Kieran Pitchers[1], Alison Gray[1], Claire Baker[2], Jon Owen[2], Ben Maddison[2], Robert Atterbury[1], Kevin Gough[1]

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

Abstract

Antimicrobial peptides (AMP) are a burgeoning field of research in the search for alternatives to antibiotics. AMPs are short, cationic, and amphipathic peptides which are found in virtually all forms of life. Phage display is a technique to create modified filamentous phage particles which have a peptide of interest fused to a capsid protein, thereby displaying the peptide on their surface. Phage display has been used to mine for potential AMPs due to the vast array of peptide sequences that can be created. In this work a random 16-mer peptide pIII phage display library was created via a novel inverse PCR method. The library was subsequently panned against Salmonella enterica serovar Typhimurium and an Escherichia coli serotype O2, two poultry pathogens of note. The sub-libraries were screened at panning round 5 against Lactobacillus acidophilus. Using a Z-score of two, sequences enriched in the non-pathogenic, gram-positive bacteria were removed from the final selection process. Selected peptides were synthesised and characterised in terms of inhibitory and bactericidal activity against two serotypes of E. coli and two serovars of S. enterica.

A143

Genetic Optimisation of Bacteria-Induced Calcite Precipitation

Timothy Hoffmann, Bianca Reeksting, Kevin Paine, Susanne Gebhard

University of Bath, Bath, United Kingdom

Abstract

Microbes in the built environment are mostly associated with biodegradation, biocorrosion, and bioweathering, rather than their lesser-known positive impacts. However, over recent years, bacteria have been at the basis of innovative technologies arising in civil engineering that capitalize on a process known as bacteria-induced calcite precipitation (BICP). This finds application, for example, in self-healing concrete. This technology uses encapsulated, alkali-tolerant, spore-forming bacteria embedded into concrete to repair cracks that appear during aging of built structures. BICP is a process whereby a microenvironment is created as a by-product of bacterial metabolism, which favours the precipitation of calcium cations and carbonate anions in the form of mineral calcite. This process is dependent on changes in pH, availability of cell surface nucleation sites, and ion concentrations. Current approaches that use bacteria in these technologies select for BICP-capable strains from the environment that further exhibit specific characteristic required for their respective application (pH/salt tolerance). In this project, we explore the genetic optimization of BICP for application in self-healing concrete. Our work offers an approach to identify the basic components needed for BICP to occur and a way to mobilize these into better-suited chassis organisms for application. Our results show that upregulating the ureolytic pathway offers a promising mechanism whereby BICP can be introduced into a non-precipitating strain. The ultimate goal is to create a new generation of bio-concrete that increases the lifespan of cementitious structures and thus decreases the high maintenance costs and high carbon dioxide release associated with concrete production and building.

A144

Differential amplicons for the evaluation of RNA integrity extracted from complex environmental samples

Fabien Cholet

University of Glasgow, Glasgow, United Kingdom

Abstract

Reliability and reproducibility of transcriptomics-based studies are dependent on RNA integrity. Microfluidics-based techniques targeting rRNA are currently the only approaches to evaluate RNA integrity. However, the relationship between rRNA and mRNA integrity is unknown. Here we present a new integrity index, the Ratio amplicon, R_{amp}, to monitor mRNA integrity based on the differential amplification of RT-Q-PCR amplicons of the glutamine-synthetase A (*glnA*) transcript. We showed, in a suite of experimental degradations of RNA extracted from sediment, that while the RIN generally reflected the degradation status of RNA the R_{amp} mapped mRNA degradation better. Furthermore, we examined the effect of degradation on transcript community structure by amplicon sequencing of the *16S rRNA, amoA* and *glnA* transcripts. We successful sequenced transcripts for all three targets even from highly-degraded RNA samples. While RNA degradation changed the community structure of the mRNA profiles, no changes were observed for the 16S rRNA transcripts profiles. Since both RT-Q-PCR and sequencing results were obtained, even from highly degraded samples, we strongly recommend evaluating RNA integrity prior to downstream processing to ensure meaningful results. For this both the RIN and R_{amp} are useful, with the R_{amp} better evaluating mRNA integrity in this study.

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A145

How bacteria float: identification of regulators of gas vesicle morphogenesis in *Serratia* sp. ATCC 39006.

Amy Hill, George Salmond

University of Cambridge, Cambridge, United Kingdom

Abstract

Serratia sp. ATCC 39006 (*S*39006) is the only known member of the family Enterobacteriaceae able to produce gas vesicles (GVs) naturally. GVs are hollow, intracellular, proteinaceous nanostructures that enable bacterial flotation. Environmental and physiological inputs regulating GV production include oxygen tension and quorum sensing, operating through regulatory proteins encoded within two contiguous GV operons. To identify novel regulators of GV morphogenesis, random transposon mutagenesis was performed and over 70,000 transconjugants screened to isolate 450 putative GV mutants. Random Primed PCR (RP-PCR) was used to define the corresponding transposon insertion sites for 19 mutants. In addition to known regulators of GV production including *gvrA, gvrB, gvpC,* and *gvpN,* nine novel putative regulators were defined. These included genes encoding the alternative sigma factor, RpoN, transcription factor DksA, and RNA binding protein Hfq. Using β-glucuronidase gene fusions in *gvpA1* and *gvrA* (the first genes of the two GV operons) the impact of transposon insertion on GV gene transcription was determined. Disruption of some genes caused pleiotropy, showing impacts on production of prodigiosin, carbapenem and plant cell wall degrading enzymes as well as swimming and swarming motility. RpoN is predicted to regulate GV production through binding with GvrA, an NtrC family regulator, to initiate transcription of the GV genes.

A146

Blessing in disguise: A protocol to quantify biofouling effects on drinking water electrochemical sensors is a gateway to investigate biofilm using electroanalytival methods

Aldyla Raditya, Danny O'Hare

Imperial College London, London, United Kingdom

Abstract

Provision of clean drinking water is regarded as being the most significant positive intervention in human health1 and it plays a significant role in supporting global health. Population growth, economic development and climate change all drive urbanisation2 and increase demand for clean water and the infrastructure for its delivery. As demand for clean water increases, so will the pressure to ensure its safety. Indwelling sensor networks offer real-time, long-term and intelligent monitoring system,3 and would enable optimisation of networks for quality. Electrochemical sensors are inexpensive and simple to construct and operate.4 However, prolonged exposure of the sensors to water causes biofouling which compromise their performance even in weeks or days,5 making early detection of performance failure critical.

By using a combination of electroanalytical methods6 (cyclic voltammetry, chronoamperometry, electrochemical impedance spectroscopy and hydrodynamic voltammetry) we can quantify the mass transport and kinetics effects of the first six days old biofilm layer to electrode reactions. We used agarose hydrogels and cellulose acetate layer as model biofilms to provide rapid and simplified characterisation of early fouling effects which then compared with lab grown biofilm of *Pseudomonas fluorescens*. We present a protocol for *in situ* diagnosis of electrochemical sensors fouling during early stages of biofouling. We also demonstrate that the behaviour of lab grown biofilm can be approached by using a simple model made of hydrogels (i.e. agarose) or a dried layer of an organic solution (i.e. cellulose acetate) which provides a potential application in other fields beyond environmental sensors development such as infectious disease and novel antibiotic researches.

A147

Diversity of Mycorrhizal Fungi in the Bee Orchid (*Ophrys apifera).*

wazeera abdullah, Meriel Jones

Integrative Biology, Liverpool, United Kingdom

Abstract

Abstract:

Orchid seeds require a fungal symbiont for successful germination. The Illumina MiSeq platform was used to carry out analysis of the fungal community in the soil from around bee orchid plants (*Ophrys apifera*). Soil samples from three sites on the University of Liverpool campus were investigated using Illumina amplicon sequencing of the ITS region to identify potential mycorrhiza fungi. The data were initially analysed using Qiime1, although this has not been officially supported since the end of 2017. Qiime2, the replacement, is the same as Qiime1, but with more features such as more streamlined workflows. Also, it is open source and tools can be added as plugins (https://qiime2.org). Taxonomic assignment was used for analysis of the read sequences to define Operational Taxonomic Units (OTUs). All sequences were clustered at 97% similarity and then reads were binned against a reference collection of the UNITE database which is a curated rDNA sequence database for *Ascomycota* and *Basidiomycota* and only involves high-quality sequences of well-identified fungi. There were significant differences between the fungal communities of the three sites as shown by using Kruskal-Wallis (KW) tests for comparing the alpha diversity of fungal OTUs within groups.

A148

Root-associated archaea: Investigating the niche occupied by ammonia oxidising archaea within the wheat root microbiome

Sam Prudence , Sarah Worsley, Lucas Balis, Colin Murrel, Laura Lehtovirta-Morley, Matt Hutchings

University of East Anglia, Norwich, United Kingdom

Abstract

Root associated microbiomes (RAMs) are complex communities which provide benefits to host plants via disease suppression, abiotic stress relief and increased nutrient bioavailability. Most RAM studies have focussed on bacteria and fungi, archaea have largely been overlooked as many studies fail to utilize archaea-specific 16S primers. However, there are reports of archaea being detected and isolated from the rhizosphere and endosphere of crop species, and one report of a plant-growth promoting (PGP) ammonia oxidising archaeon (AOA). Here, we aimed to assess the role of AOA within the wheat (*Triticum aestivum*) RAM. We applied archaea-specific primers & 16S amplicon sequencing to profile the archaeal community associated with wheat roots grown in agricultural soil. To assess PGP capacity we treated wheat seeds with a concentrated inoculum of model AOA *Candidatus* Nitrosocosmicus franklandus C13. In contrast to prior reports this had no impact on plant biomass, indicating N. franklandus may be a passive member of the wheat RAM. Stable isotope probing (SIP) experiments have confirmed that bacterial species metabolise *Arabidopsis thaliana* root exudates. Fractions are being examined to assess whether archaeal species can do the same, and a similar SIP experiment will be performed in wheat. An enrichment culture experiment using root exudates will also be applied to identify and isolate archaea capable of metabolising wheat root exudates. Here we show that AOA are present within the wheat RAM; to understand the niche occupied by these microbes we must further probe how they interact with host metabolites, and whether they contribute to host fitness.

A149

Antimicrobial resistance (AMR) and marine plastics: Can food packaging litter act as a dispersal mechanism for AMR in oceanic environments?

Rachel Moore[1], Cherie Millar[2,1], John Moore[2,1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Northern Ireland Public Health Laboratory, Belfast City Hospital, Belfast, United Kingdom

Abstract

Contamination of the world's oceans with plastic has now emerged as an important global environmental problem. Apart from their physical contamination and subsequent environmental ramifications, there has been a paucity of data considering their potential role in acting as a dispersal mechanism for antibiotic-resistant bacteria, residing on the surface of these materials. Simultaneously, antimicrobial resistance (AMR) has now emerged as a global public health crisis. A study was therefore undertaken investigating AMR of bacteria colonising the outer surfaces of food-packaging plastic litter from coastal locations around Northern Ireland (NI), specifically examining TOTAL AMR (intrinsic + acquired) to 12 antibiotic agents, representing seven classes of antibiotics. NI coastal locations (n=10) were sampled for plastic food-packaging litter, where plastic materials (n=31) were collected aseptically from intertidal zones. Colonising bacteria were harvested from 25cm^2 areas and plated onto Standard Plate Count agar/aerobic/22^{o}C/3 days. Antibiotic susceptibility was determined using standard disk diffusion against the following classes of antibiotics [antibiotic agent]:- Aminoglycosides [gentamicin], Beta-lactams [ampicillin, amoxicillin, ceftazidime, cefpodoxime, piperacillin/tazobactam, meropenem, representing penicillins, 3rd generation cephalosporins, penicillin/non-beta-lactam beta-lactamase inhibitor & carbapenem], Fluoroquinolones [ciprofloxacin], Macrolides [erythromycin], Oxazolidinones [linezolid], Tetracyclines [minocycline] & Trimethoprim/sulfamethoxazole. Total resistance to the macrolide, b-lactams [penicillins & 3rd generation cephalosporins], oxazolidinones] and trimethoprim/sulfamethoxazole, with susceptibility to b-lactam [carbapenem] (16.1%), gentamicin (25.8%), b-lactam [piperacillin/tazobactam] (51.6%), ciprofloxacin (62.1%) and minocycline (82.8%) was recorded. This study demonstrates that bacteria colonising food plastic litter in the sea may be a source of multidrug resistance to antibiotics, several of which have been classified as “CRITICAL” by the WHO.

A150

Low cost interventions for disinfection of potable water in developing countries

Pilar Fernandez-Ibañez[1], M. Inmaculada Polo-Lopez[2], Azahara Martinez-García[3], Brandon Reyneke[4], Wesaal Khan[4], Charles Mujanya[5], Jacent Asiimwe[5], J.A. Byrne[1]

[1]NIBEC, Ulster University, Jordanstown, United Kingdom. [2]CIEMAT-Plataforma Solar de Almería, Almeria, Spain. [3]CIEMAT-Plataforma Solar de Almería, Almería, Spain. [4]Dep. Microbiology, Stellenbosch University, Stellenbosch, South Africa. [5]School Food Technology and Nutrition, Makerere University, Kampala, Uganda

Abstract

Two billion people rely on fresh water sources that are faecally contaminated and half a million people die every year due to water and sanitation-related diseases, where they lack access to safe water. Therefore, the development of household water treatment and safe storage technologies (HWTS) to deliver safe potable water at household level is important. These technologies should be effective, available, affordable, and acceptable to the communities.

This paper will discuss various HWTS that can effectively provide safe drinking water as determined by the WHO harmonized testing protocol for HWTS. According to this protocol, the technology shall be evaluated for microbiological performance against four specific reference pathogens, *E. coli*, two surrogate bacteriophages (MS-2, phiX-174) and *Cryptosporidium parvum*infectious oocysts. The technologies will be proven as highly protective if they can reduce 4-log the bacterial concentration, 5-log bacteriopaghes, and 4-log protozoa. The first field results will be presented concerning large-scale solar reactors for the disinfection of harvested rainwater based in South Africa and Uganda. These reactors provide treated water to over 500 pupils in two primary schools in Uganda, and to 54 people in two South African communities. The paper will also introduce the SAFEWATER project, which aims to develop low-cost systems for drinking water treatment at household level for rural communities in Colombia and Mexico.

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Detection of Carbapenemase producing *Enterobacterales* in hospital effluent and municipal wastewater in an urban area in Ireland.

Niamh Cahill[1,2], Louise O'Connor[1,2], Bláthnaid Mahon[1,2], Áine Varley[1], Elaine McGrath[3], Phelim Ryan[1], Martin Cormican[1,2,3], Dearbháile Morris[1,2]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland , Galway, Ireland. [2]Ryan Institute Centre for Health from Environment, National University of Ireland, Galway, Ireland. [3]Carbapenemase-Producing Enterobacterales Reference Laboratory, Department of Medical Microbiology, University Hospital Galway, Galway, Ireland

Abstract

Carbapenemase-producing *Enterobacterales* (CPE) represent a significant health concern as certain strains are resistant to almost all antibiotics. The aim of this research was to examine hospital effluent (HE) and municipal wastewater from an urban area in Ireland for CPE. Samples of HE (n=5), and wastewater pre (n=5) and post (n=4) entry of HE to the municipal wastewater stream were collected over 9 weeks (May-July 2017). Samples were screened for CPE using Brilliance CRE agar (Oxoid). Suspect CPE were identified using MALDI-TOF, and underwent antimicrobial susceptibility testing (EUCAST). Suspect CPE were examined for carbapenemase-encoding genes; bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{VIM} and bla_{IMP}, by real-time PCR. Fourteen CPE (2 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*, 4 *Citrobacter freundii*, 7 *Enterobacter cloacae*) were detected in HE. Twelve harboured one carbapenemase-encoding gene (bla_{OXA-48} (n=7), bla_{KPC} (n=2), bla_{IMP} (n=2), bla_{VIM} (n=1)), while two (*Enterobacter cloacae*) harboured two genes; bla_{IMP} and bla_{OXA-48}. During the same period, in the hospital where HE was collected, 8 bla_{OXA-48}, 4 bla_{VIM} and 1 bla_{IMP} were detected in clinical samples. In post-hospital samples, 8 CPE were detected (2 *Klebsiella pneumoniae* (1 bla_{OXA-48}, 1 bla_{IMP}), 1 *Klebsiella oxytoca* (bla_{VIM}), 3 *Citrobacter freundii* (2 bla_{KPC}, 1 bla_{OXA-48}), and 2 *Enterobacter cloacae* (bla_{OXA-48})). One CPE (NDM-producing *Escherichia coli*) was detected in pre-hospital wastewater. HE is a larger source of CPE in comparison to municipal wastewater. In this era of CPE, there is a new element of risk associated with discharging untreated HE. Testing of HE may have applications in monitoring for unrecognised CPE dissemination in hospitals.

A152

The Selective Sequestration of Bacterial Populations Using Polymeric Glycomaterials

Joshua Petch, Stephan Heeb, Miguel Camara, Giuseppe Mantovani

University of Nottingham, Nottingham, United Kingdom

Abstract

The ability to selectively sequester bacteria from a mixed population is a desirable aim across a range of fields. Already some technologies exist to attempt to meet these aims spanning microfluidics and automated flow cytometry to antibody conjugated magnetic nanoparticle precipitation. However, these technologies are generally limited to the confinement of small populations in defined locations. Furthermore they may not be able to differentiate morphologically similar but phenotypically divergent cells. We aim to produce a novel technology to isolate bacteria from mixed populations by utilising polymeric carbohydrate ligands which bind to inducible bacterial adhesion proteins. To achieve this aim an inducible mutant of the *fim* operon in *Escherichia coli* has be constructed, thus allowing for switchable production of the mannose binding organelle, Type 1 fimbriae. Furthermore, we have previously observed that mannosylated polymers selectively bind to Type 1 fimbriated *E.* coli and to our induced mutant. Novel mannose functionalised polymers are being synthesised via a reversible addition-fragmentation chain transfer (RAFT) scheme. These polymers contain a catechol terminus which may be conjugated to magnetic Fe_3O_4 nanoparticles thus facilitating selective bacterial sequestration by magnetic separation. The successful development of this polymer-based bacterial sequestration platform could potentially enable the equivalent of immunoprecipitation in large-scale fermentation processes or the precise manipulation of living cells in laboratory scale procedures.

A153

Metagenomic analysis reveals significant seasonal variations in the epiphytic bacterial communities associated with different parts of the brown seaweed *Laminaria digitata*.

Maureen Ihua[1], Alan Dobson[1,2], Dagmar Stengel[3]

[1]University College Cork, Cork, Ireland. [2]Environmental Research Institute, University College Cork, Cork, Ireland.
[3]National University of Ireland Galway, Galway, Ireland

Abstract

Brown seaweeds such as *Laminaria* species are a rich source of polysaccharides such as laminarin and fucoidan which have a variety of functional food and animal feed applications, as well as alginates with demonstrated biological and pharmacological activities. Macroalgal surfaces are rich in carbon-based constituents which provide a suitable environment for growth and colonization by diverse bacterial communities.

Several environmental and non-environmental factors can influence the composition and abundance of epibacterial communities associated with seaweeds. In addition to the biological, physical and chemical properties of the macroalgal surface, seasonal variations have been found to play a significant role in the structure of the associated microbial communities. Variations in macroalgal epibacterial communities have also been observed within different parts of the host algal species. However, to date, in-depth studies on bacterial communities associated with macroalgal species, their ecological role and interactions with the algae are still scarce.

To gain an insight into the diversity and composition of the microbial communities associated with the brown alga *Laminaria digitata*, the communities derived from different parts of the alga including the blade, meristem, stipe and holdfast; were investigated using metagenomic Illumina sequencing of 16S rRNA gene amplicons. Seasonal variations in the microbial populations were found in samples taken from the Irish coast in different seasons between 2017 and 2018. This metagenomic-based investigation provides a detailed view of the seasonal variations in the bacterial populations associated with *Laminaria digitata* and helps provide further insights into potential interaction between this macroalga and its epiphytic bacterial communities.

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Multiple-pathway remediation of mercury contamination by a versatile selenite-reducing bacterium

Xiaonan Wang[1,2,3], Zhanfei He[4], Hongwei Luo[4], Ming Zhang[4], Daoyong Zhang[4], Xiangliang Pan[1,4], Geoffrey Gadd[2]

[1]Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi , China. [2]University of Dundee, Dundee, United Kingdom. [3] University of Chinese Academy of Sciences, Beijing, China. [4]Zhejiang University of Technology, Hangzhou, China

Abstract

Mercury contamination is a global concern because of its high toxicity, persistence, bioaccumulative nature, long distance transport and wide distribution in the environment. In this study, the efficiency and multiple-pathway remediation mechanisms of Hg^{2+} by a selenite-reducing strain of *Escherichia coli* was assessed. This bacterium can simultaneously reduce Hg^{2+} to Hg^{+} and Hg^{0} and selenite to selenide. This provides a multiple-pathway mechanism for removal of Hg^{2+} from water in addition to general biosorption. It was found that at an initial Hg^{2+} concentration of 0.2 μM, 93.2 ± 2.8% of Hg^{2+} was removed from solution. Of the total Hg removed, 3.3 ± 0.1% was adsorbed to the bacterium, 2.0 ± 0.5% was bioaccumulated, and 7.3 ± 0.6% was volatilized into the ambient environment. Most of the Hg (80.6 ± 5.7%) was removed as HgSe and HgCl precipitates and Hg^{0}. In this process, selenite was reduced to selenide which reacted with Hg^{2+} to form insoluble HgSe. In addition, Hg^{2+} was reduced to Hg^{+}, which formed solid HgCl, and Hg^{0}. This is the first report on bacterial transformation of Hg^{2+} to HgSe, HgCl and Hg^{0} via multiple pathways. It can be suggested that selenite-reducing microorganisms are promising candidates for mercury bioremediation of contaminated wastewaters, as well as simultaneous removal of Hg^{2+} and selenite.

A155

Occurrence of Extended-spectrum β-lactamase (ESBL)/AmpC producing bacteria in wastewater treatment plant effluent

Thi Thuy Do, Aidan O'Flaherty, Fiona Walsh

Maynooth University, Maynooth, Ireland

Abstract

Extended-spectrum β-lactamase (ESBL)/AmpC producing bacteria are one of the critical priority resistant bacteria that contributes to treatment failure and increased death rates. In this work we aimed to study the role of wastewater treatment plants (WWTPs) as reservoirs of ESBL/AmpC producing faecal coliforms. The effluent samples were collected from two WWTPs and faecal coliforms were isolated from all samples using the membrane filtration method. Bacterial isolates were subjected to antimicrobial susceptibility testing toward cefotaxime and ceftazidime. The isolates that showed a resistance phenotype to these antibiotics were considered as putative ESBL/AmpC producing bacteria. These bacteria were subjected to the AmpC test using a protocol with phenylboronic acid. The AmpC negative strains in the AmpC test served as samples for multiplex PCR containing primers specific for bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$. In total, 498 faecal coliforms were isolated from WWTP effluent samples. For the antibiotic susceptibility testing 99 isolates were considered as ESBL/AmpC producing bacteria. Among them, 26 isolates were found to be positive in the AmpC test. The PCR results revealed that 49 isolates carried bla_{TEM}, 6 bla_{SHV12}, 1 $bla_{CTX\text{-}M1}$ and 5 $bla_{CTX\text{-}M15}$. The ESBL/AmpC producing faecal coliforms in WWTP effluent are discharged to the receiving water environment. These data need to be considered when analysing the risk of WWTP effluent to the environment and to human health, as many of the bacteria identified are not analysed in assessment of risk of pollution from WWTPs globally.

A156

The Optimization of Microbial Induced Calcium Carbonate Precipitation in Soil Improvement Using Engineered Bacteria

Jamie Haystead

Northumria University, Newcastle upon Tyne, United Kingdom

Abstract

Poor soil conditions limit the building of new infrastructure, which is needed for an ageing and expanding population. Current soil strengthening techniques such as chemical grouting have detrimental effects on the environment from greenhouse gas production, soil pH modification and groundwater contamination, therefore there is demand for a sustainable approach to this process. Microbial-induced calcium carbonate precipitation (MICCP) is a technique that utilises the ability of bacteria to precipitate calcium carbonate ($CaCO_3$), which can be used for a variety of applications including binding adjacent soil particles and filling the pore spaces of soils to increase mechanical properties. Commonly used bacteria include *Sporosarcina pasteurii* and *Bacillus subtilis*. A range of factors influences MICCP which presents challenges with process optimisation. These factors need to be optimised in the laboratory before they can be applied for engineering purposes. The overall aims of my research are to optimise urease production in *S. pasteurii* and *B. subtilis* and to investigate the distribution and binding of these bacteria with various sand particles, by means of syringe and glass column set ups. These bacteria will be compared with engineered bacteria which can overproduce urease to investigate the impact on precipitation efficiency. Factors to control bacteria biofilm formation to influence the morphology of $CaCO_3$ will be investigated to determine the impact of various crystal shapes on soil properties. Ultimately, raw data generated from the project will be used for predicting biocementing at a lab scale for building computational models.

A157

How are bacteria involved in the detrimental health outcomes caused by exposure to air pollutants?

Louise Corscadden, Dr Julie Morrissey, Professor Julian Ketley, Professor Peter Andrew, Professor Paul Monks, Dr Joanna Purves

University of Leicester, Leicester, United Kingdom

Abstract

With an annual death toll of 7 million, the effect of air pollution on human morbidity and mortality is a major global problem. Air pollution associated with infectious respiratory disease is responsible for a mortality rate of 1.5 million annually. However, the impact of air pollution on bacterial behaviour is largely unknown. Our work has shown for the first time that black carbon (BC), a major component of air pollution, increases dissemination of colonising *Streptococcus pneumoniae* and *Staphylococcus aureus* in *in-vivo* infection models, and also alters biofilm structure, composition, and function. However, the biological mechanisms responsible for dissemination in the host and biofilm alterations by BC are unknown. BC is known to elicit an oxidative stress in eukaryotic tissues, therefore we hypothesise that the *S. aureus* oxidative stress response may play an important role in the bacterial response to BC. Our data shows that exposure to BC is toxic to *S. aureus* and that oxidative stress response of mutant strains show increased sensitivity to BC than the wildtype strain. Transcriptional analysis demonstrated that the expression of key oxidative stress genes in the oxidative stress response pathway are induced in the presence of BC. These findings demonstrate that BC has a metabolic effect on *S. aureus* and that the oxidative stress response is required for bacterial survival to BC. Furthermore, the induction of the *S.aureus* oxidative stress response may be important for increased dissemination in the host through adaptation of bacterial cells to the host immune response.

A158

The Impact of Manure Application on the Microbiome of Grassland

Ciara Tyrrell[1,2], Catherine Burgess[2], Fiona Brennan[3], Fiona Walsh[1]

[1]Maynooth University, Co.Kildare, Ireland. [2]Teagasc Food Research Centre, Dublin, Ireland. [3]Teagasc, Johnstown Castle, Co. Wexford, Ireland

Abstract

Manure spreading onto land is an important agricultural process. Manure is recycled as organic fertiliser; however it can introduce manure-derived antibiotic resistant bacteria into the environment. Grassland consists of approximately 70% of global agricultural land and is a vital source of food for livestock. Despite the important role grassland plays in food security, the impact of manure application on its resistome and microbiome is relatively unknown. Antibiotic resistance is a multifactorial issue, involving an intertwining relationship between animals, humans and the environment. Therefore, it is critical to fully understand all potential routes of antimicrobial resistance (AMR) transmission. As the microbiome of grassland is an under-researched area, it is a possible source of AMR transmission to animals which may enter the food chain. A pot trial mesocosm experiment was carried out to investigate the impact of manure application on the microbiome of the phyllosphere of perennial ryegrass *(Lolium perenne).* Pig slurry was applied to six pots of *L.perenne* and grass and soil samples were taken two weeks following manure application. Following sonication, viable bacteria were isolated from the soil, manure and grass by plating on selective agars supplemented with antibiotics. Isolates were screened for antibiotic resistant bacteria by antibiotic susceptibility testing. DNA was extracted from the soil, grass and manure and underwent microbial community compositional analysis by 16srRNA sequencing on the Illumina Miseq platform. The results from this mesocosm experiment will contribute to a further field trial to investigate the impact various manure types have on the microbiome and resistome of grassland.

A159

Fungal Transformation of Cobalt-Bearing Minerals and Metal Bioprocessing Applications

John Ferrier, Geoffrey Gadd

University of Dundee, Dundee, United Kingdom

Abstract

Geoactive fungi such as *Aspergillus niger* play a significant role in bioweathering processes and element cycling. These organisms are able to secrete a range of organic acids, such as oxalic acid, into their microenvironment. This enables them to mediate mineral dissolution, leading to metal solubilization and precipitation in the form of secondary biominerals. In this investigation, such biotransformation processes were explored as a means of cobalt bioprocessing, an E-tech element identified as being of key strategic importance, in addition to other mineralogically related metals. A range of Co-bearing mineral phases were investigated, including a Co-bearing lithiophorite [$(Al,Li)MnO_2(OH)_2$] and erythrite ($Co_3(AsO_4)2{\cdot}8H_2O$), in addition to seafloor ferromanganese nodules. Bioleaching and bioprecipitation studies were carried out to investigate the ability of *A. niger* to leach cobalt and related metals from Co-bearing minerals, and to precipitate them in biomineral form as a means of cobalt biorecovery. The objective of the work is to investigate the natural biotransformation of cobalt-bearing minerals, to investigate the factors that influence cobalt bioprocessing and to optimise the maximal yield of cobalt biominerals.

A160

Amino acid secretion influences the size and formation of copper carbonate nanoparticles in biomass-free fungal culture supernatants

Feixue Liu, Laszlo Csetenyi , Geoffrey Gadd

University of Dundee, Dundee, United Kingdom

Abstract

The ureolytic activity of *Neurospora crassa* results in an alkaline carbonate-rich culture medium which can precipitate soluble metals as insoluble carbonates. Such carbonates are smaller, often of nanoscale dimensions, than metal carbonates synthesized abiotically which infers that fungal excreted products can markedly affect particle size. In this work, it was found that amino acid excretion was a significant factor in affecting the particle size of copper carbonate. Eleven different amino acids were found to be secreted by *Neurospora crassa*, and L-glutamic acid, L-aspartic acid and L-cysteine were chosen to examine the impact of amino acids on the morphology and chemical composition of copper carbonate minerals. Powder X-ray diffraction (XRPD), scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) were used to characterize the obtained copper carbonate samples. Copper carbonate nanoparticles with a diameter of 100 – 200 nm were produced with L-glutamic acid, and the presence of L-glutamic acid was found to stabilize these particles in the early phase of crystal growth and prevent them from aggregation. Such processes may indicate a potential means to engineer the size and morphology of metal carbonate nanoparticles using biomass-free fungal systems. FTIR and TGA revealed that the amino acid moieties were intimately associated with the copper mineral particles. In addition, component analysis of the final products of TGA of the copper minerals showed the ultimate formation of Cu_2O, suggesting a novel synthesis method for producing this useful Cu-containing material.

Environmental and applied microbiology forum
Presentations: Monday and Tuesday evening

A161

Characterization of *Enterococcus faecium* SP15, a novel isolate with probiotic and therapeutic potential

Fauzia Aziz[1,2,3], Muhammad Khan[2], Safia Ahmed[4], Ian Jones[1], Simon Andrews[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. [3]University of Poonch Rawalakot, Azad Kashmir, Rawalakot, Pakistan. [4]Quaid -i-Azam University, Islamabad, Pakistan

Abstract

Aims: To characterise the genome and enterocin content of *Enterococcus faecium* SP15, a new isolate from natural spring water.

Methods and Results: *Enterococcus faecium* strain SP15 was isolated from natural spring water and its identity confirmed using 16S rDNA sequence. The bacterial strain produced antimicrobial compounds (enterocins) against pathogenic bacteria including *Listeria monocytogenesis* as determined by the agar spot method. In addition, an anti-cancer activity was observed on human cancer cell line HT-29 by cytotoxicity assay (MTT) and apoptosis study. A draft genome sequence revealed the presence of several enterocin genes capable of activity against a panel of pathogenic bacteria including *L.monocytogenesis.* The active production of enterocins was also supported by the presence of peptides in part purified fermentates following trypsin digest and mass spectrometry. The genome showed no classical virulence factors or hemolysins and was free of antibiotic resistance genes. To confirm enterocin related killing, select enterocin genes were cloned and expressed as His-tagged proteins in *E.coli*. Purified enterocin retained activity in *L.monocytogenes* overlay assay.

Conclusion: *E. faecium* SP15 is a promising strain for probiotic use and/or food preservation. Purified enterocins retain their activity suggesting them a template for structure-function studies and future improvements as antimicrobial and cancer cell therapeutics.

Significance and Impact of the Study: New antibacterial and anticancer agents are required to combat antibiotic resistance and a limited drug repertoire. Enterocins from *Enterococcus faecium* SP15 could fulfil these goals in addition to use of the strain as a whole organism probiotic.

A162

Understanding autotrophic bacterial community structure and function along a naturally occurring iron deposit gradient.

Mark R Cunningham[1], Yi-Chen Chiang[1], Neeleema Seetaloo[1], Shamsudeen Dandare[1], David Griffiths[2], John W McGrath[1], Katrina A Macintosh[1], Deepak Kumaresan[1]

[1]School of Biological Sciences and the Institute for Global Food Security, The Queen's University of Belfast, Belfast, United Kingdom. [2]School of Geography and Environmental Sciences, Ulster University, Coleraine, United Kingdom

Abstract

Naturally occurring iron deposits in upland streams have previously been studied in the context of their chemical composition, epilithic biomass, and impacts upon invertebrate community structure over an iron gradient in the Sperrin Mountains, Northern Ireland. The Sperrin Mountains consist of metamorphosed schist and unconsolidated glacial drift with peat/peaty podzol (mainly humic) soils. Anthropogenic influences on the study sites are limited,with only low intensity sheep farming and localised coniferplantation forestry: there is no evidence of mining occurring now or in the past in the study catchments. The occurrence of autotrophic, iron-oxidising bacteria in the deposits was inferred by a previously published study; however, their presence was not confirmed. In this study, we present data pertaining to the bacterial community structure and function along this naturally occurring iron deposit gradient. 16S rRNA gene polymerase chain reaction (PCR) and stable isotope probing (SIP) with ^{13}C bicarbonate were carried out to identify autotrophs and their activity levels relative to spatial and temporal influences. The sequenced species revealed groups from the phylum *Proteobacteria, Acidobacteria* and *Clostridia*. Species involved in iron cycling such as *Geobacter* and *Magnetococcus* from *Deltaproteobacteria* were present. Several *methylotrophs* were also detected in *Alphaproteobacteria*. The data obtained reveals the variety of functional autotrophic bacteria that can be found in non-acid mine drainage iron deposits.

A163

Penetration of the Air-Liquid Interface is Key to the Wrinkly Spreader Success

Robyn Jerdan[1], Anna Kuśmierska[2], Nia White[1], Andrew Spiers[3]

[1]Abertay University , Dundee, United Kingdom. [2]University of Łódź , Łódź , Poland. [3]Abertay University, Dundee, United Kingdom

Abstract

The adaptive radiation of Pseudomonas fluorescens SBW25 populations in static liquid microcosms results in the appearance of the biofilm–forming Wrinkly Spreader. This adaptive mutant is able to colonise the high O2-rich region at the top of the liquid column established by the metabolic activity of earlier wild-type colonists, and by doing so, enjoys a significant fitness advantage over non-biofilm–forming competitors including the ancestral Pf. SBW25. Although the underlying molecular biology and evolutionary ecology of the Wrinkly Spreader is well understood, we have recently questioned the need for expensive biofilm–formation to colonise the air-liquid (A-L) interface, as O2-directed flagella-mediated swimming (aerotaxis) should be sufficient to maintain cells in this region. Our investigations show that swimming can overcome displacement by Brownian diffusion and microcurrents within the liquid column. However, it is not sufficient to explain the high levels of enrichment at the A-Linterface shown by Wrinkly Spreader cells. A comparison of the liquid surface tension of wild-type and Wrinkly Spreader cultures, supernatants, and washed cells, suggests that the Wrinkly Spreader produces a surface-active compound weakly associated with the cell which helps penetration of the A-L interface and allows cells to remain in the high-O2 region without further expenditure of energy. Our results suggest that this penetration is key to the following biofilm–formation which supports higher populations at the A-L interface and that this explains the adaptive advantage of the Wrinkly Spreader.

A164

Fungal biodeterioration of copper metal

Jiayue Zhao, Geoffory Gadd

University of Dundee, Dundee, United Kingdom

Abstract

In this work, geoactive fungi including *Aspergillus niger*, *Beauveria caledonica* and *Paecilomyces javanicus*, were used to investigate their biocorrosion and deteriorative effects on copper metal to gain an understanding of the roles that fungi may play in biodeterioration of such a material in the built environment. It was clearly demonstrated that the test fungi possessed a high tolerance to copper metal. New biominerals resulted from fungal interactions with copper metal mainly arising from organic acid excretion. Copper oxalate was formed by oxalate excretion from the fungi and different patterns of bioweathering and biomineralization were generated on the copper surfaces. In addition, copper could be dissolved by certain fungi, result in significant biodeterortive effects such as etching and pitting.

These results provide compelling evidence for deteriortion of copper metal by fungi and that organic acids, particularly oxalate, play an important role in this process. Such properties of metal biocorrosion and deterioration indicate the potential significance of fungi in biodeterioration of metal substrates and the importance of considering methods of protection and preservation in the built environment.

A165

Use of cellular and high throughput genetic approaches to unravel the antibacterial mechanism of honey

Maria Masoura, Mathiew Milner, Tim Overton, Pete Lund, Kostas Gkatzionis

University of Birmingham, Birmingham, United Kingdom

Abstract

The medical importance of honey has been extensively demonstrated. Although high osmolarity, acidity and hydrogen peroxide (H_2O_2) proved to be the most prevalent factors in honey's activity, the underlying antimicrobial mechanism remains obscure. Our aim is to provide insight into the physiological changes and genetic responses in honey-treated bacteria, thus improving our understanding of this natural product as a potential novel antimicrobial.

A model honey composed of sugars, gluconic acid, and H_2O_2as they are accumulated in honey after enzymatic reaction happens, was used the investigation of honey's activity. The bactericidal action of the model was tested on *E.coli* K-12 strain MG1655. Flow cytometry (FC) and Atomic Force Microscopy (AFM) identified physiological changes such as membrane potential, blebbing, and cell lysis. Reactive Oxygen Species (ROS) accumulation was observed in individual cells by FC. Transposon Directed Insertion Sequencing (TraDIS) identified mutants' fitness over a time course of *E.coli* treatment by model honey. The loss of selenocysteine (*selAB*) and formate dehydrogenase (*fdhDE*) mutants, proved the redox- balancing activity as essential for the repression of ROS in stressed cells. High susceptibility of energy metabolism (*atpABD*) and peptidoglycan synthesis (*prc*) mutants, indicated the strain unable to maintain the reductive cell environment necessary for cellular activities, post honey exposure.

Our findings identified some of the honey's targets when acting as an antimicrobial. The synergies observed support the use of honey as an antimicrobial; however, the identification of mutations that led to enhanced resistance to honey is an important finding that needs further study.

A166

Sustainable natural production of vitamins for human consumption in long Space missions using synthetic ecology approaches.

Ellen Harrison

Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom

Abstract

The MELiSSA (Micro Ecological Life Support System Alternative) project aims to create a closed loop system, capable of providing all the necessary food, water and oxygen for astronauts on long Space missions. The MELiSSA loop is comprised of four compartments, with compartment IV_A containing photoautotrophic bacteria, such as *Arthrospira platensis*, a good source of oxygen, edible biomass and micronutrients. One important example of the latter is cobalamin (B_{12}) deficiency of which can lead to pernicious anaemia and neurological systems, and thus would be detrimental to astronauts on long Space missions. The molecule is not made by plants or fungi, so the ultimate source of B_{12} in the environment is prokaryotes, and its synthesis requires over 20 enzymatic reactions. Many eukaryotic algae also require cobalamin for growth, and some species have been shown to accumulate the vitamin when grown in coculture with cobalamin synthesising bacteria. The aim of this project is to extend existing knowledge of algal-bacterial mutualisms involving cobalamin. Eukaryotic species such as *Haematococcus pluvialis* and *Chlorella vulgaris,* both certified as safe for human consumption, along with *A. platensis* will be investigated with different bacterial partners to maximise cobalamin accumulation. Not only will this research help provide adequate nutrition on long Space missions, it will also support nutritional supply on Earth with the rise of veganism, as well as, aiding in understanding the dynamics of algal-bacterial mutualisms, which is of interest in terms of nutrient cycling in the environment.

A167

Effect of temperature increase in bacterial and fungal communities of chlorinated Drinking Water Distribution Systems

Carolina Calero Preciado, Joby Boxall, Víctor Soria-Carrasco, Isabel Douterelo

University of Sheffield, Sheffield, United Kingdom

Abstract

Drinking Water Distribution Systems (DWDS) are diverse ecosystems where the majority of microorganisms live forming biofilms, which can alter the water quality if they are mobilised to the bulk water. Biofilm communities can be affected by the increase of temperature due to climate change, thus compromise the distribution of safe water.

To understand the effect of temperature on biofilms in DWDS, biofilm was developed for 30 days at 16°C and 24°C using a full-scale experimental DWDS facility. Samples were collected at the end of the experiment from removable coupons inserted into the pipes. DNA was extracted and the 16S rRNA and ITS rRNA genes were sequenced and analysed, for the bacterial and fungal diversity respectively.

Differences in bacterial and fungal diversity at both temperatures were observed at family level. At 16°C bacterial community was dominated by Comamonadaceae (21.48%), Pseudomonadaceae (16.41%) and Sphingobacteriaceae (12.99%). However, at 24°C the most abundant family was Pseudomonadaceae (50.60%) followed by Sphingomonadaceae (9.59%) and Sinobacteraceae (7.82%). Fungal diversity showed that at 16°C the most abundant family was Nectriaceae (68.9%), followed by Helotiales (24.5%) and Filobasidiales (1.5%). However, at 24°C the community was dominated by Nectriaceae (98.15%) and the following families showed a low relative abundance, Rhizopodaceae (0.95%) and Cryptomycota (0.24%).

Temperature is a key factor for microbial growth in DWDS and affect the composition of the microbial communities. Temperature increase leads changes and a loss in complexity in bacterial and fungal communities of biofilms, which can affect the water quality.

A168

Role of oxalate in fungal precipitation and biorecovery of lanthanum

Xia Kang, Geoffrey Gadd

School of Life Sciences, Dundee, United Kingdom

Abstract

Lanthanum is an important member of the rare earth elements (REE) that are a global strategic resource and have many technological applications ranging from microelectronics manufacturing to the production of clean and renewable energy. *Aspergillus niger* is widely used in industrial fermentations due to its production of multiple secondary metabolites including citric and oxalic acids. Since it is a ubiquitous soil inhabitant and produces geoactive agents, it can play a role in the biotransformation of metal-containing minerals. Previous studies found that *A. niger* is capable of mineral solubilization and secondary mineral formation, many metals being precipitated as oxalates. However, there is limited knowledge about the biotransformation of La mediated by fungi. The aim of this project was to explore the mechanisms and factors determining the interactions between *A. niger* and La. In this study, fungal growth on La-supplemented solid media was carried out and it was discovered that crystalline deposits were formed around fungal colonies in the presence of $LaCl_3$. These biogenic crystals were recovered and subjected to examination for their elemental composition, morphological features and mineral phases using energy dispersive X-ray analysis (EDXA), scanning electron microscopy (SEM) and X-ray diffraction (XRD) respectively. These confirmed the biotransformation of lanthanum and identified the products as lanthanum oxalate $[La_2(C_2O_4)_3{\cdot}10H_2O]$, which was further transformed into La_2O_3 by thermogravimetric (TG) treatment. Geochemical modelling also supported these results. Our findings provide a new aspect for the bioprecipitation and biorecovery of REE from solution using fungal culture systems.

A169

MICROALARM: “A new system for rapid quantification of the total bacteria in water samples”

Miguel Ángel Fernández-Fuentes[1], Elena Soria[1], Adela Yáñez[1], Marc Jofre[2], Cedric Hurth[2], Pedro Martínez[2], Valerio Pruneri[2,3], Vicente Catalán[1]

[1]LABAQUA, S.A., Alicante, Spain. [2]ICFO - Institut de Ciències Fotòniques, Barcelona, Spain. [3]ICREA - Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

Abstract

Background

Analytical methods applied in diagnostic microbiology laboratories are usually performed manually and have several drawbacks, the long time-to-results (48-72 hours) being the major one.

Rapid detection methods may overcome the disadvantages of traditional microbiological methods and achieve fast detection, which will help preventing the spread of waterborne pathogens and outbreaks of waterborne diseases.

The objective of the MICROALARM project is to validate a platform for monitoring the quantification of the total bacteria present in water samples.

Methods

The MICROALARM system integrates a microfluidic cartridge where the microorganisms from the sample are labelled to facilitate the detection and measurement by a fluorescence sensor. The prototype system has been validated according to ISO 16140-2:2016 in the laboratory through the comparison with conventional (culture isolation) and alternative methods (flow cytometry) to determine whether the method is suitable for quantifying microorganisms in water samples.

Results

The system will be capable of processing, labelling and quantifying automatically the total bacteria of the water sample using a membrane-permeable fluorescence marker. The system has been validated at the laboratory scale using a wide range of water matrices, then installed and validated in different facilities with satisfactory results.

Conclusion

The MICROALARM system is designed to be portable, thus suitable for on-site applications. It will be a low-cost solution for rapid microbiological analysis. The system will avoid water sampling and transport to the laboratory while enabling on-site quantification of the total bacteria in a reduced timeframe for early decision-making.

A170

Genomic Trends on the Biogenic $CaCO_3$ production in the genus *Bacillus*

Philippa Loftus[1], Andre Antunés[2]

[1]Edge Hill University, Ormskirk, United Kingdom. [2]Edge Hill University, Ormskrik, United Kingdom

Abstract

The biogenic production of $CaCO_3$ via Microbially Induced Calcite Precipitation (MICP) has been reported in many microbial strains. This process has a wide variety of current and potential applications, namely in civil engineering, agriculture and bio-remediation. The number of species used in such applications remains limited and seems to be biased towards ureolytic strains. Urea degradation is the best documented process leading to MICP, however, there are several alternative processes, possibly more relevant, which are mostly overlooked. In general, and despite being widely reported, the MICP process is still poorly understood, and has been chronically understudied from a genomic perspective.

Here we report on the genus-wide analysis of MICP capability, centred on genomic-based analysis, and focusing on the genus *Bacillus*. This genus harbours several species capable of MICP and is the most widely used regarding its biotechnological application. The very high number of species within this genus, and availability of whole genome sequence data for several makes it an ideal target for this analysis. Our preliminary results uncover a diverse range of MICP-associated genes, identifies similar genomic profiles within phylogenetic subgroups, and questions the importance of urease activity for $CaCO_3$ production in the genus *Bacillus*. This study is the first of its kind and provides key insights into the genomic basis of MICP, while testing the feasibility of a genomic-based prediction method for fast identification of new strains with such capabilities, which would be applicable to other genera and be particularly useful for downstream applications.

A171

Bacterial Biosensor for Detection of Endocrine Disrupting Chemicals

Su-Hyun Ryu, Yu-Rae Kim, Jang-Wan Son, Youn-Ha Ok, Jin-Won Lee

Hanyang university, Seoul, Korea, Republic of

Abstract

Endocrine disrupting chemicals (EDCs) are becoming a social problem because of their ability to interact with human nuclear hormone receptors producing abnormal signals in the endocrine system. Despite their danger, EDCs such as bisphenol A, nonylphenol, and phthalate are widely used in our lives and industries. Although a lot of methods for detecting EDCs have been developed by many researchers, they are either expensive or time-consuming. Therefore, we developed bacterial cell-based biosensor using a bacterial two-hybrid system. When estrogens bind to ERα, ERα can interact with its coactivator and regulates its target genes. Using this characteristic, ERα ligand binding domain (LBD) and its coactivator, RIP140 are introduced into bacterial two-hybrid system. When EDCs or estrogens bind to ERα LBD, ligand dependent specific interaction between ERα LBD and RIP140 produces LacZ expression. Using this biosensor, estrogens such as 17β-estradiol, estriol, and estrone can be detected in nanomolar range and EDCs such as bisphenol A and nonylphenol can be detected in micromolar range. Also, EDCs in household products such as a receipt paper were detected using our system. Interestingly, estrogenic compounds in a bisphenol A-free receipt paper were also detected. They were estimated to be bisphenol S or bisphenol F, and they were detected in micromolar range using our biosensor. In conclusion, we developed the bacterial biosensor which can detect estrogens, EDCs, and EDCs in household products with low cost, fast detection, and simple method. And we suggest that ‘BPA-free’ is no longer a word of safety.

A172

Selenium and tellurium oxyanion reduction by yeasts

Lara Codognotto , Geoffrey Michael Gadd

University of Dundee, Dundee , United Kingdom

Abstract

Geomicrobiology Group, School of Life Sciences, University of Dundee*, Dundee, DD1 5EH, Scotland, UK*

Selenium and tellurium are two metalloids essential for future green energy technologies due to their associated photovoltaic and photoconductive properties. In addition, selenium and tellurium oxyanions can be toxic in the environment and can potentially affect human health. This work aims to examine some geochemical influences on Se/Te reduction carried out by selected yeast strains to identify what limitations there are to the process, and their importance. Several yeast strains, capable of selenite or tellurite reduction, were isolated from environmental soil samples on solid media containing selenite or tellurite, reduction being detected by the colour change of colonies to red (Se) or black (Te). Such reduction resulted in the formation of nanoparticles of elemental Se^0 or Te^0. Growth was assessed in the presence of selenite or tellurite and minimum inhibitory concentrations determined. Rates of selenite and tellurite depletion were determined in different growth conditions and the production of elemental Se^0 or Te^0 was analysed using energy dispersion X-ray analysis (EDXA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). This work furthers understanding of selenium and tellurium transformation by yeasts also suggests potential routes for Se/Te biorecovery by the formation of Se/Te nanoparticles.

A173

Investigations into *Apium graveolens* as a novel antimicrobial agent

Sophie Hutchinson[1], Catherine Duckett[1], Graham Stafford[2], Thomas Smith[1]

[1]Sheffield Hallam University, Sheffield, United Kingdom. [2]University of Sheffield, Sheffield, United Kingdom

Abstract

Celery *(Apium graveolens)* seed has been widely used within traditional medicines. A number of antimicrobial compounds have been discovered in celery seed extract (CSE) one of which contains potent antifungal activity. This is of particular interest in view of the shortage of effective antifungal drugs currently available. This study aims to identify and characterise antimicrobial compounds extracted from celery seed to explore as potential novel antimicrobials.

CSE was extracted and fractionated from fresh celery seeds using a solvent extraction and sicila gel column chromatography. Thirteen distinct subfractions, as judged by their TLC profiles, were obtained from the CSE. Various subfractions showed antimicrobial activity against important Gram-positive and Gram-negative pathogens, together with a variety of pathogenic fungi, for example; *Staphylococcus aureus, Helicobacter pylori, Aspergillus niger, Candida albicans, Fusarium solani* and *Trichophyton* ssp.. We have identified four distinct antimicrobial molecules which are present within CSE. Among these, one is active only against *S. aureus* and three have both antibacterial and antifungal activity.

One of the subfractions has been shown to have an antimicrobial effect against a range of *C.albicans* mutants including an isolate which can be locked in either a hyphal or yeast state. This demonstrates the potential suitability of these compounds for treatment of complex infections in a clinical setting. The haemolytic activity and cellular toxicity levels of these subfractions are also currently being examined.

A174

Biodiversity and biochemical characterization of bacteria from Terrestrial Subsurface Environment

NAZEERULLAH RAHAMATHULLAH[1], THAJUDDIN NOORUDDIN[2]

[1]Gulf Medical University, Ajman, UAE. [2]Bharathidasan University, Tiruchriappalli, India

Abstract

Forty-one aerotolerant subsurface bacterial colonies were isolated from terrestrial subsurface environment at various depth from 5 feet to 50 feet at 5 feet intervals. Out of 41 colonies, 32 morphologically distinct bacterial colonies were selected for gram staining reaction, various sugar utilization pattern and scanning electron microscopic studies. Most of the bacterial colonies were gram-positive rods; some are gram-negative rods, gram-positive cocci and coccobacilli, and gram-negative coccobacilli respectively. Seven bacterial colonies NTN33, NTN34, NTN35, NTN36, NTN01, NTN02 and NTN03 isolated from 40 to 50 feet depth were neither gram positive nor gram negative, and no distinct morphological shape but gigantic structure have identified in that bacteria. The bacterial size were identified on the bacteria NTN36, NTN35, NTN01, NTN30, NTN34, NTN02 as 4.5µm, 4.1µm, 3.5µm, 3.4µm, 3.2µm and 2.2µm. Gigantic rod surrounded by capsular sheath and gigantic rod with footprint like appearance were identified in the bacteria NTN02 and NTN36; those are isolated from 45 and 50 feet depth. Intracellular granules were also found in bacteria NTN34, NTN35 and NTN01. In total, 24 different sugars had used to analyse the sugar utilization pattern of that 32 bacteria. Most of the bacteria have utilized only 03 different types of sugars either maltose, galactose and glycerol or lactose, trehalose and mannose. However, the bacteria NTN16, NTN12, NTN06, NTN24, NTN27, NTN28, NTN34 and NTN02 had utilized 14 different sugars. Nevertheless, very few bacteria have utilized the L-arabinose, α-methyl-D-gluconate and inulin. None of the bacteria had utilized the sugar sodium gluconate, salicin, glucosamine and dulcitol.

A175

Microbial decomposition of porcine tissue in organic compost at different temperatures

Branka Franicevic, Jonathan Fletcher, Andrew Wilson, Rob Janaway

University of Bradford, Bradford, United Kingdom

Abstract

Laboratory experiments were conducted to determine the effect of temperature (20°C and 30°C) on the rate of tissue decomposition in organic compost for a period of 28 days. Porcine tissue was used as a substitute for human body parts in burial microenvironment. Measurements of the decay include porcine tissue mass loss, soil pH, the metabolic activity of soil microbes, viable count and Gram stain of associated microflora. Incubation temperature had a significant impact on the post-mortem decay rates and stages at 30°C in comparison to the samples incubated at 20°C. Bacterial enumeration demonstrated microbial burden to be higher at higher temperatures, suggesting accelerated decomposition. Gram staining identified mostly Gram-negative bacteria, decomposers that might have originated from the organic meat as opposed to soil bacteria. There was a significant difference in soil pH levels during harvest times and at the end of the experiments in both microenvironments. It is an indication that measured pH of the depository may reveal whether the body parts were recently buried. A fluorescein diacetate (FDA) method that measured metabolic activity in compost demonstrated the highest release of fluorescein during harvests at 30°C. A non-parametric Friedman test of differences among repeated measures rendered significant results ($\alpha = 0.00$) showing differences in microbial activity according to the temperature level. The results suggest that depositional microenvironment would be significantly modified by the decaying organic matter with the rise of temperature. This way, the temperature would make an impact on the decay of buried remains.

A176

Gut microbiome of poison arrow frogs and their potential role in toxin sequestration

Karen Siu-Ting[1], Jess Friedersdorff[2], David Carreño[3], Ben Thomas[2], Jamie Newbold[4], Mary O'Connell[5], Christopher Creevey[1]

[1]Queen's University, Belfast, United Kingdom. [2]Aberystwyth University, Aberystwyth, United Kingdom. [3]Leicester University, Leicester, United Kingdom. [4]SRUC, Edinburgh, United Kingdom. [5]University of Nottingham, Nottingham, United Kingdom

Abstract

Poison frogs secrete alkaloid toxins in their skin as defence mechanisms against predators. Numerous studies have shown that the origin of alkaloid toxins in the skin is through “sequestration from diet”, i.e. uptake and storage of toxins or their chemical precursors, mostly from consumed arthropods. There exists the intriguing possibility that the gut microbiome of these frogs may play a role in this process. We address this question by looking at the organism together with its associated microbial communities, an effective symbiotic relationship between host and microbiome that could have allowed phenotypic adaptation of the host to a toxic diet. We sequenced the Bacterial and Archaeal 16S rRNA regions of the gut microbiome of 7 Poison frog species and 9 outgroup frog species caught in the rainforest of Eastern Peru. Frog species were selected based on sharing similar microhabitats and comparable individual sizes. A comparative analysis of the microbiome composition across all our samples allowed us to identify a core group of abundant symbiotic microbes unique to poison frogs in spite of intrinsic variation within species. We speculate this group could be associated to their ability to sequester toxins and we carried further metagenomic sequencing to allow us to determine possible functions that may be involved in toxin processing in these frogs.

Environmental and applied microbiology forum
Presentations: Monday and Tuesday evening

A177

High-Resolution 16S Biogas Upgrading Communities: Contrasting *in situ* and *ex situ* Setups

Jamie A. FitzGerald[1,2], Marcus Voelklein[1,2], Marcus J Claesson[1], Jerry D. Murphy[1,2], Alan D.W. Dobson[1]

[1]University College Cork, Cork, Ireland. [2]The MarEI Centre,, Cork, Ireland

Abstract

As biogas from anaerobic digestion becomes an increasingly attractive biofuel, the need to improve the quality of biogas has come to the fore. Biological upgrading focuses on adding enough hydrogen to an anaerobic biogas reactor to allow methanation of the remaining carbon dioxide by methanogenic Archaea (in situ upgrading). Alternatively, biogas and hydrogen can be mixed in the absence of feedstock, in a reactor operated exclusively to facilitate methanogenesis (ex situ upgrading). This novel technology can encounter inhibition at high loading rates of hydrogen: however, in contrast to anaerobic digestion, the dynamics of this thermophilic functional microbial community are sparsely characterised.

High-resolution 16S rDNA community profiles from four anaerobic biogas upgrading reactors were constructed to determine how feedstock, hydrogen, and CO_2 influence biomethanation. Presence/absence of a feedstock led to large differences between in situ and ex situ communities, determining the dominant methanogen genera, and encouraging distinct populations of hydrolysing and fermenting *Firmicutes*. Although high hydrogen flow rates (~37L/day) caused a collapse in methanogenic *Methanothermobacter* populations in situ, ex situ hydrogen rates greatly exceeded these levels (~400L/day) without collapse of *Methanobacterium*, despite some observed instability and proliferation of likely homoacetogens. Subsequent reduction of hydrogen rates ex situ (259L/day) appeared to create a niche for hydrogen production, indicated by increased abundance of various syntrophic fermenters known to supply biogenic hydrogen. In either upgrading setup, instability due to increased hydrogen levels manifested as a disruption of fermenting and hydrolysing populations prior to disruption of methanogens.

Environmental and applied microbiology forum
Presentations: Monday and Tuesday evening

A178

Carbapenemase-producing *Enterobacterales* and extended-spectrum β-lactamase-producing *Enterobacterales* detected in Irish recreational waters, 2016-2017

Bláthnaid Mahon[1,2], Carina Brehony[1], James Killeen[1], Elaine McGrath[3], Niamh Cahill[1,2], Louise O' Connor[1,2], Aine Varley[1], Sinead Ryan[1], Martin Cormican[1,2,3], Paul Hickey[4], Shane Keane[4], Ann Dolan[5], Martina Mulligan[6], Bryan Ruane[6], Dearbháile Morris[1,2]

[1]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland, Galway, Galway, Ireland. [2]Ryan Institute Centre for Health from Environment, National University of Ireland, Galway, Galway, Ireland. [3]Carbapenemase-Producing Enterobacterales Reference Laboratory, Department of Medical Microbiology, University Hospital Galway, Galway, Ireland. [4]Environmental Health Service, HSE West, Galway, Ireland. [5]Galway County Council, Galway, Ireland. [6]J. Murphy & Sons Limited , Galway, Ireland

Abstract

The role that the environment plays in the dissemination of antimicrobial resistance is not well understood. The aim of this study was to examine seawater, freshwater and sewage for the presence of carbapenemase-producing *Enterobacterales* (CPE) and extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-PE), which are organisms of clinical significance. Overall, 66 samples were taken from 21 sample points at two sites (Site A and Site B) between May 2016 and September 2017. A total of 30L of seawater or freshwater and 200mL of sewage were collected per sample. Samples were examined for CPE and ESBL-PE, and isolates obtained were tested for susceptibility to 14 antimicrobial agents. Suspect CPE and ESBL-PE were examined for β-lactamase encoding genes by real-time PCR. A total of 26 CPE were obtained. The majority (n=24), were identified in samples taken at Site A. NDM-producing *Enterobacterales* were detected continuously in recreational waters at Site A for more than 14 months. Evidence indicates that it was the same strain of *Escherichia coli* and *Klebsiella pneumoniae* repeatedly detected. Untreated human sewage being continuously discharged at Site A was identified as the source of CPE. OXA-48-producing *E. coli* (n=1) and OXA-48-producing *Klebsiella pneumoniae* (n=1) were detected in two separate seawater samples taken at Site B. The source of CPE at this site is unknown. ESBL-PE were detected in 62% of samples taken, with $bla_{\text{CTX-M Group-1}}$ as the predominant CTX-M variant identified. The findings of this study show that the role that the aquatic environment plays in the spread of antimicrobial resistance warrants further investigation.

A179

Tracking plasmid-mediated antibiotic resistance from environmental reservoirs to the food chain.

Sinéad Murphy[1], Aoife Joyce[1], Vera Vollenweider[2], David Drissner[2], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Co. Kildare, Ireland. [2]Agroscope, Zurich, Switzerland

Abstract

It has been well documented that antibiotic resistance (AR) is a clinical concern that affects both human and animal health but AR in the environment and food-chain is not as well understood. AR bacteria can occur naturally in soil, water and organic fertilizers used in agriculture so there is a risk that AR can pass to humans via the food-chain. This study focuses on lettuce cultivation undergoing four treatments (Normal irrigation water + normal soil, normal irrigation water + manure, UV irrigation water + normal soil,UV irrigation water + manure)to determine the mechanisms by which the AR is transferred to the plants over the growth period of the lettuce(7 time-points - week 0 to week 6).Plasmids (n=318) have been isolated from irrigation water (n=36), soil (n=45) and lettuce (n=42) samples using the exogenous isolation method for week 0 and week 6 initially. Antibiotic susceptibility testingto amikacin, cefotaxime, ciprofloxacin, imipenem, kanamycin, tetracycline has been carried out. Multi-drug resistance profiles were established for soil taken at timepoint 0 and lettuce taken at timepoint 6. Extracted plasmid DNA was sent for metagenomic analysis to determine which genes are involved in the transfer of AR at the interfaces.The results of the sequencing showed that there are multiple AR genespresent,including Tet, Sme, Cmy, Oxa and ANT(4')-Ib, that confer resistance to bacteria. The identification of multi-drug resistance in soil and lettuce samples is concerning and highlights the need to determine the mechanisms leading to antibiotic resistance in food.

A180

Assessing the Impact of chemically engineered surface modifications with respect to attachment, survival and the development of microbes at the cellular level.

Ross Mulhall, Sean Goodman, Rasmita Raval, Thomas Hasell, Jessica Smith, Heather Allison

University of liverpool, Liverpool, United Kingdom

Abstract

Recently scientific and industrial interest has grown in relation to antimicrobial surfaces. This interest is mainly due to the persistent and prevalent microbial contamination of industrial and medical surfaces. A critical issue is the dissemination of bacterial colonies across biotic and abiotic surfaces, from surface contact-contact interaction. This ability to colonise materials presents a major problem for cross-contamination and pathogenic bacterial proliferation, resulting in wide-spread distribution and mutation, presenting increased risk to human health. There are two approaches to prevent bacterial spread – disinfection and antimicrobial surfaces. The use of disinfectants presents pollution to the surrounding environment, and the increased development of resistant microbial strains. The beneficial design of antimicrobial polymers enables contact-killing, without the release of biocides into the environment. This project aimed at synthesising sulfur polymeric materials through inverse vulcanisation, with the aim of producing functional antimicrobial materials. As of yet, there is no publication evaluating the antimicrobial effect of diisopropenyl benzene (DIB) and dicyclopentadiene (DCPD) co-polymers as bulk solids, even though elemental sulfur is known to exhibit antimicrobial effects. There is growing demand for materials with antimicrobial capabilities, especially in medical environments, where the epidemiology of hospital acquired infections is of great research interest. The aim of this study was to evaluate the antimicrobial properties of both S-DIB and S.DCPD using *Escherichia coli* (DSM 1576) and *Staphylococcus aureus* (DSM 346) against an internationally recognised standard (ISO 22196). To gain further in-depth analysis, confocal microscopy was employed to access the surface impact on bacterial cells.

A181

Antibiotic resistance transfer in *Enterococcus faecalis* via pheromone-induced conjugation.

Mary McCarron, James Dooley, Ibrahim Banat, Joerg Arnscheidt, William Snelling

Ulster University, Coleraine, United Kingdom

Abstract

It is acknowledged that the environment facilitates antibiotic resistance development and spread but not much research has been undertaken to understand the mechanisms involved. This study focuses on antibiotic resistance transfer by enterococci, normal inhabitants of the mammalian gut and important healthcare associated pathogens, under a variety of environmental conditions. These are novel environmental strains, isolated from a farm in Monaghan, Ireland. The primary aim of this project is to quantify factors controlling the horizontal gene transfer (HGT) of antibiotic resistance genes via pheromone-induced conjugation. The main objectives include; identifying enterococcal isolates capable of transferring antibiotic resistance genes under laboratory conditions; using whole genome sequencing (WGS) to characterise genetic events involved in conjugal transfer; developing markers of transfer efficiency; and developing environmentally relevant models to measure enterococcal HGT. The transfer of vancomycin resistance genes from a donor to a recipient cell have been demonstrated and transfer efficiencies range from 1.09×10^{-1} to 9.74×10^{-5}. This data shows that some donors are better at donating vancomycin resistance genes than other donors and some recipients are better than other recipients at receiving them. Preliminary data also shows the transfer of trimethoprim, tetracycline and erythromycin and we can see that within a localised environment *Enterococcus faecalis* can spread resistance genes very differently. In conclusion this project hopes to further our knowledge of the mechanism of pheromone-induced conjugation in environmental Enterococci and to show that environmental enterococcal strains can become multi-drug resistant just as easily as clinical isolates.

A182

Investigating methane mitigation in beef cattle fed with natural additives

Mariana Ornaghi[1,2], Rodolpho do Prado[1], Linda Oyama[2], Sharon Huws[2], Ivanor do Prado[1]

[1]State University of Maringá, Maringá, Brazil. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

Antibiotics are extensively fed to beef cattle as they act as rumen modulators, improving animal efficiency and decreasing methane emissions. However, current recommendations by health agencies to limit/ban antibiotic use in animal production call for alternatives such as natural additives. In this study, we aimed to evaluate the efficacy of natural additives in methane mitigation. Bulls (½Angus and ½Nellore)16±2.2 months old, with average body weight of 385±20.7 kg were fed a basal diet (70% concentrate, 30% corn silage) offered ad libitum for 62 days in a feedlot and randomised on five treatments (8 bulls/treatment): control treatment, and addition of 1.5, 3.0, 4.5, or 6.0 g/day/animal of a blend of natural additives containing 37.5% each of clove essential oil, the commercial blend containing vanillin, eugenol and thymol, 12.5% and 12.5% of castor and cashew oils). Methane production from rumen fluid was estimated based on the theoretical fermentation balance for observed molar distribution of VFAs in the rumen. DNA extracted from rumen fluid were sequenced and analysed for methane genes within the MG-RAST database. The natural additives linearly reduced methane production (76%, $P<0.02$). Evaluation of *Archaea* abundance showed a reduction (79%, $P<0.05$) in the major methane producing genera: *Halorhabdus, Ferroplasma, Methanoplanus, Picrophilus*. A reduction of *Fibrobacter* and *Lactobacillus* (71%), the greatest producers of acetate releasing hydrogen for methane formation was also observed. Our findings suggest that natural additives such as essential oils may be useful in the mitigation of greenhouse gases such as methane in animal production.

Environmental and applied microbiology forum
Presentations: Monday and Tuesday evening

A183

Application of Remote Real-time Monitoring of Biological Airborne Particles to Characterise Different Hospital Environments

Mehael Fennelly[1,2], David O'Connor[3], Noel Murphy[4], Colman Casey[5], Barry Plant[4], Niamh O'Sullivan[6], Stig Hellebust[7], John Sodeau[7], Mike Prentice[2,8]

[1]Environmental Research Institute University college Cork, Cork, Ireland. [2]Department of Pathology University college Cork, Cork, Ireland. [3]School of Chemical and Pharmaceutical Science Dublin Institute of Technology, Dublin, Ireland. [4]Adult Cystic Fibrosis Centre Cork University Hospital, Cork, Ireland. [5]College of Medicine and Health University college Cork, Cork, Ireland. [6]Our Ladys Children Hospital Dublin, Dublin, Ireland. [7]School of Chemistry University college Cork, Cork, Ireland. [8]APC Microbiome Institute University College Cork, Cork, Ireland

Abstract

Conventional microbial sampling of air in hospitals is usually carried out using settle plates or impaction air samples. This provides little information about intermittent contamination events and is unhelpful for source attribution. Direct continuous bioaerosol sampling is an established technology used to characterise ambient external air. Portable instruments such as the Wideband Integrated Bioaerosol Sensor (WIBS) combine laser particle size and shape detection with signals of biological origin (fluorescence from amino acids and NAD(P)H) characteristic of viable bioaerosols. Monitoring is continuous for weeks at a time and data collected remotely over the internet. We present evidence of the utility of WIBS analysis in characterising air in hospitals in three different environments: operating theatres (plenum ventilated and ultraclean), a respiratory ward, and a specialist cystic fibrosis outpatients. The airborne particle profile was quantitatively and qualitatively different in each environment. Plumes of biologically-relevant airborne particles were detected and source investigation of failing conventional counts in an operating theatre aided by the continuous record. Nebulised drugs contributed a detectable effect on airborne particles which lasted for several hours on the ward despite air changes. A significant effect of plasma air treatment on airborne particles in the ward was detected by WIBS and not conventional cultures. Continuous monitoring may in future allow objective standard setting for airborne particles in different hospital environments and facilitate rapid detection of airborne infection risks.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A184

An audit of the post-exposure management of rabies in Addenbrooke's Hospital, Cambridge.

Anna Smielewska[1], Reem Santos[2], Kyriaki Ranellou[1,3], Hongyi Zhang[1], Hamid Jalal[1]

[1]Cambridge University Hospitals NHS Foundation Trust Laboratory, Public Health England, Cambridge, United Kingdom. [2]Cambridge University Hospitals, Cambridge, United Kingdom. [3]Department of Pathology, University of Cambridge Addenbrooke's Hospital Cambridge, Cambridge, United Kingdom

Abstract

Rabies is a clinically significant disease, caused by a neurotropic virus that is transmitted in the saliva of infected animals. The incubation period is exceptionally variable, followed by viral encephalitis, and almost always death. Overall rabies infection kills approximately 50,000 to 70,000 people/year. Control of the disease can be achieved by eliminating the animal reservoir or effective pre-exposure and post-exposure prophylaxis (PEP). Effective human rabies vaccines exist for pre-exposure immunization. These are normally recommended for people in high-risk occupations and travellers to rabies-affected areas.

Although rabies is no longer endemic in the UK, it remains a significant problem in returning travellers. There are approximately 2000 PEP courses issued/year, 85-90% for returning travellers and approximately 10-15% for bat exposure in the UK.

In this audit, we have evaluated the quality of the rabies PEP management in Addenbrooke's Hospital, Cambridge over 3 years. The objectives of the audit were the correct risk assessment of the patient, timely prescription of PEP, as well as effective communication between clinical teams and record keeping. The standards were drawn from previously published literature.

Although the standards relating to patient safety were met and exceeded, some drawbacks in communication and record keeping, as well as some non-auditable issues were identified. Additionally, a review of annual case numbers and peak periods of PEP referrals was used to identify key staff training periods. A shared experience with other issuing centres across the country could be used to optimise the approach to this highly clinically significant issue.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A185

A retrospective regional audit of Cytomegalovirus (CMV) laboratory diagnostics in Crohn's/Colitis patients in Northern Ireland - *'towards a diagnostic algorithm'*

Susan Feeney[1], Graham Turner[2], Seamus Murphy[3], Tony Tham[4], George Jacob[5], Graham Morrison[6], Peter Watson[2], Paul Kelly[2], Lynsey Patterson[7], Peter V Coyle[1]

[1]Regional Virus Laboratory, Royal Victoria Hospital, BHSCT, Belfast, United Kingdom. [2]Royal Victoria Hospital, BHSCT, Belfast, United Kingdom. [3]Southern Health Socal Care Trust (SHSCT), Newry, United Kingdom. [4]South Eastern Health and Social Care Trust (SEHSCT), Belfast, United Kingdom. [5]Northern Health and Social Care Trust (NHSCT), Antrim, United Kingdom. [6]Western Health and Social Care Trust (WHSCT), Derry, United Kingdom. [7]Public Health Agency, Belfast, United Kingdom

Abstract

Introduction: Concurrent cytomegalovirus (CMV) in inflammatory bowel disease (IBD) related colitis, (Ulcerative Colitis (UC) or Crohn's Disease (CD) is an important yet complex scenario associated with high rates of colectomy and other morbidity. This regional audit aimed to identify baseline standards for a virological diagnostic-based approach for the identification of those at risk of CMV reactivation (flare) in the setting of IBD.

Methods: Retrospective, cross-sectional study over a three year time-period, January 2010 - March 2013, involving all five Northern Ireland HSC Trusts. Sample cohort n=277 IBD patients of which n=106 further grouped as SRC 'severe acute colitis and/or steroid refractory colitis'. Seven audit standards were assessed including specimen(s) submission for CMV diagnostic analysis, antiviral therapy, ascertainment of colectomy rate, and result communication protocol.

Findings: Audit primarily found a need to better define test requesting protocols (optimal sample required for optimal serology and molecular diagnostics), and a need to work towards timeliness in both test requesting and result reporting. This audit also identified the high risk patient group (SRC) when test positive for CMV (PCR and/or histopathology) three times more likely to undergo colectomy (OR 3.16, 95% CI (0.74, 13.21; P=0.06).

Conclusions: Diagnostic-based patient identification is optimal to identify those most at risk of CMV reactivation in the IBD setting. The serological IgG profile is key in risk assessment of potential reactivation, together with quantitative CMV PCR in colonic tissue, with/without supportive histopathology, as the most sensitive and timely means of identify those high risk patients.

A186

Development of a single domain antibody-based ELISA for poliovirus

Keith Grehan[1], Lee Sherry[1], Helen Fox[2], Andrew Macadam[2], Nicola J Stonehouse[1], David J Rowlands[1]

[1]University of Leeds, Leeds, United Kingdom. [2]National Institute for Biological Standards and Control, Hertfordshire, United Kingdom

Abstract

Poliomyelitis is a devastating disease caused by infection with poliovirus (PV). The development of two vaccines and the global vaccination programs have reduced the prevalence of the virus such that eradication is a viable goal in the near future. Recent work is aimed at developing a VLP-based vaccine that would remove the need to culture wild type PV for production. However, post-eradication there will be a need for cost effective tools to ensure that ongoing surveillance is effective and sustainable. VHHs are single-domain antigen-binding fragments that are derived from the heavy chain antibodies produced by camelids. These antibodies are encoded by a single gene and so can be readily expressed in bacteria and easily modified to aid purification or modulate functioning.

VHHs are highly specific and stable under a variety of conditions that denature conventional antibodies. VHHs may, therefore, offer a novel set of tools that can be incorporated into a variety of assays. Here we describe incorporation of VHHs into a non-competitive sandwich ELISA. Currently, detection and vaccine evaluation employs serotype-specific antibodies which immobilise the polio antigen. The captured antigen is then detected by a monoclonal antibody that is specific to the native antigen. Our results indicate that the replacement these antibodies with a VHH can yield similar results and provide a consistent and reproducible assay that is no longer dependent on antibodies. Furthermore, as VHHs can be produced in bacteria this offers the potential for a post-eradication assay for PV that is sensitive and cost effective.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A187

Use of hyperattenuated poliovirus as a replacement for Sabin or wild type strains for laboratory assays in a post eradication world

Helen Fox, Sarah Carlyle, Philip Minor, Andrew Macadam

NIBSC, Potters Bar, United Kingdom

Abstract

Poliovirus serotype 2 (PV2) has been declared eradicated and was removed from the live vaccine programme in 2016. WHO have developed the Global Action Plan (GAPIII) to describe containment conditions for laboratory work with poliovirus after eradication, and in the UK, all PV2 has been reclassified to hazard group 3 (HG3). Laboratory work involving live poliovirus will continue to be required years after eradication for the purposes of: vaccine testing; environmental surveillance; immunoglobulin testing; and for the development of new public health interventions. Compliance with GAPIII and HG3 containment conditions would impose financial burden and failure would lead to disruption to essential activities. Development of safer poliovirus strains for use outside of containment could overcome these issues.

The S19 poliovirus strain, designed to be hyperattenuated and extremely genetically stable, was used as a cassette for the introduction of capsid proteins of other strains and serotypes (Knowlson, *et al*. 2015), originally for vaccine production. S19 viruses are unable to infect transgenic mice (by intraspinal inoculation) or non-human primates (by mouth) at very high doses and are unlikely to infect humans at biological temperature. PV2 S19 strains have recently been approved for use outside of GAPIII containment requirements by the Containment Advisory Group.

Validation of strains for laboratory assays and a highly sensitive QC assay based on NGS will be described.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A188

Macrolide-resistant *Mycoplasma genitalium* in Male and Female Patients Attending a Northern Irish Genitourinary Medicine Clinic

Ciara Cox[1,2], James McKenna[2], Conall McCaughey[2], Alison Watt[2]

[1]Queens University Belfast, Belfast, United Kingdom. [2]Regional Virus Laboratory, RVH, Belfast, United Kingdom

Abstract

Background: *Mycoplasma genitalium* (MG) can cause urethritis in men and pelvic inflammatory disease in women. Despite the increasing rate of MG resistance to first-line macrolide treatment due to 23S rRNA gene mutations, the availability of testing remains limited in UK laboratories. This study obtained preliminary data on the rate of macrolide resistant strains circulating in N. Ireland.

Methods: Clinical specimens from 1052 patients attending a Genitourinary Medicine (GUM) clinic in Belfast from April 2017 to April 2018 were screened using an in-house MG PCR assay. MG positive samples were subsequently tested using *ResistancePlus*™ MG assay (Speedx, Australia) to detect 23s rRNA gene mutations associated with macrolide resistance.

Results: Amongst all study samples, 3.9% (n=41) tested positive for MG. Out of these 41.5% (n=17) had a 23S rRNA mutation. The rate of MG was highest in rectal swabs (8.13%) followed by vaginal swabs (4.96%) and then male urines (2.85%). The rate of 23s rRNA mutations associated with macrolide resistance in positive vaginal swabs was 21.4% whereas the rate amongst urine and rectal swabs was at least 50%.

Conclusion: Data from this study adds to the evidence base to perform MG testing in risk groups in N. Ireland to improve patient outcomes and reduce antimicrobial resistance.

A189

Retrospective point-prevalence study of Enterovirus D68 (EV-D68) detection in the symptomatic paediatric population presenting to the Children's Hospital Emergency Department, RBHSC, Northern Ireland.

Annamarie Balgos, Ciara Cox, James McKenna, Susan Feeney

Royal Victoria Hospital BHSCT, Belfast, United Kingdom

Abstract

Enterovirus D68 (EV-D68) detection has recently been associated with severe neurological symptoms in adults and children in European countries. The spectrum of disease by EV-D68 ranges from asymptomatic to acute respiratory symptoms, hospitalisation, and sporadically to neurological symptoms, including acute flaccid paralysis (AFP) and acute flaccid myelitis (AFM), and death.This has led to increased vigilance for detection of enterovirus D68, especially in cases that present with the more severe clinical syndromes. In normal cases, enterovirus is transmitted by faecal-oral routes and/or respiratory routes, however in the case of EV-D68 it is almost exclusively passed through respiratory transmission. An infection of EV-D68 is generally distinguished by its rapid onset of disease. The incubation period of the disease between 3 to 5 days, which is unlike the other enteroviruses which usually have an incubation period of around 10 days. This study describes a retrospective analysis of 150 non-invasive respiratory specimens (nose/throat or throat swabs) collected from paediatric outpatients presenting to Childrens Hospital Emergency Department with respiratory and/or CNS symptoms including fever and seizure. Specimens have been collected from November 2017- November 2018. All specimens tested by four RT-PCR assays for Pan-Enterovirus, EV-D68 specific, human Rhinovirus and RNaseP (quality control). Results on prevalence of EV-D68, as single or co-infection, are presented. Results indicate low prevalence of EV-D68 in the Northern Ireland symptomatic paediatric population. As EV-D68 is an emerging infection it is critical to remain vigilant particularly in the case of neurological presentation.

A190

Lyme Disease in Northern Ireland- a look back on serological testing

Louise McCorry, Conall McCaughey

Belfast Health and Social care trust, Belfast, United Kingdom

Abstract

Background:

Lyme disease is an illness caused by the spirochete Borrrelia burgdoferi. NICE guidance suggests an approach to diagnosis resting on clinical presentation, presence in an epidemiologically plausible area, and positive serology. It is however, well documented that serological diagnostics in European Lyme disease is problematic.

There is a movement for Lyme advocacy, a voice that emphasises the poor diagnostics in this infection. With the spotlight on Lyme disease, we are increasingly seeing patients presenting in Northern Ireland across a broad range of specialities. Currently there is minimal data looking at the local epidemiology of Lyme disease.

We present previously unpublished data looking at the demographics of those testing positive for Borrelia burgdoferi antibodies.

Methods:

We collected data from all patients tested for Lyme disease in Northern Ireland from 2013- 2018. We excluded negative results. A randomised selection of 300 cases were chosen. We recorded patient age, gender, origins of testing, home postcode, tick bite history, relevant travel history, duration of symptoms, timings of testing, antibody results including confirmatory testing and treatment.

Results:

Of the 300 selected patients 241 (80%) were IgM positive and 83 (30%) IgG positive using DiaSorin, Liason CLIA. The average age of presentation was 39 years old. There was a predominance of females tested (62%). 32% of tests originated in primary care. Of the IgM positives only 41 (17%) were confirmed by C6 peptide or lineblot in the reference laboratory (RIPL, Porton Down).

The results highlight the likely low seroprevalence of Lyme disease in Northern Ireland.

A191

Validation of a hepatitis B virus (HBV) Next Generation Sequencing (NGS) pipeline at Barts Health NHS Trust

Jon Hubb, Mark Hopkins, Sophie May, Anna Jeffery-Smith

Barts Health NHS Trust, London, United Kingdom

Abstract

At Barts Health NHS Trust, historically HBV genotyping and resistance testing is performed using nested PCR followed by Sanger sequencing. With the development of NGS assays for HIV and HCV, in order to harmonise workflows an NGS HBV pipeline was developed.

Primers were designed for the whole genome amplification of HBV. Plasma samples were extracted for nucleic acid using a Qiagen Qiasymphony. Whole genomes of HBV were amplified in a single round PCR and quantified using a Qubit fluorometer. Libraries were prepared using the Nextera XT library preparation kit and sequencing was performed on an Illumina Miseq. Sequencing reads were assembled into consensus sequences using a Linex-based pipeline. Resistances and genotypes were determined using the HBV Grade website. Minority variants were variants <20% of the overall population.

Pan-genotypic PCR primers were designed in the nick region of the partially double stranded HBV genome. In-silico analysis allowed primers to be designed to bind multiple genotypes. Sensitivity analysis of primers was investigated for the most common circulating genotypes. A panel of HBV positive and external quality assurance samples showed good comparability to Sanger sequencing results. Inter- and intra-assay variability showed the assay was robust and fit for purpose. No resistance associated minority variants were observed.

The development an NGS pipeline for analysis of HBV allowed the harmonisation of diagnostic pathways within the virology sequencing laboratory. Introduction of this test would allow data to be gathered to assess the importance of minority variants in HBV infection.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A192

Identification of prognostic protein biomarkers for Dengue disease severity through an integrated 'omics analysis of patient serum

Philip Lewis[1], Andrew Davidson[1], Brian Schwem[2], Elihu Aranday-Cortes[3], Majo Galarion[2], Colleen Pangilnan[2], Carol Leitch[3], Ana Filipe[3], David Lee[1], Viravarn Luvira[1], David Matthews[1], Andrew Dowsey[1], John McLauchlan[3], Raul Destura[3]

[1]University of Bristol, Bristol, United Kingdom. [2]University of the Philippines Manila, Manila, Philippines. [3]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Dengue is the most important arthropod-borne viral disease of humans and endemic in the Philippines. Dengue infection produces a wide spectrum of clinical presentations, but the factors contributing to differential disease severity are not entirely understood. Although most cases of dengue can be managed at home, the lack of a diagnostic test to predict severe disease with hemorrhagic and plasma leakage complications, forces patients and healthcare providers to seek hospital admissions for "safety purposes", saturating an already overwhelmed healthcare system.

Our overall objective, by adopting an integrated 'omics approach, is to correlate disease severity with serum protein biomarkers, viral sequence variations, and changes in the host transcriptome, using serum samples from dengue patients in the Philippines with well-defined clinical outcomes.

We have analysed 170 serum samples across two clinical cohorts using 10-plex, and 11-plex Tandem Mass Tagging (TMT)/LC-MS/MS, from patients with; dengue without warning signs, dengue with warning signs, severe dengue, pyrexia of unknown origin and healthy controls. The proteomic analysis has identified and quantified >1400 serum common proteins across both cohorts. In addition to giving us greater insight into serum proteome changes between healthy and dengue-virus infected patients, we have also identified changes between differing severities of dengue fever. These proteomic changes have further been confirmed by Western blotting, and represent potential biomarkers that can be investigated further for their use in clinical diagnosis to identify the patients most at-risk of developing severe dengue disease.

Virology workshop: Clinical virology
Presentations: Monday and Tuesday evening

A193

Investigating the impact of influenza point-of-care testing - what are the benefits?

Emily Goldstein[1], William Harvey [2], Matthew Maycock[3], Emilie Bellhouse[3], Rory Gunson[1], Brian Choo-Kang[3]

[1]West of Scotland Specialist Virology Centre, Glasgow, United Kingdom. [2]Institute of Biodiversity, Animal Health and Comparative Medicine , Glasgow, United Kingdom. [3]Glasgow Royal Infirmary , Glasgow, United Kingdom

Abstract

Point-of-care testing (POCT) for influenza viruses is being used increasingly in NHS hospitals. In the 2017/18 influenza season, NHS Greater Glasgow and Clyde implemented the Cepheid GeneXpert System for the detection of influenza A, B and respiratory syncytial virus in 7 wards across 5 hospital sites.

To evaluate the impact of influenza POCT in Glasgow Royal Infirmary during the 2017/18 influenza season, we retrospectively compared data from 150 influenza A infected patients. We analysed data from 100 patients who were diagnosed using a laboratory based in-house respiratory PCR assay (50 patients from 2016/17 and 50 patients from the 2017/18 season) and 50 patients who were diagnosed using POCT in the 2017/18 season.

The aim of the study was to investigate whether POCT impacted patient management and outcome. General linear models were used to test for an association between POCT and a number of outcomes, whilst accounting for host factors. These outcomes included: 1) time from review to influenza result 2) admission status 3) length of hospital stay 4) patient outcome 5) duration of antibiotics 6) antiviral treatment 7) patient isolation and 8) the number of duplicate influenza tests performed.

In addition to investigating the impact of POCT, we used this opportunity to explore additional factors that may affect the outcomes of patients infected with influenza A, including: age, sex, imaging results and underlying risk factors.

A194

Studies on Amylase from Protoplast Fusants of Aspergillus species Using Response Surface Methodology.

Tolulope Adeleye[1], Sarafadeen Kareem[2], Olawale Dairo[2], Olusegun Atanda[3]

[1]Federal University of Agriculture Abeokuta, Abeokuta, Bermuda. [2]Federal University of Agriculture Abeokuta, Abeokuta, Nigeria. [3]McPherson University, Abeokuta, Nigeria

Abstract

Improved amylases were developed from protoplast fusants of two amylase-producing *Apergillus* species. Twenty regenerated fusants were screened for amylase production on Remazol Brilliant Blue agar. Crude enzymes were produced by solid state fermentation on rice bran were assayed for activity. Three variable factors (temperature, pH and enzyme type) were optimized for amylase activities of parent and selected fusants on a rice bran medium by solid state fermentation. The variables assessed were optimized using the Central Composite Design (CCD) of the Response Surface Methodology (RSM). Amylase activities at room temperature and at 80ºC showed *Aspergillus* designates, T5 (920.21 U/ml, 966.67 U/ml), T13 (430 U/ml, 1011.11 U/ml) and T14 (500.63 U/ml, 1012.00 U/ml) as preferred fusants. Amylases produced by the fusants were observed to be active over the range of pH studied. Fusants T5 and T14 had an optimum acidic and alkaine pH respectively. Optimization studies revealed enzyme T5 at pH 4 and temperature of 40ºC as optimum for amylase production. The statistical tools employed, predicted and compared the optimal conditions for enzyme activities of amylases from parent and fusant strains of *Aspergillus* revealing the desirability of the fusants over the parents in industrial applications.

A195

Sero-epidemiology of scrub typhus among suspected cases in selected areas of Nepal

Rajendra Gautam, Keshab Parajuli, Jeevan Bahadur Sherchand

Institute of Medicine, Kathmandu, Nepal

Abstract

Introduction:
Scrub typhus is an acute, febrile, infectious disease which is caused by *Orientia tsutsugamushi*. In the current days of Nepal huge burden of scrub typhus have been found, mainly Southern Nepal.

Methodology:
Blood samples were collected from the suspected patients of scrub typhus who is having acute febrile illness. Detection of Immunoglobulin M (IgM) antibody to *Orientia tsusugamushi* was performed by using Scrub Typhus Detect™ kit, In Bios International USA.

Results: A total 1585 cases, 358 (22.58%) were positive for IgM Antibodies to *Orientia tsutsugamushi*. Multivariate analysis demonstrated that the following factors were significantly associated with the scrub typhus. 1. Females (odd ratio [OR] =2.037, P=<0.001, confidence interval [CI] =1.465-2.831) 2. Rural residential location (odd ratio [OR] =0.431, P=0.001, confidence interval [CI] =0.261-0.714), 3. House near grassland (odd ratio [OR]=3.279, P=<0.001,confidence interval [CI]=1.932-5.563), 4. Presence of mouse inside the house (odd ratio [OR] =5.462, P=<0.001, confidence interval [CI] =4.048-7.371), 5. Working in the field (odd ratio [OR] =9.845, P=0.004, confidence interval [CI] =2.068-46.954).

Conclusion:
The study indicated that Scub typhus is a big burden of Nepal, where we have identified the prevalence rate was 23%. Use of IgM ELISA test will help for early diagnosis and it is urgent to investigate to save the life of people who lives endemic areas of Scrub typhus in Nepal.

A196

Alterations in the Gut Microflora Balance of Neonatal Wister Rats Induced With Aflatoxin M1 in Milk

Amina Badmos, Flora Oluwafemi

federal university of Agriculture, Abeokuta, Nigeria

Abstract

Aflatoxin M1 is a metabolite of the most potent aflatoxin, (AFB1) and thus, has been treated and rendered not so toxic, on this basis, its study has been taken for granted. It is the aim of this study to ascertain the toxic nature of AFM1 by determining its effects on microbial flora in the gut of neonatal rats. A dosing experiment was conducted on the neonates, where they were divided into groups and treated with different concentrations of AFM1 using uncontaminated milk as a carrier medium into the rats. The rats were sacrificed; the small and large intestine were harvested and cultured on appropriate selective media for growth of microorganisms. Results show samples from the control group had an uninterrupted microbial community, while the treated group, with increasing doses of AFM1 decreases and depletes the microflora in the gut samples. Lactic acid bacteria were also significantly depleted by AFM1. These findings suggest the capability AFM1 in modifying the gut microbiota in a dose-dependent manner which might result in serious health hazards in neonates.

A197

Regulation of *cabA*, a Calcium-Binding Protein Gene, Essential for Biofilm Development in *Vibrio vulnificus*

Seung-Ho Hwang[1], Jin Hwan Park[2], Sang Ho Choi[1]

[1]Seoul National University, Seoul, Korea, Republic of. [2]University of California, Santa Cruz, Santa Cruz, USA

Abstract

Biofilm is a structured community that protects bacteria from various stresses such as nutrient limitation, temperature change, antimicrobial agents, and host immune defenses. A pathogenic marine bacterium *Vibrio vulnificus* forms biofilms to colonize and persist in environmental niches. *V. vulnificus* CabA is an extracellular matrix protein crucial for the structural integrity of robust biofilms. BrpT and BrpR are transcriptional regulators involved in biofilm development in *V. vulnificus*. To examine the roles of the two regulatory proteins in *cabA* regulation, the isogenic mutants deficient in BrpT, BrpR, or both BrpT and BrpR were constructed from a parent strain JN111. Transcript analysis revealed that both BrpT and BrpR activate *cabA*, and BrpR activates *brpT* at the transcriptional level. While BrpT activates *cabA* in the absence of BrpR, BrpR activates *cabA* only in the presence of BrpT, suggesting that BrpT and BrpR function sequentially in a regulatory cascade. Electrophoretic mobility shift assay and DNase I protection assay, together with the deletion analysis of the *cabA* promoter P_{cabA}, indicated that BrpT activates *cabA* by direct binding to the specific sequences of P_{cabA}. Crystal violet staining revealed that the level of biofilm formation was reduced in the isogenic mutants relative to that in JN111 under elevated c-di-GMP condition. Microscopy analyses of the colony morphology and biofilm structure produced by JN111 and its isogenic mutants showed that only JN111 developed rugose colony morphology and robust biofilms. The combined results indicated that BrpT and BrpR positively regulate the *cabA* expression in a regulatory cascade, leading to the development of well-structured biofilms.

A198

Making antimicrobial susceptibility visible with flow cytometry

Tim Inglis[1,2], Teagan Paton[2], Kieran Mulroney[1], Christine Carson[1]

[1]University of Western Australia, Perth, Australia. [2]PathWest Laboratory Medicine, Perth, Australia

Abstract

Background. Antimicrobial therapy exploits critical vulnerabilities in microbial physiology. Conventional methods of antimicrobial susceptibility determination rely on microbial inhibition or death to obtain a measurable endpoint. Proliferation of antimicrobial resistance mechanisms as an evolutionary response to extensive exposure delays antimicrobial susceptibility determination in clinical laboratories. Bacterial cell population analysis provides insight into early physiological response to antimicrobial exposure and could theoretically enable susceptibility testing at greater speed and accuracy.

Method. Representative Gram negative and Gram positive bacteria were exposed to a range of antimicrobial concentrations spanning the EUCAST breakpoint and analysed by flow cytometer after 3-4hr incubation with antimicrobials and 5min incubation with SYTO9 dye. Flow cytometer output files were analysed using a machine learning pipeline to determine minimum inhibitory concentrations (MICs). Broth microdilution MICs were conducted in parallel.

Results. MICs were obtained by flow cytometry for the majority of antimicrobials tested against *Klebsiella pneumoniae* and *Staphylococcus aureus*, based on visualisation of an antimicrobial concentration dependent effect. Exceptions were those combinations that placed a predicted effective concentration outside the tested range (i.e. very sensitive or very resistant). The broth microdilution MIC differed from the flow cytometry method for Ceftriaxone.

Conclusion. Machine learning methods enabled classification and visualisation of complex multidimensional flow cytometry data from antimicrobial-exposed bacterial populations, determination of a visible concentration dependent effect, and in most combinations, a predicted effective concentration. Discrepant results may reflect physiological differences between methods. MICs were determined with unprecedented speed by visualising measurable effects on bacterial physiology that occur before cell death.

A199

The role of acetyl-phosphate in the pathogenesis of *Neisseria gonorrhoeae*

Ernesto Felix Diaz Parga, Jonathan G. Shaw

University of Sheffield, Sheffield, United Kingdom

Abstract

Acetyl-phosphate (AcP), an intermediate from the phosphotransacetylase-acetate kinase (PTA-AK) pathway, has shown to be critical in pathogenic bacteria for the general metabolism and synthesis of virulence factors. Lysine acetylation is a post-translational modification (PTM) that occurs enzymatically and non-enzymatically by the addition of an acetyl residue from acetyl coenzyme A and AcP, respectively. *Neisseria gonorrhoeae*, the etiologic agent of gonorrhoea, has been shown to use AcP for lysine acetylation, however, the role that AcP has in the pathogenesis and how acetylation is regulated has not been discerned.

The concentration of AcP was altered in *N. gonorrhoeae* MS11 by interrupting the genes involved in the PTA-AK pathway, *pta* and *ackA*, and the gene that encodes for a lysine deacetylase family protein, *kdac*. AcP concentrations were increased in Δ*ackA* and decreased in Δ*pta* resulting in modulation of lysine acetylation. Growth on glucose, lactate or pyruvate were investigated. In aerobic conditions, Δ*ackA* mutant solely grew in glucose, while the Δ*pta* mutant grew in glucose and lactate. In microaerophilic conditions, Δ*ackA* and Δ*pta* mutants solely grew in presence of glucose. The virulence of Δ*ackA* and Δ*pta* was tested by infecting larvae of *Galleria mellonella.* WT killed 50% population (n=15) after 6 days and Δ*ackA* after 24 h, however, Δ*pta* after 6 days it only killed 10%.

Taken together, our results show AcP as an important metabolite for the metabolism and virulence of *N. gonorrhoeae*.

A200

Cooperating under stress - overlapping roles for *Salmonella* chaperones

Claire Hews, Gary Rowley

University of East Anglia, Norwich, United Kingdom

Abstract

The ability of the Gram-negative pathogen *Salmonella* to withstand stress is crucial to its survival and ability to cause infection. During its lifecycle, *Salmonella* encounters many environments and associated stressors including changes in pH and heat, antimicrobial compounds and reactive oxygen species. Envelope stress response (ESR) pathways sense and respond to perturbations in outer membrane and periplasmic proteostasis induced by such conditions. Many of the genes regulated by these pathways encode chaperones, important for refolding or degrading damaged proteins.

We aim to understand the contribution of the stress-induced small heat shock proteins (sHsps) IbpA, IbpB, AgsA, and the *Salmonella* specific putative chaperone STM1250, to the ESR. We predict that functional overlap exists between these four proteins and that their roles are not restricted to surviving heat shock. To investigate this we have subjected single and combined deletion mutants to conditions known to disrupt the outer membrane. We have shown that, unlike the single mutations, the quadruple mutant is attenuated in macrophages, more sensitive to H_2O_2 and the cationic antimicrobial peptide polymyxin B, and has reduced survival in serum.

Our findings indicate a new role for sHsps and STM1250 in maintenance of outer membrane integrity and tolerance to extracytoplasmic stress. We will now perform functional activity assays on purified STM1250 to decipher the mode of action of this uncharacterised protein. Improved understanding of the role of chaperones during infection and survival of environmental stress, as well as their overlapping functions, will identify whether these proteins are novel therapeutic targets.

A201

Biofertilizer / biopesticide potentiality of zinc solubilizing Pseudomonas aeruginosa FA-9 and Enterobacter sp. FA-11 isolated from the wheat rhizosphere grown in arid zone

Muhammad Abaid Ullah, Fauzia Yusuf

COMSATS University, Islamabad, Pakistan

Abstract

Bacterial strains were obtained from wheat rhizosphere and screened for zinc solubilization on agar plates. Two strains FA-9 and FA-11 were found efficient for Zn solubilizing activity and were identified as *Pseudomonas aeruginosa* and *Enterobacter* sp. by 16S rRNA and gyrase (*gyrB*) genes analysis, respectively. The strain FA-9 produced a clear zone diameter of 63 mm, 60 mm and 51 mm on agar plate amended with different zinc ores. The strain FA-11 produced a zone diameter of 17 mm with zinc carbonate and 20 mm with zinc oxide while no zone was observed with zinc phosphate. Both strains showed no visible activity with ZnS ore. Similarly, FA-9 and FA-11 increased maximum soluble zinc content (102 µg mL^{-1} & 45 µg mL^{-1}) from zinc carbonate ore as compared to zinc oxide ore (102 µg mL^{-1} & 45 µg mL^{-1}) in liquid broth. It was noted that both strains exhibited less potential (7µg mL^{-1} & 0.57 µg mL^{-1}) to solubilize ZnS ore in liquid broth. A comparison between agar plate assay and liquid broth quantification shows that agar plate assay does not present the solubilizing potential of ZSB precisely. The strains FA-9 and FA-11 produced auxin with L-tryptophan (3.25 µg mL^{-1}& 2.86 µg mL^{-1}) and without L-tryptophan (1.23 µg mL^{-1} & 1.02 µg mL^{-1}). Both strains expressed exo-polysaccharides (EPS) and siderophores activity along with phosphate (P) solubilization, ACC deaminase and antifungal activities. The ACC deaminase and N-fixation activity was confirmed by the amplification of *acdS* and *nifH* genes respectively.

A202

Proteomic Analysis Of Escherichia coli Associated with Urinary Tract Infections

Ghadah Alsharif, Abdulla Alsubhi, Ahmed Mirza

King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Urinary tract infection (UTI) is considered to be one of the most prevalent bacterial infections in the world. It affects urinary tract system including bladder and kidney. Uropathogenic *Escherichia coli* is more prevalent in females due to their anatomical structure as well as they are more susceptible to recurrent infections. Every woman out of three is affected by UTIs. Gram-negative bacteria are a major cause, particularly *Escherichia coli (E. coli). E. coli* was considered a main causative agent for 80-90% of community-acquired infection and for about 40% of nosocomial UTI. Moreover, it is responsible for 25% of recurrent infection. Proteomic can be used to analyze and identify complete components of proteins. It can be used to distinguish between bacteria based on synthesized proteins. In addition, proteomic is applicable to identify possible targets of therapy. My study aims To compare protein profiles of *E. coli* from different UTI patients and identify possible unique protein signature for future biomarker studies. Six Urine samples with *E. coli* were taken from females aged from 15 to 50 years old. Samples loaded onto Sodium dodecyl sulfate Polyacrylamide gel electrophoresis for separation. Samples with abundant proteins profiles on SDS-PAGE, were selected for run on two dimensional gel electrophoresis. Gels were compared to each other to look for interesting protein spots. Many differences were observed in protein profiles of *E. coli* isolates in both 1D SDS-PAGE and 2DGE. Two bacterial proteins identified as possible candidate were (OmpA) found in Gram negative bacteria and RNA polymerase-binding transcription factor DksA mostly found in *E. coli*

A203

Investigation of the FetMP-FetABCDEF (Ftr1-P19) Iron-Uptake System of Campylobacter jejuni

Dhama Alsallami, Simon Andrews

university of Reading, Reading, United Kingdom

Abstract

Gut colonization by *C. jejuni*, a common gastrointestinal pathogen, depends upon its iron-acquisition ability. P19 is part of a *C. jejuni* iron-uptake system encoded by a conserved cluster of eight genes (*ftr-p19-cj1660-5*) that is well expressed under *in vivo* conditions. P19 is a metal-binding protein thought to deliver iron to with Ftr1 (a ferric permease), for high-affinity iron transport. The function of *cj1660-5*is unknown; they encode a second potential transporter and two predicted periplasmic thioredoxins. The aim of this study is to further explore the functions of the P19 system in *C. jejuni*.

The *ftr1-p19* or *cj1660-65*gene sets were used to complement *E. coli*JC32 (lacking iron uptake systems). This resulted in growth enhancement under acidic pH and low iron conditions. Knockout of *ftr1*, *cj1660* or *cj1663* resulted in a significant growth inhibition for *C. jejuni* in Muller-Hinton broth (low iron) at acidic pH, which was reversed with the addition of iron. Complementation reversed the iron-restriction phenotype. Chicken gut (caecum) colonization experiments indicated weak colonization levels at 3 days post-infection (dpi) for all three mutants (7.6 $\log_{10}$cf ~4 $\log_{10}$cfu/g) but at 7 dpi the *cj1660* and *cj1663* mutants had adapted to give colonization levels similar to those of the wildtype, and indeed the recovered isolates grew similarly to the wildtype under iron restriction (genome sequencing is underway). Thus, the P19 system is required for normal colonization of the chicken caecum (pH ~5.7) and the *cj1660-65*genes contribute to growth under iron restriction at low pH.

A204

Cyclic di-nucleotides – what is their role in biofilm formation and pathogenicity of *Fusobacterium nucleatum*?

Maria Muchova[1], Daniel J. Slade[2], Gary A. Cameron[3], Michael R. Milward[1], Paul R. Cooper[1], Sarah A. Kuehne[1]

[1]School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom. [2]Department of Biochemistry, Virginia Tech, Blacksburg, USA. [3]University of Aberdeen, The Rowett Institute, School of Medicine, Medical Sciences and Nutrition, Aberdeen, United Kingdom

Abstract

Introduction

Cyclic di-nucleotides (CDNs) act as important second messengers in bacteria, regulating multiple cellular functions, including biofilm formation. *Fusobacterium nucleatum* is a key player in disease-associated biofilms in periodontitis (gum disease). Previous studies revealed the importance of CDNs in the virulence of other dental pathogens such as *Porphyromonas gingivalis*, but their function in *F. nucleatum* virulence remains elusive.
Here, we aim to elucidate their importance in the pathogenicity of *F. nucleatum*.

Methods

Using bioinformatics, we identified a putative dual adenylyl/guanylyl cyclase in the genome of *F. nucleatum* ATCC 23726, the only currently genetically tractable strain. Consequently this target gene was deleted from the chromosome. Wild-type and mutant strains were grown in single- and multi-species biofilms, the amount of biomass quantified by crystal-violet assay and the biofilm topography analysed using scanning electron microscopy. Additionally, intracellular CDNs were quantified using LC-MS/MS.

Results

Differences in biofilm formation comparing wild-type, mutant and further *F. nucleatum* subspecies will be presented. Furthermore the level of CDN production of those strains will be shown.

Conclusions

Disease-associated biofilms in periodontitis affect over 50% of the adult UK population. The disease can be debilitating, potentially leading to tooth loss and the bacteria involved have also been associated with systemic diseases such as cardiovascular disease, arthritis or certain types of cancer. Understanding the involvement of CDNs in the pathogenicity of *F. nucleatum* and its interaction with various periodontal pathogens might provide new insights into prevention and treatment of periodontitis and other conditions.

A205

Carbon, Nitrogen and Phosphorous assimilation in *V. cholerae* – a case of imperfect nutrient sensing.

Shridhar Paranjape[1,2], Shashidhar R[1,2]

[1]Homi Bhabha National Institute, Mumbai, India. [2]Bhabha Atomic Research Centre, Mumbai, India

Abstract

Vibrio cholerae experiences frequent feast and famine conditions. In the absence of all the three nutrient components (Carbon, Nitrogen, and Phosphorous), the cells could manage starvation and survive up to 6 months with only 1 log reduction in viability. Addition of carbon or nitrogen to the starvation media resulted in 3 log reduction in cell number. Simultaneous addition of carbon and nitrogen (Phosphorous starvation) reduced the cell viability to a below detectable level. The suboptimal growth conditions; non-metabolizable source of carbon and nitrogen; variable C: N ratio; inhibition of metabolism and cell division, increased the cell survival under phosphate starvation. However, when all the three components are limited, the cells did not initiate active metabolism and could conserve the energy for long-term survival. This observation suggests that integration of carbon, nitrogen and phosphorus sensing is imperfect in *V. cholerae* and it cannot down-regulate the metabolism during phosphorous limitation. The carbon and nitrogen could prime the cells to accelerate the rate of metabolism, irrespective of the presence of phosphorous, thereby creating an energetically unfavorable situation. The lack of crucial component phosphorus fails to activate a stringent response that results in increased futile cycling of nutrients, loss of ATP and cell death. The Vibrio genus was found to be less efficient in surviving phosphate starvation than *E. coli* and *S*. Typhimurium. The two-component system CreC (a response regulator of phosphorous) is absent in *V. cholerae* and may be responsible for the lack of stringent response to phosphorus starvation.

A206

Baby feeds bifido: carbohydrate metabolism preferences of *Bifidobacterium* reflect changing baby diet

Magdalena Kujawska[1], Shabhonam Caim[1], Ian O'Neill[1], Anne McCartney[2], Lesley Hoyles[3], Lindsay Hall[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of Reading, Reading, United Kingdom. [3]Nottingham Trent University, Nottingham, United Kingdom

Abstract

Background: The genus *Bifidobacterium* is frequently enriched in the gastro-intestinal tract of breast-fed infants, with abundance proposed to decline in response to dietary change, i.e. weaning. Adaption of *Bifidobacterium* to this early life nutritional environment is through a large repertoire of glycosyl hydrolases (GH), including specific enzymatic clusters that enable digestion of human milk oligosaccharides (HMOs). Notably, genetic organisation and size of gene clusters varies significantly between *Bifidobacterium* species. In this study, we assessed the genetic and metabolic diversity of 76 *Bifidobacterium longum* isolates recovered from healthy term infants over time (from birth to 1.5 years of age).

Methods: In silico analysis included phylogenetic, pan-genome analysis, and genome-wide functional annotation with identification of GH families and HMO gene clusters. Growth assays were used to confirm carbohydrate utilisation capabilities.

Results: *B. longum* strains displayed genomic diversity and varied in their carbohydrate utilisation profiles between and within individual infants. Whilst there were some similarities in functional potential among strains, further analysis revealed subspecies-related differences (i.e. *longum* vs. *infantis*) with respect to GH families and enzymatic clusters encoded over time, which correlated with dietary changes.

Conclusion: The intraspecific genomic diversity of *B. longum* isolates and their carbohydrate utilisation profiles indicate a strong link between host diet and *Bifidobacterium* species/strain, which rapidly responds to a changing nutritional environment i.e. moving from breast milk to a solid food diet. These data provide new insights into bifidobacterial adaption in the infant gut and may allow rational development of new dietary therapies for this important development window.

A207

Replication control by the DarTG DNA modification system of *Mycobacterium tuberculosis*

Rachel Butler[1], Suzie Hingley-Wilson[1], Shan Goh[2], Sharon Kendall[3], Gytis Jankevicius[4], Ivan Ahel[4], Christophe Tang[4], Graham Stewart[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Hertfordshire, Hatfield, United Kingdom. [3]Royal Veterinary College, London, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

ADP-ribosylation is a chemical modification of macromolecules that occurs in all domains of life. Jankevicius *et. al.* (2016) recently characterised a novel ADP-ribosyl transferase in prokaryotes, which forms part of the toxin:antitoxin system DarTG. DarT recognises the sequence TNTC on ssDNA and modifies the second thymidine, acting as a sequence specific ADP-ribosyl transferase. DarT from *Thermus aquaticus* is toxic when overexpressed in *Escherichia coli.* DarG, the antitoxin, inhibits the activity of DarT by directly interacting with the toxin, and by acting as a DNA ADP-ribose glycohydrolase, allowing for reversible ADP-ribosylation.

The DarTG system is conserved in *M. tuberculosis*, and *darG* antitoxin is essential in this organism. In *M. tuberculosis* complex organisms *darTG* is situated immediately downstream of the *dnaB*-encoded replication helicase, which generates ssDNA during chromosome replication. Global analysis of transcription start sites and operon prediction indicates that *dnaB* and *darTG* are co-transcribed, suggesting that in *M. tuberculosis* DarTG has a role during DNA replication, potentially regulating bacterial growth.

In this study we have utilised the CRISPR interference technique to inhibit expression of *darG* in *M. tuberculosis* and *M. bovis* BCG. We show that inhibition of *darG* expression causes a rapid loss in culturability. Using time-lapse confocal microscopy in a microfluidic device, we demonstrate that these non-culturable cells continue to elongate but fail to divide. Disruption of *darT* in *M.tuberculosis* confers increased *in vitro* growth. Our data suggest a crucial role of the DarTG toxin:antitoxin system in control of growth and cell division of the *M. tuberculosis* complex.

A208

Glucose metabolism via the Entner-Doudoroff Pathway in a select subgroup of *Campylobacter jejuni*

Nourah Almimoni, Sheila Macintyre

University of Reading , Reading , United Kingdom

Abstract

*Campylobacter jejuni*is the most common cause of bacterial gastroenteritis worldwide. It is often considered a 'commensal' in chickens where it rapidly colonises the caecum of young chicks and is present in around 80 % of farmed poultry. *C. jejuni*has a small genome and most strains are unable to transport or metabolize glucose. Instead they use amino acids, TCA cycle intermediates and short chain fatty acids as energy and carbon sources. Recently, a WGS project identified an unusual group of *C. jejuni*(RG-2 group) within a bank of *C.jejuni*strains isolated from farm associated Norway rats. These strains had acquired an entire locus (*glc*) of seven genes which enable uptake and metabolism of glucose via the Entner Doudoroff (ED) pathway. This project is addressing the impact of these genes on metabolism and niche survival of these strains. Campylobacter are microaerophilic bacteria. Good growth of *C. jejuni*NCTC11168 and of a*glc*negative Norway rat *C. jejuni*isolate was recorded in the presence of 5 % O_2up to 13 %in a Whitley M35 workstation. In contrast, two *glc*positive strains of *C. jejuni*, Dg275 and Dg95, exhibited the unusual ability to grow well in an atmosphere of 16 % oxygen, as monitored by CFU and OD_{600}. Current studies are also focusing on the contribution of glucose utilization via the ED pathway to influence survival and growth in oxygen.

A209

CRP i.e. cAMP receptor protein provides a competitive edge to *Salmonella* Typhimurium in a microbial community set-up

Kirti Sawant, Ravindranath Shashidhar

Bhabha Atomic Research Centre, Mumbai, India

Abstract

The survival of enteric pathogens in the multi-nutrient environment involves interaction with other organisms. The fact that competition for nutrient resources has shaped the networking of metabolic pathways is well known. The cAMP receptor protein i.e. CRP is a keystone regulatory protein, connecting various metabolic pathways. Our aim was to study the importance of CRP in nutrient uptake and utilization in *Salmonella* Typhimurium under intra-species and inter-species nutritive competition. The *crp* gene knockout (Δ*crp*) was co-cultured with the wild-type or other pathogens. The Δ*crp* failed to compete with the wild-type *Salmonella* Typhimurium, *Escherichia coli*, *Vibrio cholerae* and *Staphylococcus aureus* in nutrient intensive media. However, the survival of the co-cultured Δ*crp* was unaffected in nutrient-poor media. These results suggest that CRP is necessary for the effective acquisition of readily available nutrients as found in rich media in co-culture. The role of released antimicrobials or surface proteins of the co-cultured strains was also overruled by culturing the mutant in the supernatant of these organisms and separating the cultures in the same media respectively. The co-cultured Δ*crp* showed an enhanced survival when overall metabolism was reduced with low temperature and antibiotics like chloramphenicol. A circumstantial evidence that CRP manages the global and limits the futile metabolism is provided by this study. The absence of CRP doesn't affect the survival of the standalone culture. However, the role of this protein becomes obvious in a bacterial community setup. Therefore, CRP is crucial for *Salmonella* to survive in an intestinal and external environment.

A210

Biochemical Screening Approach to Identify Regulatory DNA-binding Proteins

Julio Cesar Ayala, Jorge Antonioi Benitez, Anisia Ailva-Benitez

Morehouse School of Medicine, Atlanta, USA

Abstract

The interaction between transcription factors, promoter elements and RNA polymerase is crucial to bacterial adaptation to physical and chemical changes in the environment. Transcription factor discovery has strongly relied on the isolation of regulatory mutants followed by biochemical confirmation. However, highly pleiotropic regulatory mutations can often be deleterious, unstable and difficult to select, or could directly or indirectly affect gene expression at multiple levels. Here, we describe a biochemical approach to identify trans-acting regulatory proteins independent of their cellular function that could be used as an alternative to genetic screens. The method consists in (*i*) incubating bacterial lysates with an immobilized DNA encompassing a promoter as defined by RNA-Seq data (*ii*) pull down of bound proteins and (*iii*) liquid chromatography tandem mass spectrometry (LC/MS/MS). We tested the usefulness of this approach by identifying proteins binding to the *Vibrio cholerae rpoS* promoter that drives the expression of the general stress response regulator RpoS. The approach identified several proteins binding to the *rpoS* promoter that included the factor for inversion stimulation (FIS), and the master quorum sensing regulator HapR. Binding of both purified proteins to the *rpoS* promoter was confirmed by electrophoresis mobility shift assays. The role of *fis* and *hapR* on *rpoS* expression was examined in strains containing a chromosomally-integrated *rpoS-lacZ* fusion. Deletion of *fis* had little effect while HapR appeared to enhance *rpoS* expression.

A211

Electrophotonics: Multimodal sensors for bacteria identification and phenotyping.

Steve Thorpe, Thomas Krauss, Gavin Thomas, Steven Johnson

University of York, York, United Kingdom

Abstract

A comprehensive understanding of bacteria phenotypes requires tools that are able to characterise structure and function across multiple length scales, from communities and individual cells down to single molecules. Multimodal sensing combines multiple transduction technologies in parallel to probe different properties simultaneously, and thereby increase the range of measurable interactions, the amount of information that can be extracted, and improve detection accuracy. Electrophotonics is a multimodal sensing approach that combines electrochemical and photonic techniques in a single, integrated device that provides enhanced quantitative measurements of chemical reactions. We present a new electrophotonic device based on a Si_3N_4 guided mode resonant (GMR) structure with an integrated indium tin oxide (ITO) electrode. The GMR structure is sensitive to refractive index changes at the sensor surface, enabling label free, real time detection of biomolecules, microorganisms and imaging of molecular interactions with micrometre-scale resolution to provide spatial information about surface binding interactions. The ITO electrode has been shown to be compatible with voltammetry-based techniques for interrogating redox behaviour along with electrochemical impedance spectroscopy. We demonstrate the wide range of microbiological applications of electrophotonic technology including the characterisation of redox active protein electron transfer and surface adsorption, bacterial adherence and growth on chemically functionalised surfaces, and label free parallel detection of clinically relevant biomarkers. We believe that the multimodal measurements with this novel technology can provide new approaches to investigate and understand microbial biology.

A212

Molecular studies on iron metabolism, redox stress and pathogenicity in *Bartonella*

Sheama, Alali , Simon Andrews

School of Biological Sciences, Whiteknights, University of Reading, Reading , United Kingdom

Abstract

For bacteria, mechanisms of resistance to redox stress are utilised to increase survival. Although *B. henselae* has the ability to resist redox stress, its genome sequence is characterised by a paucity of genes responsible for redox-stress resistance, particularly hydrogen peroxide degradation systems. However, our results show a surprisingly high resistance to peroxides, given the lack of peroxide disposal systems. To determine how *B. henselae* achieves resistance to H_2O_2 stress, the potential role of MbfA (membrane-bound bacterioferritin), which in other α-Proteobacteria is believed to function as an iron exporter, was investigated. The results show that *B. henselae* has the ability to export iron and that this export activity is promoted by H_2O_2 as export was inhibited by exogenous catalase and anaerobiosis. The form of iron exported was largely ferric. The impact of the iron export process on the resistance to, and degradation of, H_2O_2, by *B. henselae* was determined and the results showed that *B. henselae* mediates a rapid consumption of exogenously supplied H_2O_2. This degradation was entirely inhibited when iron chelators were included along with the H_2O_2. The resistance of *B. henselae* to NO was also tested since NO is generated by phagocytic host cells along with H_2O_2, and is suggested to potentiate the toxicity of H_2O_2 towards engulfed bacteria through inhibition of haem-dependent catalases and alkylhydroperoxidases. Our results suggest that NO does not cause a marked increase in H_2O_2 toxicity for *B. henselae*, in contrast to *E. coli* (a haem-catalayse/peroxidase dependent bacterium).

A213

Recycling of recalcitrant organic matter at the full-ocean depth by SAR202 bacteria

zhan-fei wei

:Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, SanYa, China

Abstract

SAR202 in Chloroflexi phylum plays an important role in degradation of organic compounds in the oligotrophic deep ocean. Due to lack of high-quality genomes from all the depths of water column, the metabolic capacities of the SAR202 subclusters in the marine environment were still obscure in deep-sea waters. In this study, we exhibit the dynamics vertical distribution of the SAR202 subclusters in the global ocean. A total of 11 high-quality metagenome-assembled genomes (MAGs) with a wide range of sizes were obtained for two SAR202 subclusters in the Mariana Trench. Genomic comparison revealed the presence of vitamin B_{12} (VB12) synthesis only in the subcluster III. A large number of genes encoding the oxidase and degradation pathways indicate that the SAR202 bacteria were capable of metabolizing chitin, aromatic compounds, dimethyl sulfoxide and osmolytes in the environment. With the predicted genes responsible for sulfite oxidation and export, the SAR202 probably also conducted important biogeochemical cycles. The *in situ* transcriptional activities of these pathways at 7,100 m depth were supported by metatranscriptomics data. This study highlights genetic plasticity, *in situ* activities and ecological role of SAR202 in deep sea zones.

A214

Disruption of the *mce* operon from Streptomyces affects spore resistance and results in precocious germination

Emily Addington [1], Gabriel Uguru[1], Laura Clark[2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of York, York, United Kingdom

Abstract

Streptomyces coelicolor is a non-pathogenic soil saprophytic bacterium and is a model organism for antibiotic production. This species contains a single copy of a nine gene cluster known as the mammalian cell entry (*mce*) operon. This operon was originally characterised in *Mycobacterium tuberculosis* as an important virulence factor acting in invasion and survival within macrophages and encodes an ABC transporter for cholesterol import. As the function of the *mce* operon in *S. coelicolor* is currently unknown, this study aims to characterise the operon through deletion of the *mce* locus and resulting impact on bacterial morphology and survival. SEM images demonstrate that spores of a *mce* deletion mutant (*Δmce*) display a wrinkled, and 'fragile' phenotype, with spores appearing to germinate whilst on the spore chain. Heat kill assays show that the deletion of the *mce* operon result in *S. coelicolor* spores which are less tolerant to temperatures of 60, 70, 80, 90 and 100°C compared to WT *S. coelicolor* spores. Heat activation of *Δmce* spores was also consistently absent at all temperatures tested. The spores of a *Δmce* mutant also exhibit a precocious germination phenotype seen on SEM images confirmed with germination assays.

A215

Metabolic recuperation in valine production by mutant strain

Misbah Jamil

university of punjab, Lahore, Pakistan

Abstract

The contemplation of present research work to get over scantiness of amino acid by amelioration ofmicroorganism . As a matter of fact the use of chemical and physical factors symbolize a new era in the excerption of microorganism for proficient of producing a desired product . In this workbiosynthetic pathway of valine and their regulation was observed in Bacillus cereus , Corynebacterium and Pseudomonas fluoresence . The contrivance of operation of ems mutants was analyze in different fermentation media at 24 , 48 , 72 and 96 hours incubation under optimum condition by acidic ninhydrin method. optical density was recorded by spectrophotometer 470nm for Valine . The effects of carbon and nitrogen sources and growth factors on the production of valine were studied . In this studies mutant evince that mutation confabulate upon the bacteria the ability to produce valine many times more than its parental strain within the same span of incubation period .The objective of this work to step up headway to search unconventional sources to overcome nutritional exhortation of valine .

A216

Genomic Mining of Thermophilic Actinobacteria from compost for Novel Antimicrobials

Gillian Halket[1], Carol Munro[2], Paul Herron[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom

Abstract

Actinobacteria have provided a rich source of novel antimicrobial compounds throughout the antibiotic era. Thermophilic Actinobacteria growing at higher temperatures (>50°C), have not been extensively studied, despite producing important antibiotics such as thermomycin and anthramycin. We are testing the hypothesis that thermophilic Actinobacteria produce new and unusual antimicrobials at higher temperatures, potentially leading to the discovery of novel heat stable compounds; especially those active against life-threatening fungal infections in humans such as invasive aspergillosis caused by *Aspergillus fumigatus,* which has developed resistance to current treatments. Compost is a rich source of thermophilic Actinobacteria responsible for generating the heat required for decomposition and yet this niche has been overlooked in terms of natural product discovery. *A. fumigatus* is a fungus that also lives in compost and also contributes to the composting process and we reasoned that Actinobacteria living in this environment might display activity against pathogenic strains of the fungus. Samples from a series of "windrows" at a commercial green waste processing facility yielded 13 thermophilic Actinobacteria, and strains of *Aspergillus fumigatus*. The phylogeny and species identity of the bacterial strains were determined by 16S rRNA sequencing. Candidate strains were screened for the ability to inhibit ESKAPE pathogens as well as *A. fumigatus*, using agar overlays and MIC assays. Selected strains were analysed by whole genome DNA sequencing and likely antimicrobials predicted. Compound identification using mass spectrometry and metabolic profiling has been undertaken on strains that display antibiotic activity, providing a path for the development of new antimicrobials for clinical use.

A217

D-serine: trick or treat?

Natasha Turner

Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom

Abstract

D-serine is an amino acid that has become a focus in recent years due to its unique role in many biological processes. It is a host metabolite in humans with diverse roles in neurotransmission and signalling. Previous work in our group showed that D-serine can play a critical role in controlling expression of pathogenic virulence factors in bacteria, specifically *Escherichia coli*, as well as impacting microbial community composition through niche specificity. In enterohaemorrhagic *E. coli* (EHEC), the presence of D-serine results in down regulation of the type 3 secretion system (T3SS), resulting in inability to colonise. In contrast, uropathogenic *E. coli* (UPEC) can catabolise this metabolite and colonise the bladder where D-serine is present in high concentrations, leading to a urinary tract infections (UTI). Hence, in different pathotypes, D-serine can act as a positive (treat) or negative (trick) environmental stimulus.

UPEC and neonatal meningitis *E. coli* (NMEC) strains often carry the DsdXCA operon which allows for the metabolism of D-serine. This locus is responsible for the detoxification of D-serine, allowing for tolerance of D-serine as a carbon source. My work has focused on understanding the role of D-serine as a signal for gene expression in UPEC and NMEC through the action of the regulator, DsdC. Using global approaches I have characterised the binding sites of DsdC across the chromosome revealing new insights into how this protein contributes to UPEC and NMEC pathogenesis. The work is important as it helps us understand how specific pathogens sense their environment and cause disease.

A218

Biofilm formation in non-tuberculous mycobacteria

Anja Dokic, Apoorva Bhatt

Institute for Microbiology and Infection, Birmingham, United Kingdom

Abstract

Mycobacterium abscessus belongs to the group of non-tuberculous mycobacteria (NTM) and it predominantly causes chronic infection in lungs of immunocompromised patients. It poses a global threat due to its high resistance to most commercially available antibiotics. Similarly to other NTMs, *M. abscessus* forms a biofilm which has been shown to increase bacterial survival in host cells and resistance to antibiotics. Given the severity of disease that *M. abscessus* causes, and the difficulty observed in treatment, the molecular genetics and mechanisms behind biofilm formation warrant exploration. Using fluorescence microscopy, we analyzed the macromolecules in the extracellular matrix (ECM) of *M. abscessus;* revealing differences between ECM composition in surface-attached and pellicular biofilms. By conducting fractionated lipid extraction and thin layer chromatography from planktonic and biofilm cultures, we were able to show altered lipid profiles during biofilm maturation, characteristic of NTMs. These results illustrate an initial biochemical and microscopic characterization of *M. abscessus* biofilms.

A219

Elucidation of ABC-transporter functionality in Corynebacterium glutamicum

Stephen Bethell[1], Karin Krumbach[2], Lothar Eggeling[2], Jan Marienhagen[2], Gurdyal Besra[1], Luke Alderwick[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Edgbaston,, Birmingham, United Kingdom. [2]IBG-1: Biotechnologie, Wilhelm-Johnen-Straße, 52428, Jülich, Germany

Abstract

Mycobacterium tuberculosis, and closely related members of the Corynebacterineae suborder produce a range of complex lipid-linked glycoconjugates that include phosphatidylmannosides (PIMs), lipomannan (LM) and lipoarabinomannan (LAM), two important host immunomodulators. The biosynthesis of these glycoconjugate molecules has been largely delineated, however, the proteins and processes responsible for their transport from the inner to outer leaflet of the cytoplasmic membrane are yet to be elucidated. Here we describe the phenotypic response to genetic deletion of a pair of putative ABC-transporters in *Corynebacterium glutamicum*, a surrogate organism used to study mycobacterial cell physiology. A double deletion mutant results in a significant depletion in the export of $AcPIM_2$ from the inner membrane to the external bacterial surface with concomitant alterations in the LM and LAM profile. Together our data suggest that these genes are responsible for the transport of $AcPIM_2$ across the inner membrane leading to complete maturation of cell envelope lipoglycans.

A220

Functional analysis of the FeoABC iron uptake system of *E. coli*

Salem AL-Aidy[1,2], Prof.Simon Andrews[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Wasit/Faculty of Medicine, Wasit, Iraq

Abstract

FeoABC as the major ferrous iron uptake system in *E. coli* and homologues are found in ~60% of sequenced bacteria. The encoding *feoABC* operon is induced by anaerobiosis and by iron deficiency, and is required for gut colonisation by *E. coli*, and for virulence in several pathogens. The operon encodes three proteins: FeoA, a small, cytoplasmic SH3 protein; FeoB, the ferrous permease consisting of an N-terminal, cytoplasmic G-protein domain and a C-terminal polytopic permease domain composed of two 'Gate' motif domains; and FeoC, a small Fe-S-containing protein thought to control FeoB stability in response to oxygen. A major aim of this work is to determine whether Feo can function aerobically and, if not, how is Feo activity limited by the presence of oxygen.

To further characterise the Feo system, His- and FLAG-tagged variants of FeoA and FeoB were generated. Inducible, compatible plasmids containing various combinations of the wildtype and tagged feoA, feoB and feoC genes were transformed into *E. coli* JC32.

To further understand the mechanism of FeoB iron uptake, nine highly-conserved FeoB residues within the permease domain were altered by SDM. The FeoB-C403S, -C432S, -C677S and -E582G variants failed to exhibit FeoAB-enhanced growth under iron restriction indicating an essential role for these residues. In contrast, FeoB-E488G showed normal FeoAB activity.The FeoB-C772S/H773G and -C763S/C764S (residues in the C-terminal cytoplasmic tail of FeoB) variants showed greater FeoC dependence in terms of the FeoAB-mediated enhanced low-iron growth effect; this indicates that FeoC may interact with C-terminal cytoplasmic tail of FeoB.

A221

Enigma of Gastric Microbiome: Diversity, Genomic and Transcriptomic Insights into *Ochrobactrum* spp. (A Non *H. pylori*, Urease positive bacterium)

Kushal Gohil[1,2], Mahesh Dharne[1,2]

[1]CSIR National Chemical Laboratory, Pune, India. [2]Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

Abstract

The dominance and pathogenicity of *Helicobacter pylori* in the gastric niche is eminent. Urease test is used to diagnose the infection of *H. pylori.* However, nowadays several reports indicated the emergence of organisms other than *H. pylori* (Non *H. pylori* bacteria– NHPs). We have reported urease positive, *Ochrobactrum intermedium* from the non-ulcer dyspeptic individual. Therefore such urease positive bacteria raise a question on the reliability of urease test. Since 2005, nearly 35 cases are reported for the presence of *Ochrobactrum* spp. from clinical specimens. Their high level of resistance to antibiotics and phylogenetic relationship to anthropozoonotic pathogen, *Brucella* makes them a probable human pathogen. Therefore, it is necessary to study their prevalence, survival mechanism and function in the human stomach. To check their pervasiveness in the Indian population, 218 urease positive gastric biopsy samples were processed and 62 *Ochrobactrum* spp. isolates were identified with and without *H. pylori*. Population study of *Ochrobactrum* spp. was also done using multilocus sequence typing (MLST) which revealed 45 sequence type and clonal population. As the prevalence of *Ochrobactrum* spp. is approx. 30%, their survival mechanism was studied under *in vitro* gastric conditions (acidic pH, urea, microaerophilic environment), using microarray and RNA seq methods. The differential gene expression analysis showed 2 different acid resistance mechanism, i.e. Urease dependent acid resistance mechanism like *H. pylori*, and Amino acid dependent acid resistance mechanism. Our finding necessitates further detailed investigation of such NHPs in gastric environment and role of such bacteria in gastric niche and warrants further refinement of urease based diagnosis.

A222

Novel Nitrogen-Containing Heterocyclics with Bactericidal Activity Against *Mycobacterium tuberculosis*

Alice Lanne[1], Yixin Cui[2], Mariwan Hama Salih[2], Philip Craven[2], Katie Dale[1], Helena Dodd[2], Geraint Jones[3], Xinran Lu[2,4], Gavin Milne[3], John Ritchie[3], Yiming Zhao[2], John Fossey[2], Luke Alderwick[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom. [2]School of Chemistry, University of Birmingham, Birmingham, United Kingdom. [3]Sygnature Discovery, Nottingham, United Kingdom. [4]School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, China

Abstract

Tuberculosis (TB) is the leading cause of global morbidity and mortality caused by an infectious disease, with over 10 million new cases emerging in 2017. This global emergency is exacerbated by the emergence of multi-drug and extensively-drug resistant TB, therefore new drugs and new drug targets are urgently required. From a whole-cell phenotypic screen we identified a series of nitrogen-containing heterocyclic compounds that elicit potent anti-mycobacterial activity with MIC values <10 uM against *Mycobacterium tuberculosis*. Interestingly, this series of compounds demonstrate no detectable drug resistance in mycobacteria. Mode of action and target deconvolution studies suggest that these compounds inhibit mycobacterial growth by interfering with late-stage mycolic acid biosynthesis. In addition, these compounds exhibit a suitable toxicological and PK/PD profile that paves the way for further development as an anti-TB chemotherapy.

A223

Structure and function of the antimicrobial resistance-related DedA family of integral membrane proteins.

Christopher Mulligan

University of Kent, Canterbury, United Kingdom

Abstract

Antimicrobial resistance is a major global health concern. As well as the need for new drug discovery, combatting resistance itself is a promising approach that could render previously resistant pathogens susceptible to antimicrobial activity.

The DedA family of integral membrane proteins are involved in the resistance of clinically relevant pathogens to several different antimicrobials. Recently, a DedA family member was shown to be essential for resistance to colistin, which is a drug of last resort, in *Klebsiella pneumoniae*. In addition, DedA family members have been shown to be essential for viability in several bacterial species. Despite the apparent importance of this protein family and their potential as targets for inhibition in therapeutic development, there is very little known about their structure, function and physiological role.

Here, we have used an *E. coli*model to explore the relative contributions of different *dedA*genes to susceptibility to antimicrobials and other stressors, including variable pH and cation concentration. To explore the structure of DedA proteins we have undertaken a substituted cysteine accessibility measurement (SCAM) study and an alanine scan of conserved residues identifying residues essential for function.

The information gleaned from these studies will enlighten our understanding of this fascinating protein family and may pave the way for future inhibitor development.

A224

Expression, purification and antimicrobial activity of recombinant pediocin PA-1 M31L, a PA-1 derivative with enhanced stability

Taís Mayumi Kuniyoshi[1,2], Paula M. O' Connor [1,3], Sara Arbulu[1,3], Beatriz Mesa-Pereira[1,3], Ricardo Pinheiro de Souza Oliveira[2], Collin Hill [4,3], Paul Ross[1,3,4], Paul D. Cotter[1,3]

[1]Teagasc Food Research Centre Moorepark, Fermoy, Ireland. [2]University of São Paulo, São Paulo, Brazil. [3]APC Microbiome Ireland, Cork, Ireland. [4]Department of Microbiology, University College Cork, Cork, Ireland

Abstract

Pediocin PA -1 is class IIa bacteriocin that displays efficient antimicrobial activity against pathogenic *Listeria* spp. This bacteriocin is known to lose activity during long periods of storage especially at non optimal pH, thus reducing its usefulness for the pharmaceutical and food industries. Loss of activity has been attributed to oxidation of the methionine residue at position 31, however, replacement of this residue by leucine results in a peptide with activity equivalent to that of the native peptide. In this work, the heterologous expression of the structural (with Met31 to Leu substitution), accessory and transport genes from pediocin PA-1 operon was carried out in *Escherichia coli* Tuner^{TM}(DE3) cells. The sequences of all genes were redesigned using codon bias for the host and were cloned into an expression vector that allows control of plasmid copy number. The heterologous expression of pediocin Met31Leu was optimized for temperature, induction time, IPTG concentration and plasmid copy number and was evaluated via antimicrobial activity assays against *Listeria innocua* DPC3572. Maximum activity (2560 AU/mL) was achieved using low plasmid copy number and 6 hours of induction at 37^{o}C with 1mM of IPTG. Recombinant pediocin PA-1M31L was successfully purified in 5 steps (> 95% purity) as confirmed by mass spectrometry (4606.27 Da) with a yield of 0.725 mg per liter of culture. This variant showed a similar spectrum of activity to the native pediocin PA-1 and is an interesting alternative for industrial applications due to its greater stability.

A225

Temperate Stx-bacteriophage 24B subverts fatty acid synthesis pathways that promote a bacterial response to antimicrobials

Darren Smith[1], Giles Holt[1], Simon Bridge[1], Adnan Tariq[1], Heather Allison[2], Alan McCarthy[2]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Shigatoxigenic Esherichia coli (STEC) infection is a global health concern. A key virulence determinant of STEC infection is the carriage of an AB-holotoxin that is encoded and disseminated by dsDNA, lambdoid-like bacteriophages. This toxin is responsible for the severe pathophysiology of infection including; haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC). Shigatoxin-encoding bacteriophages or Stx-phages carry an accessory genome, where most genes have no associated function yet they are commonly conserved between phages of that type. Bacteriophage 24B is a well-studied, clinically relevant Stx-phage as it was isolated from an outbreak of STEC infection and shares genomic similarity to other Stx-phage's e.g 933W. The accessory genome of phage 24B is conserved when compared to other reported stx-phages from clinical incidence and therefore must be important in the biology of the bacteriophage or bacterium as a lysogen. Using metabolomics alongside transcriptomics data we illustrate that 24B subverts bacterial cell metabolism during growth and when challenged with antimicrobials offering a selective advantage to the lysogen. We here focus on phage subversion of both the biotin and fatty acid synthesis pathways in the lysogen's cellular metabolism and how this compares to alteration of fatty acids presented at the wall that may play a role in antimicrobial resistance (AMR). Importantly this alteration in AMR is not associated with a phage-encoded gene and therefore we hypothesise that this selection is based upon subversion of the bacterial cell physiology.

A226

A possible alternative for chromosome dimer resolution in *E. coli*

Cameron Croft, Cindy Macpherson, David Summers

University of Cambridge, Cambridge, United Kingdom

Abstract

Dimer formation is a serious threat to the stable maintenance of ColE1-like plasmids. Dimers form infrequently by homologous recombination but accumulate rapidly by having two origins of replication. This results in elevated plasmid loss and a reduction in host cell growth rate. Plasmid dimers are resolved to monomers by the XerCD recombinase plus accessory proteins ArgR and PepA, acting at the *cer* recombination site. The circular chromosome of *E. coli* also forms dimers infrequently, and consequent failure of chromosome partition results in filamentation, SOS induction, and failure of cell division. Site-specific recombination is required for dimer resolution during cell division in a process facilitated by XerCD acting at the *dif* (deletion induced filamentation) site near the *E. coli* chromosome terminus. ArgR and PepA accessory proteins are nonessential, but the septum-associated protein FtsK is necessary for dimer segregation, suggesting the XerCD/*dif* complex interacts with division septums. Our preliminary work had revealed homology between *cer* and a 170-bp chromosomal site (*tcs*, terminal region *cer*-like site) 1.2 min from *dif*. The *tcs* site and surrounding region was cloned into plasmids, dimer plasmids were purified and then assayed for *tcs*-mediated recombination. Our results demonstrate that a construct with a 500-bp *tcs* insert supports XerCD-mediated recombination, whereas smaller constructs were recombination-deficient. The absence of plasmid monomerization in mutant strains indicates that ArgR and PepA are required for recombination. Additionally, the *tcs* knockout strain displayed moderate filamentation of cells. Given the similarities between *cer*, *tcs*, and *dif*, these results suggest that *tcs* could facilitate chromosome dimer resolution.

A227

Mycobacterium smegmatis sliding colonies can expand using fluid filled protrusions

Eric Pollitt

University of Sheffield, Sheffield, United Kingdom

Abstract

Mycobacterium smegmatis can spread over soft surfaces by sliding motility and can form either round or dendritic colonies. Sliding motility is a form of passive motility where growth and reduction of surface adhesion enable the bacteria to push each other outwards. We investigated *M. smegmatis* sliding motility as it has previously been shown to produce pointed dendrites, not normally associated with sliding motility, which is largely associated with round or frond like colonies. Pointed dendrites have been associated with unusual behaviours in other bacterial species. We adapted a published sliding motility assay to obtain two different types of sliding colony. We dissected the colonies and analysed them under several different types of microscope. We also observed them using timelapse microscopy. In addition to the previously observed round sliding colonies we also produced colonies with previously unobserved protrusions that differed from the previously reported dendrites. These protrusions contained a central channel filled with a mobile liquid containing a suspension of *M. smegmatis*. This liquid was retained by the colony even when it was inverted. Expansion of the fluid enables the extension of the protrusions without any perceptible bacterial growth. The observation of these protrusions represents a novel finding of how passive sliding motility can occur. The fluid component may be important as in other bacterial species wetting agents are often important virulence factors/antibiotics that can be hard to detect in other situations.

A228

Post-transcriptional regulation of gene expression in Escherichia coli experiencing sustained nitrogen starvation

Josh McQuail, Amy Switzer, Ramesh Wigneshweraraj

Imperial College London, London, United Kingdom

Abstract

While transcriptional reprogramming is perhaps the most well understood form of controlling gene expression in response to nitrogen starvation in bacteria, how post-transcriptional regulation (PTR) of gene expression contributes to this adaptive response remains elusive. Small regulatory RNAs (sRNAs) are the major post-transcriptional regulators of gene expression in bacteria. They regulate gene expression by base pairing to target mRNAs, leading to enhanced translation or inhibition of translation and/or alteration of mRNA stability. To form productive interactions with target mRNAs, most sRNAs require an RNA chaperone. In many bacteria of diverse lineages, the RNA chaperone Hfq plays a central and integral role in the PTR of gene expression by stabilising sRNAs and promoting their interactions with cognate mRNAs. Comparative analysis of the transcriptomes of Escherichia coli at different stages of nitrogen starvation reveal that levels of sRNA vary throughout starvation. We used Hfq as a surrogate to study sRNA-mediated PTR of gene expression during sustained nitrogen starvation. Our results indicate that sRNAs-mediated PTR of gene expression plays a major role in the adaptive response to sustained nitrogen starvation. Intriguingly, using single-molecule PALM, we reveal that Hfq is involved in the formation of intracellular structures which functionally might resemble processing (P) bodies found in eukaryotic cells involved in mRNA turnover.

A229

"Functional Characterisation of cell wall proteins that enhance nasal colonisation by Staphylococcus aureus, through binding to host mucin"

Amenah alotaibi[1], Simon Clark[2]

[1]Reading university, Reading , United Kingdom. [2]Reading University, Reading , United Kingdom

Abstract

Human nasal colonization with Staphylococcus aureus sets the stage for subsequent systemic infection. The mechanisms responsible for colonization are not fully understood. This study characterizes *S. aureus* adhesion to nasal mucosa in vitro and observes the interaction of *S. aureus* with mucin. *S. aureus* showed significantly higher binding to mucin during stationary phase in comparison to log-phase cells. Adherence of *S. aureus srt*A mutant to mucin was not significantly different from wild-type, thus Srt A shows no influence on *S. aureus* interaction with mucin. Adherence to mucin was saturable in a dose- and time-dependent fashion. Biotin–labelled mucin bound to surface protein (55 kDa) of cell wall extracted *S. aureus* which is encoded by the sbi gene. These data suggest that adherence factors are present on the surface of *S. aureus* such as sbi. Purified recombinant Sbi was prepared and the mucin binding capacity of the protein was tested by ELISA. In order to determine the function of specific domains of Sbi in adhesion, Sbi constructs with, without the IgG-domain and with B2-glycoprotein -domain were expressed on the surface of *E.coli* BL21(DE3). The expression of Sbi with the B2-glycoprotein -domain on the surface adhesion, emphasizing the role of Sbi in mucin adhesion .A profound binding effect was observed with Sbi incubated in wells coated with host mucin. Therefore, it is proposed to investigate a novel interaction of S. aureus to host mucin in order to control *S.aureus* nasal colonization.

A230

Identification of essential residues for polyprenol phosphate mannose synthase and protein O-mannosyl transferase activities required for protein glycosylation in *Streptomyces*.

Nathaniel Holman[1], Pamela Banana-Dube[2], Tony Wilkinson[3], Manuela Tosin[2], Maggie Smith[1]

[1]Department of Biology, University of York, York, United Kingdom. [2]Department of Chemistry, University of Warwick, Coventry, United Kingdom. [3]Department of Chemistry, University of York, York, United Kingdom

Abstract

Actinobacteria have a protein O-glycosylation system that resembles eukaryotic protein O-mannosylation. Both *M. tuberculosis* and *S. coelicolor* have growth retarded phenotypes when protein-O-mannosyl transferase (Pmt), which transfers mannose from polyprenol phosphate mannose to a target protein, is absent. Moreover, *S coelicolor pmt*$^-$ mutants are resistant to φC31 phage infection and have increased susceptibility to vancomycin and multiple b-lactams. *S. coelicolor* strains that lack polyprenol phosphate mannose synthase (Ppm1), which transfers mannose from GDP-mannose to polyprenol phosphate, are even more susceptible to antibiotics and a *ppm1*$^-$ mutant in *M. tuberculosis* is lethal. Pmt and Ppm1 are therefore possible new targets for the isolation of antimicrobials to be used against *M. tuberculosis*. Our aim is to further understand the structure and function of these enzymes.

Sequence alignments and structural bioinformatics were used for *S. coelicolor* Ppm1 and Pmt to identify site-directed mutagenesis targets. Mutant alleles were introduced into *ppm1*$^-$ (DT3017) or *pmt*$^-$ (DT2008) *S. coelicolor* strains using conjugative integrative plasmids and scored for their ability to complement phage sensitivity and antibiotic hyper-susceptible phenotypes. Twenty-two highly conserved Pmt residues were each changed to alanine and six mutants failed to complement DT2008, indicating essentiality. Modelling these six residues indicated that five are positioned close to the predicted catalytic DE motif. For Ppm1, ten mutant alleles were tested and eight were essential for DT3017 complementation, with four residues positioned close to the predicted catalytic DXD motif. Whilst some of the mutations were predicted to impair catalytic activity, others may have affected localisation or substrate binding.

A231

Intrageneric competition of staphylococci reveals discrete evolutionary outcomes.

Hind ALRAJEH, M. J Horsburgh, Hanaa Ghabban

Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom

Abstract

We previously identified the diversity of antimicrobial activity in nasal microbiomes that correlates with presence and absence of *Staphylococcus aureus*. The major competitor of skin surfaces is *Staphylococcus epidermidis* that can express diverse antimicrobial activities. Ten-fold less frequently found in skin and nasal microbiomes, *Staphylococcus hominis* is relatively unstudied. The aims of the research were to develop insights into the factors of *S. hominis* that contribute to the dynamics of competition with these major skin colonising staphylococci. One prominent inhibitory strain, S*.hominis* was selected to culture with both S*.aureus* SH1000 and *S.epidermidis* with aim to use genome sequencing to reveal loci contributing to competition. *S. aureus* evolved during the competition of *S. epidermidis* and inhibitor-producing *S. hominis* to reveal two discrete phenotypes. Non-pigmented clone expressed less alpha-haemolysin and had a SNP in *agrC* encoding the receptor of the Agr quorum-sensing system. More Pigmented clone had a SNP in *sigB* encoding the accessory sigma factor required for expression of the staphyloxanthin. Distinctively, competition of *S.aureus* SH1000 with*S. epidermidis* revealed evolution of *S.aureus* that corresponded with a SNP in the *lytS* gene of the LytSR two-component system controlling murein hydrolase activity and autolysis. Pacbio genome sequencing followed by use of AntiSMASH revealed the *S. epidermidis* inhibitory strain, which enabled persistence of *S.hominis,* encoded the lantibiotic gallidermin biosynthesis operon on a 39kb plasmid. Gallidermin decreased *S. aureus* survival to competitor *S. epidermidis*, and an increased ability of *S. hominis* to maintain its population size during evolution experiments, which supported the dynamic relationship of the three staphylococci. Future studies will unravel explanations for the contributions the identified loci make to multi-species competition.

A232

A multiplatform approach to investigate the structure and architecture of the biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in response to antimicrobial treatment

James Brown, Morgan Alexander, Paul Williams, Kim Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

Bacterial biofilms are highly complex communities, composed of highly structured extracellular polymers and subpopulations of differentiated cells, such as persisters. These contribute to the resistance of bacterial biofilms to antibiotics, creating a significant issue in the treatment of infections and resulting in elevated levels of mortality and morbidity.

Here, we use a microfluidic system coupled with time-lapse microscopy and fluorescent dyes for exopolysaccharide (EPS) and extracellular DNA (eDNA) to investigate how the architecture of the growing biofilm is ordered. We show that in *Pseudomonas aeruginosa* EPS is deposited first in the initial stages of microcolony development, but that eDNA then acts as a leading edge for further microcolony expansion. We explore how this assembly is perturbed by the introduction of different antimicrobial agents.

Further, working with partners in the European Association of National Metrology Institutes (EURAMET) we are developing cross platform methods for the label-free localisation of antimicrobial agents and bacterial components within the biofilm. These platforms include 3D OrbiSIMS (secondary ion mass spectrometry) and Raman spectroscopy. Here we demonstrate the localisation of key biofilm components such as Pseudomonas Quinolone Signal (PQS) molecules in a 3D chemical map of the biofilm.

A233

Understanding virus resistance due to ISG15-loss-of-function

Andri Vasou[1], David Holthaus[1], Jelena Andrejeva[1], Connor Bamford[2], John McLauchlan[2], Richard Randall[1], David Hughes[1]

[1]University of St Andrews, St Andrews, United Kingdom. [2]University of Glasgow , Glasgow, United Kingdom

Abstract

Viral infections induce profound cellular responses resulting the expression of hundreds of IFN-stimulated genes (ISGs). Some ISGs have specific antiviral activity, while others regulate the cellular response. For most viruses, the specific antiviral ISG(s) is not known, which has potential consequences for the quest for new therapeutics.

The ubiquitin-like protein ISG15 is a major regulator of antiviral response and inherited ISG15-deficiency leads to autoinflammatory interferonopathies where patients exhibit elevated ISG expression in the absence of infection. Using CRISPR/Cas9 knockout technology, we have recapitulated these effects in cultured cells, confirming 'free' ISG15's role as a central regulator of type-I IFN antiviral response. We also show that during an antiviral response, ISG15-deficiency leads to significant physiological defects (inhibition of translation and proliferation) and resistance to parainfluenza viruses.

We asked if virus resistance was due to the direct antiviral activity of ISGs, or whether cells were non-permissive due to physiological defects. We took advantage of the knowledge that IFIT1 is the principle antiviral ISG for parainfluenza virus 5 (PIV5). Knockdown of IFIT1 restored PIV5 infection in ISG15-deficient cells, confirming that resistance was due to the antiviral response and not due to physiological state related to ISG15-deficiency. We also compared infections with related viruses where IFIT1 has known intermediate antiviral activity (PIV2) and low activity (PIV3); restoration of replication with these viruses reflected their sensitivity to IFIT1 restriction. Based on the observations in IFIT1-knockdown cells, we propose a novel platform for the identification of antiviral ISGs based on recovery of virus infection.

A234

The VP4 protein from a very virulent IBDV strain antagonises type I IFN responses to a greater extent than the VP4 from a classical strain

Katherine Dulwich[1,2], Efstathios Giotis[2], Michael Skinner[2], Andrew Broadbent[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Infectious bursal disease virus (IBDV) infects B cells in the bursa of Fabricius (BF) causing morbidity, mortality and immunosuppression in infected chickens. Classical strains (e.g. F52/70) cause 1-2% mortality whereas very virulent strains (e.g. UK661) cause over 60% mortality for reasons that remain poorly understood. We inoculated birds with either F52/70 or UK661, and found that the expression of pro-inflammatory and type I IFN-related genes was significantly down-regulated in UK661 compared to F52/70 infected birds ($p<0.05$), despite no statistically significant difference in peak virus titres between the two strains. This was also observed *in vitro* in an immortalised B cell line where UK661 caused significantly reduced IFNβ and Mx1 expression compared to F52/70 ($p<0.05$). The IBDV protease (VP4) has previously been reported to act as a type I IFN antagonist, although it remains unknown whether this is characteristic of all IBDVs or a strain-specific phenomenon. Using a luciferase reporter assay, we compared the IFNβ production in DF-1 cells in response to poly I:C stimulation in the presence of eGFP-VP4 expression plasmids, finding UK661 VP4 was able to down-regulate IFNβ production to a greater extent than F52/70 VP4 ($p<0.01$). There are 9 amino acid differences between the two VP4 proteins and we are identifying those contributing to the observed phenotype. Taken together, our data suggest that the VP4 protein in very virulent IBDV strains evolved a greater ability to antagonise type I IFN responses than classical strains which may, in part, explain their enhanced virulence.

A235

Identification of host proteins that interact with non-structural proteins-1α and -1β of PRRSV-1

Sofia Riccio[1,2], Ben Jackson[1], Julian Hiscox[2], Simon Graham[1], Julian Seago[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Porcine reproductive and respiratory syndrome viruses (PRRSV) are responsible for the most important infectious disease affecting the global pig industry, causing respiratory disease in piglets and reproductive failure in sows. Both species of PRRSV (PRRSV-1 and -2) are rapidly evolving and existing vaccines are failing to control the PRRS panzootic. PRRSV produces 16 non-structural proteins (NSPs) that are involved in viral replication and/or modulating the host immune response. Previous studies have shown that PRRSV NSP1α and NSP1β modulate host cell responses; however, the underlying molecular mechanisms remain to be fully elucidated. PRRSV-1 strains predominate in Europe but have been studied far less than PRRSV-2, therefore, this project aims to identify and characterise novel PRRSV-1 NSP1-host protein interactions. NSP1α and NSP1β from a representative PRRSV-1 subtype 1 strain (215-06) were screened using the yeast-2-hybrid (y-2-h) system and a cDNA library generated from porcine alveolar macrophages - the primary target cell of PRRSV-1. The screens identified 62 and 127 putative binding partners for NSP1α and NSP1β, respectively. Potential binding partners involved in IFN signalling, the NF-κB pathway, ubiquitination and nuclear transport have been selected for confirmation and characterisation. Identifying and characterising these novel interactions will increase our understanding of how PRRSV-1 NSP1α/β modulates the host cellular immune response, which could subsequently be exploited to rationally attenuate PRRSV-1 as a basis for improved vaccines.

A236

Murine Ifit1b is a novel cap1 binding protein which regulates viral and host translation during infection.

Harriet Mears, Teodoro Fajardo, Trevor Sweeney

University of Cambridge, Cambridge, United Kingdom

Abstract

IFIT proteins are highly expressed as part of the cell-intrinsic immune response following viral infection. IFIT1 binds directly to the 5' terminus of foreign RNA, particularly those with non-self cap structures, thereby inhibiting translation initiation. IFIT1 is highly specific for capped RNA which lacks methylation on the first and second cap proximal nucleotides (cap0), a signature of 'non-self'.

Knock-out mouse models have been extensively used to study IFIT antiviral activity, particularly in the development of attenuated flaviviruses. However, mice are not naturally permissive for a number of flavivirus infections, including Zika and Dengue viruses, necessitating the use of immunocompromised animals which are an unsuitable framework to study IFIT-flavivirus interactions. Additionally, we and others have demonstrated clear differences in murine and human IFIT function and regulation. A more complete understanding of murine IFIT biology will improve the usefulness of mouse models for understanding IFIT function.

Murine Ifit1 has been extensively characterised, due to its similarity to human IFIT1, but the roles of closely-related paralogues Ifit1b and Ifit1c are unknown. Using qPCR, we confirmed that Ifit1b and Ifit1c are expressed in murine cells following different stimuli. We show that Ifit1b binds preferentially to cap1 RNA substrates *in vitro*, while binding is much weaker to cap0 and cap2 RNA. Consequently, overexpression of Ifit1b restricted wildtype Zika virus replication in murine cells, while also modulating host translation. Therefore, we have identified a species-specific restriction factor which renders murine cells non-permissive for flavivirus infection, a characteristic which could be manipulated to improve flavivirus mouse models.

A237

Study of the regulation of human IFIT1 stability

Xin Yun Leong, Trevor Sweeney

University of Cambridge, Cambridge, United Kingdom

Abstract

Interferon-induced proteins with tetratricopeptide repeats (IFITs) are produced in both interferon-dependent or interferon-independent manners after pathogen-associated molecular pattern recognition. Four IFITs (IFIT1, IFIT2, IFIT3 and IFIT5) have been characterized in humans. They are cytoplasmic proteins with repetitive tetratricopeptide repeats, protein motifs well characterized to mediate protein-protein interactions. IFITs play several functions in cells and their antiviral roles are well established. IFIT1 principally binds non-self cap0 mRNAs and inhibit their translation. On its own, IFIT1 overexpresses poorly in cells. Co-expression with IFIT3 enhances IFIT1 protein levels. Likewise, downregulation of endogenous IFIT3 decreases the expression of IFIT1 at the protein level. The stabilization is dependent on the interaction of the C terminus of IFIT3 with IFIT1 via a specific motif in both proteins, disruption of which greatly reduced IFIT1 stability. It is currently unclear why efficient IFIT1 expression is regulated by its interaction with IFIT3 in this manner. To address this, we have begun to investigate the process of IFIT1 degradation. We have used a range of inhibitors to disrupt specific cellular protein degradation pathways. Surprisingly, inhibition of proteasome and lysosome pathways showed protection of IFIT1 from active degradation, suggesting the protein may be degraded via various routes. We will discuss our current efforts to generate stabilized IFIT1 mutants using random and insertional mutagenesis to identify motifs that may contribute to IFIT1 turnover. These findings will contribute to a deeper understanding of IFITs role during the immune response and may identify methods by which their function can be manipulated.

A238

A spatial proteomic approach to identify members involved in the IFN response to HCMV

Colin Davies[1], Martin Potts[1], Dan Itzhak[2], Katie Nightingale[1], Alex Davies[1], Robin Antrobus[1], James Williamson[1], Georg Borner[2], Mike Weekes[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Max Plank Institute of Neurobiology, Munich, Germany

Abstract

During the early stages of viral detection, signalling and translocation by key protein mediators initiate antiviral activity. For example, interferon (IFN) signals through the JAK/STAT pathway, leading to phosphorylation of STAT1/2 and movement in complex with IRF9 into the nucleus. To identify novel proteins involved in the IFN response to viruses, we have employed a variety of unbiased, spatial proteomic screens that detect translocation of proteins between different organelles on a global scale.

Using three different spatial screens in IFN-stimulated or control human foreskin fibroblasts, we quantified movement of >8000 proteins between the nucleus, cytoplasm and individual organelles. A novel method enabled simultaneous analysis of >2000 phosphoproteins. Proteins were shortlisted based on significant, reproducible movement. Results were validated by appropriate movement of STAT and IRF9 controls.

To focus on proteins involved in the IFN response that are important during HCMV replication, we used a complementary screen of proteins that are rapidly downregulated or degraded by HCMV. The results of these screens will be presented, including a shortlist of novel factors that may have new roles in the IFN response to HCMV.

Virology workshop: Innate immunity
Presentations: Monday and Tuesday evening

A239

Comparative responses of paediatric airway epithelium to viral and allergen insult.

Sheerien Manzoor[1], Lindsay Broadbent[1], Caoimhe McDermott[1], Stephen Main[2], Steve Turner[2], Jurgen Schwarze[3], Michael Shields[1], Ultan Power[1,4]

[1]Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Northern Ireland, Belfast, United Kingdom. [2]University of Aberdeen, Aberdeen, United Kingdom. [3]The University of Edinbrugh, Edinbrugh, United Kingdom. [4]Breathing Together Consortium, London, United Kingdom

Abstract

Asthma affects over a million children in the UK. Early age viral infection, allergen sensitization, atopy and genetic predisposition increases the likelihood of developing asthma. Our aim is to understand the consequences of allergen exposure (house-dust mite; HDM) and/or RSV infection on innate immune responses, cytopathogenesis and virus replication in paediatric airway epithelium.

Well-differentiated primary paediatric nasal epithelial cell (WD-PNEC) cultures derived from newborn or pre-school children were infected with RSV before or after stimulation with HDM. Virus growth kinetics, cytopathology and innate immune responses were analysed.

HDM pre-treatment did not affect the culture integrity or RSV growth. Preliminary RT-qPCR analysis revealed upregulation of *irf9* in RSV infected/HDM treated and HDM pre-treated/RSV infected cultures at 24 & 96 hours post-infection (hpi). *Isg15* was also upregulated in HDM pre-treated/RSV infected cultures at 24 hpi in newborns only. At 96 hpi, *irf9*, *isg15, ifi6, duox2* and *duoxA2* were upregulated in RSV infected cultures. Surprisingly, HDM treatment alone did not induce any significant upregulation of these genes compare to untreated/uninfected controls. However, data from more donors will be required to confirm these preliminary data. IL-29/IFNλ1 secretion was also significantly reduced following HDM pre-treatment of RSV infected cultures, while there was a trend towards reduced IL-6 secretion. These results suggest that HDM exposure modulates the innate immune responses to RSV infection of airway epithelium. The preliminary data are consistent with our over-arching hypothesis that our model of aeroallergen exposure and viral infection of airway epithelium will help elucidate mechanisms by which pre-school wheeze develops.

Virology workshop: Innate immunity
Presentations: Monday and Tuesday evening

A240

Quantitative Analysis of Protein Degradation During HCMV Infection identifies Helicase-like Transcription Factor as a Novel Antiviral Restriction Factor

Kai-Min Lin[1], Katie Nightingale[1], Benjamin Ravenhill[1], Colin Davies[1], Luis Nobre[1], Ceri Fielding[2], Lior Soday[1], Alice Fletcher-Etherington[1], Hester Nichols[2], Eddie Wang[2], Yagnesh Umrania[1], Robin Antrobus[1], Gavin Wilkinson[1], Richard Stanton[2], Peter Tomasec[2], Michael Weekes[1]

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

Abstract

Significant progress has been made quantifying how viruses up- or down-regulate host proteins or transcripts. However, a given virus may regulate hundreds of host proteins meaning that a functional approach is required to identify novel molecules most likely to be important in innate antiviral immunity.

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus often targets host antiviral factors for degradation to promote infection and replication. To identify host proteins specifically targeted for early degradation, we developed three orthogonal multiplexed tandem mass tag-based proteomic screens. Several known antiviral restriction factors, including components of cellular promyelocytic leukemia nuclear bodies Sp100 and MORC3 were enriched in a shortlist of degraded proteins.

A particularly robust novel 'hit' which was the helicase-like Transcription Factor (HLTF), a DNA helicase important in post-replication DNA damage repair. HLTF was degraded throughout early infection and potently inhibited early viral gene expression. HLTF knock-down cells exhibited significantly increased early viral gene expression, suggesting that HLTF as a critical early HCMV restriction factor. Possible mechanisms of action of HLTF during HCMV infection are either as a sensor that detects viral DNA in the nucleus, or a transcription factor that regulates viral gene expression. We will present data examining these possibilities.

Overall, our work provides powerful screening tools to identify novel HCMV restriction factors and provide a clearer view of how HCMV manipulates host proteins for immune evasion and favours its own survival. Specifically, we show that virus readily targets host DNA repair mechanisms to counteract the intricate innate antiviral immunity.

A241

Inhibition of IFN-gamma signalling by vaccinia virus protein C6: a multi-functional interferon antagonist.

Callum Talbot-Cooper[1], Yongxu Lu[1], Jennifer Stewart [1,2], Geoffrey Smith[1]

[1]University of Cambridge , Cambridge , United Kingdom. [2]Department of health, London, United Kingdom

Abstract

Vaccinia virus (VACV) encodes multiple proteins that function to inhibit both the production and downstream effects of interferons, which are critical modulators of the antiviral response. One such protein, VACV C6, inhibits both the production of type I IFN (by blocking IRF3 signalling) and the downstream effects of type I IFN (by blocking JAK-STAT signalling). Further to this, our findings suggest C6 can also inhibit the downstream effects of type II IFN of which IFN-gamma is the sole member. The observation that C6 co-immunoprecipitates with STAT1, a protein crucial in activating the downstream functions of IFN-gamma, might explain its inhibitory activity.

A242

THE ANTI-RSV ACTIVITY OF IRF9 & IFI6: TWO GENES IMPLICATED IN SEVERE RSV DISEASE

Jonathon Coey, Lindsay Broadbent, Helen Groves, Patricia Coulter, Michael Shields, Ultan Power

Queens University Belfast, Belfast, United Kingdom

Abstract

Background:

Respiratory syncytial virus (RSV) is the main cause of severe lower respiratory tract infections (LRTI) in infants worldwide and there is no vaccine currently available. Most infants who develop severe RSV disease have no underlying conditions. However, irf9 and ifi6 were expressed much higher in RSV infected well-differentiated primary nasal epithelial cells from infants with mild RSV disease compared to the severe cohort.

Methods:

Kinetics and localisation of irf9 and ifi6 were investigated using RT-qPCR and immunofluorescent microscopy following infection or treatment of BEAS-2B cells with RSV or IL-29/IFNλ1, respectively. Antiviral activity was investigated by transfecting BEAS-2B cells with either siRNA or overexpression plasmid prior to RSV infection. Fluorescent images, supernatants, RNA, and cell lysates were taken every 24 hours.

Results:

Protein/gene expression of irf9 and ifi6 were upregulated following IL-29/IFNλ1 treatment or RSV infection. siRNA knockdown of irf9 or ifi6 resulted in a significant increase in RSV infection as measured by % fluorescence coverage and viral growth kinetics. Conversely, overexpression of IFI6 resulted in a significant decrease in RSV infection. These data indicate that irf9 and ifi6 have a significant anti-RSV activity. The mechanisms by which these proteins exert their antiviral activities is currently under investigation.

Conclusions:

Our data support the concept that diminished irf9 and ifi6 expression following RSV infection is associated with the development of severe RSV disease in infants, a phenomenon which to date has proven impossible to predict for the majority of hospitalised infants.

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A243

Influenza A Virus interactions with complement factor H

Iman Rabeeah[1], Uday Kishore[2], Beatrice Nal-Rogier[2], Holly Shelton[3]

[1]Brune University London / Pirbright Institute, London/ Surrey , United Kingdom. [2]Brunel University London, London, United Kingdom. [3]Pirbright Institute, Surrey , United Kingdom

Abstract

Influenza A virus (IAV) is a major causative agent of respiratory tract infection in humans. Human IAVs cause seasonal epidemics, whilst avian IAV strains sporadically cross the host range barrier leading to zoonotic infections. The complement system is a component of the innate immune system that acts to remove pathogens including IAVs. Many bacterial pathogens and several viral pathogens have been reported to actively target components of the complement activation pathway as a defensive strategy. The aim of this project is to understand if there is an interaction between IAV and co-factor H, a critical inhibitor of the complement system and what effect any interaction may have on influenza replication. Using purified human co-factor H protein and purified human (H1N1 and H3N2) and avian (H9N2 and H5N3) IAVs we have demonstrated using ELISA assays that an interaction does occur between co-factor H and all the IAV strains tested. Far western blot analysis suggests that the interaction is with the viral Hemagglutinin (HA), which is responsible for attachment and entry of IAV to cells. Interestingly the interaction has divergent effects on the replication of the IAV strains; enhancing human H1N1 replication and restricting H3N2 replication in A549 and THP-1 cells whilst having no effect on the replication of avian H5N3 or H9N2 viruses. Understanding IAV interaction with the complement pathway could enhance our ability to produce effective vaccines.

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A244

Early life innate immune responses to RSV in cystic fibrosis airway epithelium.

Patricia J. Coulter[1,2], Helen Groves[1,2], Lindsay Broadbent[1], Jonathan Dean Coey[1], Michael D. Shields[2,1], Ultan F. Power[1]

[1]Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queens University, Belfast, United Kingdom. [2]Royal Belfast Hospital for Sick Children, Belfast Health and Social Care Trust,, Belfast, United Kingdom

Abstract

Respiratory syncytial virus (RSV) infection is the major cause of severe lower respiratory tract disease in young infants. Evidence suggests that cystic fibrosis (CF) is associated with significant morbidity following RSV infection. We and others previously demonstrated that airway epithelium is the primary target of RSV infection. However, little is known about the impact of RSV infection on CF airway epithelium. To address this, we established and characterised well-differentiated primary nasal epithelial cell cultures (WD-PNECs) from recently diagnosed CF infants to study RSV cytopathogenesis in CF airway epithelium. CF WD-PNECs were successfully generated from 11 infants and characterised by light and fluorescent microscopy. CF WD-PNECs secreted thick dry apical mucous, consistent with *in vivo* observations. Extensive cilia coverage was also evident, although cilia beat frequencies appeared lower than those evident in WD-PNECs from healthy neonates. Quantification of goblet and ciliated cells in the CF WD-PNEC cultures were similar to those of healthy cultures. Viral growth kinetics were similar for CF WD-PNECs and healthy WD- PNECs, with peak virus titres evident at 72-96 hpi. CXCL8/IL-8 and CXCL10/IP-10 secretions were upregulated following RSV infection of CF WD-PNECs, while IL-6 secretions did not change. Interestingly, our data suggested that *duoxa2* and *duox2* expression post-infection were reduced in WD-PNECs from CF compared to healthy infants. Our data suggest that this model provides an exciting opportunity to elucidate the cytopathogenic, inflammatory and molecular consequences of RSV infection of airway epithelium derived from very young CF infants.

A245

Correlative 3D X-ray and Super-Resolution Fluorescence Microscopy of Herpesvirus Assembly

Kamal Nahas[1,2], Viv Connor[1], Maria Harkiolaki[2], Colin Crump[1], Stephen Graham[1]

[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Diamond Light Source, Harwell Science & Innovation Campus, Didcot, Oxfordshire, United Kingdom

Abstract

Herpesviruses assembly is a complex multi-step process involving capsid assembly and genome packaging in the nucleus, transport into the cytoplasm (via budding into the perinuclear space followed by fusion with the outer nuclear membrane), acquisition of the proteinaceous tegument layer, and budding into intracellular endocytic compartments that contain the viral envelope proteins. Previous investigations of these processes have largely relied upon fluorescence microscopy, with limited resolution, or ultra-thin section electron microscopy, with potential artefacts caused by fixation and staining. Soft X-ray tomography of cryo-preserved samples (cryoSXT) can overcome these limitations: samples do not require staining or fixation and the highly-penetrating nature of X-rays allows the entire depth of a cell to be imaged without physical sectioning. Using the unique multi-modal imaging capabilities of Diamond Light Source beamline B24, we have combined cryoSXT with cryo-structured illumination microscopy (cryoSIM) to investigate the assembly of herpes simplex virus-1 (HSV-1) during infection of cultured cells. This allows us to correlate the ultrastructure of infected cells (cryoSXT) with the location of viral proteins and/or cellular markers (cryoSIM). By combining cryoSXT and cryoSIM we aim to identify how specific HSV-1 proteins stimulate the changes to intracellular membrane morphology that are required for efficient virus assembly.

A246

Altering the size distribution of influenza virion populations

Amy Burke[1], Jack Hirst[2], Edward Hutchinson[2]

[1]Glasgow University, Glasgow, United Kingdom. [2]MRC- University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Harry Smith Vacation Studentship

Laboratory-adapted influenza viruses produce predominantly spherical virions. In contrast, clinical and veterinary isolates produce a mixture of virions of different sizes, from 0.1 µm spheres to filaments which can reach tens of microns in length. Filamentous influenza virions were discovered in 1946, but the bulk of influenza research has analysed only spherical forms of the virus and the role of filaments in influenza infections is unclear. Functional studies of filaments require the development of methods to manipulate the ratio of spherical to filamentous virions, and we reasoned that this could be achieved by filtration.

To test this, we infected MDCK cells with the filamentous Udorn strain of influenza A virus. We collected virus-containing growth media and passed this through filters with 5 µm, 0.45 µm and 0.2 µm pores. Filtrates and unfiltered virus were compared, using Western blotting to measure their protein composition, plaque assays to measure their infectivity and negative stain transmission electron microscopy to measure individual particle sizes.

We found that filtration through a filter with 5 µm pores had little effect on composition, infectivity and the ratio of spherical to filamentous particles. In contrast, sub-micron filters, particularly those with 0.2 µm pores, caused a general depletion of virions but increased the sphere to filament ratio.

We therefore concluded that sub-micron pore sizes can be used to preferentially remove filaments from populations of pleomorphic influenza virions, providing a useful tool for subtractive studies of the contribution filaments make to influenza virus infections.

A247

Single-particle measurements reveal damage to filamentous influenza virions during laboratory handling

Jack Hirst, Amy Burke, Edward Hutchinson

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

Abstract

Most laboratory strains of influenza virus produce near-spherical virions, but clinical isolates also produce extended filaments whose biophysical properties are understudied. Most functional studies of filamentous influenza viruses do not include data on the concentration or lengths of the virions, making it hard to interpret their sometimes contradictory results. Furthermore, anecdotal reports suggest that filaments are damaged during routine laboratory handling. Therefore, to understand filament function we require a tool to assess the number and dimensions of filaments in a sample and an assessment of how filaments respond to standard handling procedures.

We initially sought to analyse filament populations using negative stain particle counting, but found that this was low-throughput and could not detect particles longer than 10 µm. Instead, we used confocal microscopy with semi-automated image analysis. This allowed a high-throughput, quantitative analysis of length distributions in filament populations. Using this, we assessed the effects of pipetting, vortexing, sonicating, clarification and freezing on filaments. Most procedures did not appreciably alter filament dimensions. Pipetting and vortexing both slightly reduced filament numbers, but their effects were only appreciable after extended treatment. In contrast, freezing substantially reduced the number and median length of filaments, as well as creating “kinks” in filaments which suggest damage to the capsid.

We conclude that confocal microscopy can provide the basic measurements needed to interpret functional studies of filamentous strains. Using this approach, we found that freezing filaments causes previously unappreciated damage, which should be considered when planning further research.

A248

Infectious Sub-Viral Particles of bluetongue virus exhibit different infectivity and use different entry mechanisms compared to whole virus particles

Marc Guimerà Busquets[1], Lyndsay Cooke[1], Lisa Stevens[2], Kerry Newbrook[1], Kathryn Moffat[1], Amelia Powell[1], Karen Chong[1], Terry Jackson[1], Karin Darpel[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

Bluetongue virus (BTV) is a vector-borne virus transmitted between susceptible ruminant hosts by *Culicoides* biting midges. BTV belongs to the genus *Orbivirus*, within the family *Reoviridae*, and its genome consists of 10 segments of double stranded (ds)RNA encoding 7 structural and 5 non-structural proteins. Similar to other members of the *Reoviridae* family, such as *Rotaviruses* and *Reoviruses*, the capsid of BTV particles can be modified by proteases from the environment to generate Infectious Sub-Viral Particles (ISVPs). ISVPs retain infectivity and may even be more infectious to specific cell populations than whole particles. The different structure of the external surface shell of ISVPs implies the involvement of different viral epitopes during ISVP cell-attachment and cell-entry, and therefore possibly alternative entry pathways compared to those of the virus particles with a full capsid, as shown for other *Reoviridae*. In this study, BTV particles were purified and ISVPs generated using different proteases (chymotrypsin and *Culicoides* vector saliva). Their infectivity on several relevant mammalian and insect cell populations was investigated, as well as the entry mechanisms used by both particle types. Whole particles and ISVPs were found to exhibit different infectivity and used different entry mechanisms in specific cell populations. In general, the role and biological implications of the different BTV particle types have not been widely studied, although it appears likely that the ability to infect cells using different cell-binding proteins/epitopes may be related to the wide range of hosts and cell types that can be infected *in vivo* by BTV.

A249

Examining receptor usage by the morbilliviruses: who, or what, is really at risk?

Carina Conceicao[1], Brian Willet[2], Dalan Bailey[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Centre for Virus Research, Glasgow, United Kingdom

Abstract

There are seven 'established' morbilliviruses: rinderpest (RPV), measles (MeV), small ruminant (PPRV), cetacean (CeMV), phocine (PDV), canine (CDV) and feline (FmoV) morbillivirus, the majority of which are thought to have narrow host ranges. The exception is CDV, which has been reported in almost all families of the order *Carnivora*, as well as non-human primates. Morbilliviruses enter cells through one of two proteinaceous receptors: signalling lymphocyte activation molecule F1 (SLAMF1), present on the surface of many immune cells; or Nectin-4, expressed on the basolateral side of various polarised epithelial cells. Given the close similarity of these viruses and their universal use of related receptors, there are realistic fears morbilliviruses may spill-over into naïve hosts, i.e. following eradication of RPV in cattle. This spill-over is not entirely unprecedented; PDV and MeV are thought to share common ancestors with CDV and RPV, respectively.

To examine morbillivirus receptor usage and predict future spill-over and zoonotic transmission events, we have developed a high-throughput, quantifiable, cell-cell fusion assay. Effector cells, expressing the viral F and H glycoproteins as well as a split-luciferase reporter, are co-cultured with target cells expressing SLAMF1 or Nectin-4 receptors and the remaining half of the reporter. Upon functional receptor engagement the effector and target cells fuse, allowing cytoplasmic mixing and reconstitution of the reporter, a process which can be quantified. Using these assays we are investigating whether the receptor usage profile of morbilliviruses represents an important factor in spill-over transmission into new hosts.

A250

Understanding assembly of infectious norovirus particles to build better vaccines

Oluwapelumi O. Adeyemi, Nicola J. Stonehouse , Morgan R. Herod

University of Leeds, Leeds, United Kingdom

Abstract

Known commonly as 'winter vomiting disease', noroviruses are a common cause of acute viral gastroenteritis. Disease outbreaks apply an annual stress to healthcare services and cost the NHS >£40-million per year. Worldwide norovirus infections are responsible for >200,000 deaths per year. One approach to prevent norovirus infections would be to develop a stable vaccine such as a virus-like particle.

Noroviruses are part of the *Caliciviridae*, a family positive-sense RNA viruses. The viral genomes are enclosed within a non-enveloped capsid that consists primarily of the major viral capsid protein, VP1, and fewer copies of the minor capsid protein, VP2. These two structural proteins are produced primarily from translation of a viral sub-genomic RNA produced during viral RNA replication. However, it is largely unclear how these two viral proteins assemble with nascent viral genomic-length RNA during replication to generate new infectious virions.

In this study, we have used the murine norovirus (MNV) model system to study assembly of infectious norovirus particles. Using immunofluorescent and proximity ligation assays, we have probed the interactions between the viral capsid proteins and the replication proteins. These interactions have been validated by immuno-precipitation assays and western blot experiments and suggested a functional interaction between capsid proteins and membrane-associated viral non-structural proteins. Using mutagenesis and labelling of viral RNA we have investigated the presence of nascent and template viral genomes in these complexes. Work is ongoing with genetic complementation assays to identify which aspects of the function of the viral non-structural proteins are required to assemble new virions.

A251

Resolving the Molecular Gymnastics of the Elusive Herpes Simplex Fusion Protein

Lorelai Vennard[1], Alana Maerivoet[1], Doina Atanasiu[2], Wan Ting Saw[2], Roselyn Eisenberg3[2], Gary Cohen[2], Juan Fontana[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Pennsylvania, Philadelphia, USA

Abstract

Herpes Simplex Virus (HSV) afflicts ~90% of the population, causing a range of diseases from oral cold sores to encephalitis. Despite the widespread effects of HSV, current drugs cannot cure infection and there are no preventative vaccines. HSV infection requires entry into the host cell, which is mediated by the fusion protein, gB. Despite various structures being available for post-fusion gB, a high-resolution model for the pre-fusion state, required to understand the fundamental mechanism of HSV fusion, is still lacking. To elucidate this structure, full length gB was previously expressed on lipid vesicles and was shown via cryo-electron microscopy techniques to adopt the elusive pre-fusion state. Strikingly, two recent studies, one characterising such vesicles, generated two different pre-fusion gB models. The discrepancy between the models can be summarised by the position of the fusion loops: are they positioned facing away from the viral membrane or towards it in the pre-fusion state? Using the gB studded vesicle production method combined with a state-of-the-art Titan Krios cryo-electron microscope equipped with a Volta Phase Plate, a direct electron detector camera and an energy filter, all to increase contrast and signal-to-noise ratio, we aim to settle the dispute as to the structure of pre-fusion gB. Revealing the pre-fusion structure could lead to possible therapeutics to a disease that can have serious complications in newborns and the immunocompromised, a growing cohort in the modern world.

A252

Elucidating conserved interactions between viral glycoproteins and cellular factors.

Thomas Rix, James Kelly, Dalan Bailey

The Pirbright Institute , Guildford, United Kingdom

Abstract

Viral glycoproteins are found on the surface of all enveloped viruses, mediating binding to host receptors and the initiation of entry events. In addition, numerous vaccines employ the same viral glycoproteins as immunogens, either vectored or recombinant in nature. During infection viral glycoproteins are thought to interact with various host-factors, facilitating their trafficking to the cell surface. However, these interactions are not currently well understood or characterised. Using a human gene expression microarray, the cellular response to expression of various viral glycoproteins (Ebola, Nipah, VSV and Measles) was assessed *in vitro*. Specifically, we found a number of genes with a fold change greater than 2 displaying significantly altered expression across all four glycoprotein transfections. A subset of these genes were selected for validation by qPCR and extended to RSV (respiratory syncytial virus) fusion protein (F) transfection. The expression of these genes was then further investigated in Measles and RSV infected cells. Our data has identified host genes with altered expression in response to a diverse panel of viral glycoproteins, potentially elucidating a conserved set of pathways important for viral glycoprotein activity. Greater understanding of the proteins and pathways involved in glycoprotein expression has the potential to identify mechanisms underpinning host susceptibility to disease as well as improving the yield of vaccine producing cells.

A253

An exploration of antigen expression of hepatitis C entry receptors on equine cells in relation to equine hepacivirus A

Mascha Sohrmann[1], Janet M. Daly[1], Julia Scott[1], Alexander W. Tarr[2], Julia H. Kydd[1]

[1]School of Veterinary Medicine and Science, The University of Nottingham, Leicestershire, United Kingdom. [2]School of Lifesciences, The University of Nottingham, Nottingham, United Kingdom

Abstract

Equine hepacivirus A (EqHV) belongs to the family *Flaviviridae* and has been identified as the hepacivirus phylogenetically most closely related to Hepatitis C virus (HCV). Like HCV, EqHV is a hepatotropic virus that has been reported in over fourteen countries from six continents. It has a high prevalence in some populations, in particular the Thoroughbred breed. However, much is still unknown about EqHV, such as its pathogenesis and mode of transmission. The main four receptors used by HCV for viral entry to human hepatocytes are Cluster of Differentiation-81 (CD-81), occludin (OCLN), claudin-1 (CLDN-1) and Scavenger Receptor Class B Member 1 (SCAR-B1). This study investigated the distribution and expression of these entry receptors on equine cells by flow cytometry, immunocytochemistry and immunohistochemistry. Using a human liver cell line (Huh-7) as a positive control, antibodies against HCV receptors appeared to cross-react with antigens on equine cells. The proportion of fetal horse kidney cells that expressed CD-81, OCLN, and CLDN-1 was 37.2%, 16.0%, and 7.0%, respectively, whereas the equine dermal cells (E.Derms) expressed CD-81 (96.0%), OCLN (1.2%) and CLDN-1 (1.7%). CD-81, OCLN and CLDN-1 were also expressed on E.Derms and Huh7 cells, as detected by immunocytochemistry and on equine liver cells and the allantochorionic region of Thoroughbred placenta by immunohistochemistry. These findings form the basis for further comparative investigation into the entry receptors used by EqHV to infect equine and human cells. Such information may inform future studies on EqHV pathogenesis and mode(s) of transmission.

A254

High throughput screening to identify host-factors involved in RSV fusion

James Kelly[1], Leanne Logan[1], Julian Hiscox[2], Stuart Armstrong[2], Sam Wilson[3], Dalan Bailey[1]

[1]The Pirbright Institute , Pirbright , United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom.
[3]University of Glasgow, Glasgow, United Kingdom

Abstract

Respiratory syncytial virus (RSV) is the major cause of childhood respiratory disease; however, currently there is no licenced vaccine available and the only therapeutic, a monoclonal antibody against the viral Fusion (F) protein, is expensive and applied sparingly. RSV particles enter cells by membrane fusion, orchestrated by F – a type I integral membrane protein. This process was recently shown to involve macropinocytosis of the particle. Separately, RSV can spread through induction of direct cell-cell fusion – again orchestrated by F.

Little is currently known about the host-factors involved in regulating or inhibiting RSV F-mediated fusion. Here, using two different high-throughput screening approaches, we have identified host-factors involved in regulating RSV fusion. Using quantitative mass-spectrometry analysis of isolated cell membrane fractions from mock and RSV-infected cells we have identified membrane proteins which are differentially regulated during RSV infection. Furthermore, using lentiviral libraries expressing individual interferon stimulated genes (ISGs) from different mammalian species we have investigated ISG-mediated inhibition of RSV fusion.

Our data provides important insights into host-factors involved in RSV spread, furthering our understanding of the fusion process and identifying potential targets for antiviral therapy.

A255

The Role of the cellular protein Dock5 as an egress restriction factor for Herpesvirus

Ben Chadwick, Sophie Schumann, Martin Fuller, Adrian Whitehouse, Juan Fontana

University of Leeds, Leeds, United Kingdom

Abstract

The herpesvirus family contains eight human pathogens that act as the causative agent of several human maladies. The herpesvirus family can be further subdivided alphaherpesviruses, betaherpesviruses, and the oncogenic gammaherpesviruses. Herpesvirus replication and capsid assembly occurs within the nucleus, from there capsids migrate into the cytoplasm. Following nuclear egress, viral capsids travel to the cellular membrane, where morphogenesis takes place. Recent analysis has demonstrated that the gammaherpesvirus Kaposi's sarcoma associated herpesvirus (KSHV)upregulates the expression of a host miR-365 to target the cellular protein, DOCK5, to enhance KSHV egress. This hypothesis is supported by the observation that inhibition of miR-365 or overexpression of DOCK5 leads to the prevention of KSHV egress and accumulation of capsids at the plasma membrane. As herpesviruses share several mechanistic features, we are now employing electron microscopy to visualise the cytoplasmic capsid accumulation and to determine how DOCK5 is involved in herpesvirus egress. This will be achieved through advanced microscopy techniques such as correlative light microscopy, cryo-electron microscopy, and a novel nanobiopsy approach known as nanopipetting. Currently we have been establishing the optimal time of capsid egress in both KSHV and Herpes Simplex Virus-1 which will aid in visualising the process through transmission electron microscopy. A better understanding of herpesvirus egress will potentially help identify novel antiviral targets against herpesviruses.

A256

Production of Influenza C HEF pseudotyped lentiviral particles

Y Tatawi

University of Kent, Kent, United Kingdom

Abstract

While influenza A and B virus contain the two glycoproteins Hemagglutinin (HA) and Neuraminidase (NA) inserted into the viral membrane, influenza C virus possesses only one spike designated Hemagglutinin-Esterase-Fusion (HEF) protein which combines the functions of both HA and NA. Like HA, it recognizes and binds to a receptor on the cell surface to initiate virus entry. However, the receptor is N-acetyl-9-O-acetylneuraminic acid. HEF is the receptor-destroying enzyme, which is the function of the neuraminidase (NA) in influenza A and B virus.

Although Influenza C virus infections are generally mild and self-limited, it comprises around 13% of influenza positive cases in hospitalized children. It is also a cause of community acquired pneumonia of children. As influenza research has been more focused on Influenza A and B, it is of interest to pseudotype influenza C HEF.

The full sequence of Inflenza C HEF (C/Tokyo/4/2014) was obtained and cloned into pi.18 expression plasmid. The second generation packaging construct pCMVΔR8.91, and the self-inactivating lentiviral vector pCSFLW expressing firefly luciferase, and pCSGW expressing GFP were used for lentiviral vector particle production. The original HEF sequence was mutated by site directed mutagenesis to inhibit esterase activity to prevent receptor destruction prior to pseudotype viral entry. Pseudotyped particles bearing the HEF glycoprotein were successfully produced and verified by fluorescence and Luciferase titration assay with titres of 5X10^7 RLU/ml. Currently, optimization is undergoing to achieve higher titres as well determining the specific protease/s utilized by HEF for its activation.

A257

A new age teaching methodology in Microbiology for Higher Education and its different aspects

Arnab Kumar Chakraborty, Malay Bhattacharya, Arnab Sen

University of North Bengal, India

Abstract

Teaching in Higher education (HE) in the field of experimental sciences especially in microbiology should be problem-based learning so that professionals and HE students get to know the practical aspect of the subject. The teaching of microbiology at any level becomes more lucid with a little historical approach, highlighting the work of pioneers such as Anton Van Leeuwenhoek, Robert Koch, Alexender Fleming, Louis Pasteur, Sergei Winogradsky and Carl Woese etc. This becomes inevitable if the target learners are from a non-biological field or with little knowledge of chemistry. This way the professionals can get the hang of it before they get any further with the subject. The very next step would be to provide a demographic overview of the subject showcasing different aspects like general, medical, environmental and evolutionary microbiology. The teaching modules should also dwell with basic concepts of organic and inorganic chemistry necessary to understand the principles of fermentation and chemoautotrophy and basic molecular biology to explain biotechnology using transgenic microorganisms and molecular phylogeny. However, in this era of cyberspace, no form of learning is restricted inside a far-off classroom or a hard to reach expert. It is equally important to make students aware of the lecture podcasts for flipped learning, learning from experts via video conference, webinars and several online courses available in different sites also made available in form of mobile application. The higher education learning should also include recent improvements in the field (especially for advanced learners) like synthetic biology with an introduction to iGEM technology and bioinformatics evaluation of microbial genomes. These aspects if taken care with highly interactive sessions can indeed improve the quality of teaching in HE.

A258

Engaging and educating the public by galvanizing the voices of female microbiologists from atop a soapbox

Geertje van Keulen

Institute of Life Science, Swansea University Medical School, Swansea, Wales, UK

Abstract

The vision of Soapbox Science, the brainchild of Drs Nathalie Pettorelli and Seirian Sumner, is brilliantly simple. Take science out to the public – literally on the street corner – and at the same time give women visibility as successful researchers across all science, technology, engineering, maths and medicine (STEMM) subjects. This novel format has been amazingly effective on both counts, and as a result has now spread across the continents. Here, I will give an account of how female microbiologists from atop soapboxes in the shopping street have engaged and educated the public in Swansea about current topics in microbiology. While the effect of organising such events has enabled women in STEMM to become more visible to the general public, it has also led to building up of confidence in speaking up, not only in public but also within disciplines, departments and higher education in general. The enthusiasm of sharing female passion for microbiology and STEMM with the general public has generated powerful voices emerging from an ever growing global network of female scientists.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B001

Genomic and epidemiological insights into chlamydial epitheliocystis

Alyce Taylor-Brown[1,2], Trestan Pillonel[3], Martina Jelocnik[1], Gilbert Greub[3], Lloyd Vaughan[4,5], Barbara Nowak[6], Adam Polkinghorne[1]

[1]University of the Sunshine Coast, Sippy Downs, Australia. [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom. [3]Institute of Microbiology, University of Lausanne, Lausanne, Switzerland. [4]Institute of Veterinary Pathology, University of Zurich, Zurich, Switzerland. [5]Pathovet, Tagelswangen, Switzerland. [6]Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, Australia

Abstract

Chlamydiae are obligate intracellular bacteria with highly reduced genomes, reflective of their abilities to sequester nutrients from the host. The host range of this phylum far exceeds that described for the *Chlamydia* genus which encompasses terrestrial animals; however, much of this diversity is uncultivated. Of particular evolutionary significance are members of the earliest diverging family, *Candidatus* Parilichlamydiaceae, comprising only uncultivated species associated with the fish gill disease, epitheliocystis. Epitheliocystis poses a significant threat to aquaculture industries globally. Due to the lack of culture systems for *Ca*. Parilichlamydiaceae, little is known about their biology, which also imposes further limitations on answering epidemiological questions.

Gill extracts from three *Chlamydiales*-positive Australian aquaculture species were subject to DNA preparation to deplete host DNA and enrich microbial DNA, prior to metagenome sequencing. We obtained three metagenome-assembled *Ca*. Parilichlamydiaceae genomes from three different host species, and conducted functional genomics comparisons with diverse members of the phylum. Using these genomes, we developed a novel *Ca*. Parilichlamydia carangidicola-specific multi locus sequence analysis (MLSA) scheme to investigate genetic diversity in this species

Genomic analysis revealed highly reduced genomes (~0.8Mbp) that share 342 orthologs with other chlamydial families, with a notable reduction in genes for synthesis of nucleotides and amino acids. MLSA revealed a high level of genetic diversity of *Ca*. P. carangidicola, with 20 genotypes distributed among 29 samples from four populations within the same aquaculture facility.

Culture-independent genomics has provided an unprecedented insight into chlamydial evolution, pathogenicity and epidemiology. This approach could be adapted for further genomic epidemiological investigations.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B002

Comparative genomic analysis of Serratia spp.

David Williams[1,2], Eva Heinz[2,3], Katarzyna Hencel[1], Kerry Pettigrew[4], François-Xavier Weill[5], Patrick Grimont[5], Matthew Holden[4], Nicholas Thomson[2,6], Sarah Coulthurst[1]

[1]University of Dundee, Dundee, United Kingdom. [2]Wellcome Sanger Institute, Hinxton, Cambridge, United Kingdom. [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [4]University of St Andrews, St Andrews, United Kingdom. [5]Institut Pasteur, Paris, France. [6]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Members of the genus *Serratia*, belonging to the family Enterobaceriaceae,are ubiquitous in the environment yet greatly understudied, particularly in a genomic context. To some extent, the nosocomial pathogen *Serratia marcescens* is an exception, nevertheless the molecular mechanisms behind its pathogenesis and wide tissue tropism are poorly understood. *S. marcescens*, a typical opportunistic pathogen, is notable for its intrinsic and rising acquired antimicrobial resistance, alongside an aptitude for inter-bacterial competition, including via deployment of a potent Type VI secretion system (T6SS).

In order to fully understand the *Serratia* genus, and the evolution of inter-bacterial competition mechanisms such as the T6SS within it, we have performed whole-genome sequencing (WGS) on a collection of *Serratia* isolates from across the genus and on a number of clinical *S. marcescens* isolates from distinct infection sites and hospitals in the United Kingdom. Analysis of these genomic sequnces, in conjunction with other published *Serratia* sequences, has revealed that a number of distinct T6SSs are found within the genus *Serratia.* Some of these T6SS are species specific, whilst others are found across several species, in a pattern implying multiple acquisition events. Complementary experimental analyses have allowed correlation of varied anti-bacterial competitive phenotypes with corresponding genotype in selected isolates of *S. Marcescens*.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B003

Whole-genome sequence analyses of Fusobacterium necrophorum

Lesley Hoyles[1], Karima Brimah[2], Pamela Greenwell[2]

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

Abstract

Fusobacterium necrophorum is a strictly anaerobic, non-spore-forming, Gram-negative bacillus encompassing two subspecies. *Fusobacterium necrophorum* subsp. *necrophorum* (FNN) is commonly found in animals, where it is the causative agent of foot rot and abscesses. *Fusobacterium necrophorum* subsp. *fundiliforme* (FNF) is found in humans and is the causative agent of Lemierre's disease, a condition associated with throat infections. Little is currently known about the genomic diversity of the two subspecies. Whole-genome sequences were generated for 18 *Fusobacterium necrophorum* isolates recovered from recurrent and persistent sort throats in UK patients. Sixty-three whole genome sequences available for *Fusobacterium* spp. were downloaded from GenBank and compared with the newly sequenced isolates. All-against-all comparisons of average nucleotide identity (ANI) were done to confirm species affiliation. Strains of FNN (*n*= 6) and FNF (*n*= 43) shared ~97 % ANI with each other, while strains of the same subspecies shared >98 % with one another. All the newly sequenced isolates belonged to FNF. All 49 *Fusobacterium necrophorum sensu stricto* genomes were subject to comparative and pangenome analyses. The strains fell into two groups, with the majority of strains clustering with the type strain of FNF. Three distinct clusters of strains were identified within the FNF group, and analyses are underway to determine their potential clinical significance. Analyses of virulence-associated leukotoxin genes are also being undertaken. It is envisaged that in-depth analyses of a large collection of *Fusobacterium necrophorum* genomes, particularly those of FNF, will provide novel insights into pathogenesis of these fastidious anaerobic bacteria.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B004

The *E. coli* gene pool: utilising data availability with a subsampling approach.

Gal Horesh[1], Eva Heinz[1], Leopold Parts[1], Jukka Corander[1,2,3], Nicholas Robert Thomson[1,4]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom. [2]University of Helsinki, Helsinki, Finland. [3]University of Oslo, Oslo, Norway. [4]London School of Hygiene and Tropical Medicine , London, United Kingdom

Abstract

Escherichia coli is a highly diverse and adapted organism. Commensal *E. coli* is a harmless member of the gastrointestinal tract, whereas pathogenic variants of the species have the ability to cause severe intestinal and extra-intestinal disease including urinary tract infections, bloodstream infections and meningitis. Pathogenicity is often the result of gain of genes which enable *E. coli* to colonise, evade and multiply within the human host. We set to utilise the availability of thousands of *E. coli* genomes in public databases to examine the diversity of the *E. coli* gene pool. Our approach is based on the construction a reference of *E. coli* genes and their abundances based on repeated subsampling of available genomes, which enables a high-level description of the prevalence of different genes in the *E. coli* population. Additionally, we can identify genetic elements which are significantly enriched within an *E. coli* lineage or pathotype compared to the general population and may be contributing to their success. The code for this approach is available to download from github and can be applied to other organisms to address other important questions.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B005

Different oral niches give rise to varying levels of genetic diversity in *Streptococcus mitis.*

Charlotte Davison, Sandra Beleza

University of Leicester, Leicester, United Kingdom

Abstract

Background: The commensal bacterium, *Streptococcus mitis* is a major coloniser of the oral cavity. This close cousin of *Streptococcus pneumoniae* has been identified to occupy a range of oral niches independent of pH, epithelial surface and redox state. We have previously observed a high level of genetic diversity in *S. mitis* with an open pan-genome and only 46% of the genome identified as core.

Aim: We aim to identify the driving force behind the high levels of genetic diversity identified in *S. mitis* and determine if this diversity can be attributed to oral location.

Methods: Samples were collected from the buccal mucosa and tongue dorsum. A total of 75 *S. mitis* isolates were whole genome and IonTorrent sequenced and subject to bioinformatic analysis.

Results: In contrast to *S. pneumoniae*, genetic diversity in *S. mitis* is predominantly driven by mutation with recombination playing only a minor role. It was also observed that isolates collected from the tongue dorsum were significantly more diverse than those collected from the buccal mucosa. Due to the mutational nature of *S. mitis* diversity, this suggests varying pressures on *S. mitis* in these two environments. Study is now underway to establish the genetic differences between *S. mitis* from these oral niches and determine how this will impact future study and study design of this significant oral microbiome component.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B006

Gut microbiota in Northern Thai populations

Siam Popluechai, Niwed Kullawong, Eleni Gentekaki

Mae Fah Luang University, Chiang Rai , Thailand

Abstract

The human gastrointestinal tract represents a collection of prokaryotic and eukaryotic microorganisms. It has been reported that gut microbiota plays a role in health and disease. Imbalance of gut microbial communities has been implicated in several non-communicable diseases including inflammatory gastrointestinal diseases, Type 2 diabetes, and obesity. However, a role of gut microbiota in adult Southeast Asians remains largely unexplored. Herein, we present gut microbiota data obtained from Thai volunteers living in Chiang Rai Province, Thailand. We use a combination of quantitative PCR, Next Generation Sequencing (NGS), and gas chromatography-mass spectrometry (GC-MS) to investigate microbial communities, individual microbial taxa, and metabolites of NCDs volunteers. We consider prokaryotic taxa and their relationships with body mass index/waist to hip ratio and diet. We find that specific taxa of lower taxonomic levels are associated with the lean and overweight, but not the obese phenotype. We also find that microbial prokaryotic communities differ significantly amongst the different study groups.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B007

High quality reference genomes for toxigenic and non-toxigenic *Vibrio cholerae* serogroup O139

Matthew J Dorman[1], Daryl Domman[1], Muhammad Ikhtear Uddin[2], Salma Sharmin[2], Mokibul Hassan Afrad[2], Yasmin Ara Begum[2], Firdausi Qadri[2], Nicholas R Thomson[1,3]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom. [2]Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh. [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Toxigenic *Vibrio cholerae* of the O139 serogroup have been responsible for several large cholera epidemics in South Asia, and continue to be of clinical and historical significance today. This serogroup was initially feared to represent a new, emerging *V. cholerae* clone that would lead to an eighth cholera pandemic. However, these concerns were ultimately unfounded. The majority of clinically relevant *V. cholerae* O139 isolates are closely related to serogroup O1, biotype El Tor *V. cholerae*, and comprise a single sublineage of the seventh pandemic El Tor lineage. Although related, these *V. cholerae* serogroups differ in several fundamental ways, in terms of their O-antigen, capsulation phenotype, and the genomic islands found on their chromosomes. Here, we present four complete, high-quality genomes for *V. cholerae* O139, obtained using long-read sequencing. Three of these sequences are from toxigenic *V. cholerae*, and one is from a bacterium which, although classified serologically as *V. cholerae* O139, lacks the CTXϕ bacteriophage and the ability to produce cholera toxin. We highlight fundamental genomic differences between these isolates, the *V. cholerae* O1 reference strain N16961, and the prototypical O139 strain MO10. These sequences are an important resource for the scientific community, and will improve greatly our ability to perform genomic analyses of non-O1 *V. cholerae* in the future. These genomes also offer new insights into the biology of a *V. cholerae* serogroup that, from a genomic perspective, is poorly understood.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B008

Local genes, for local bacteria: biogeographical variation in Campylobacter accessory genome content

Ben Pascoe, Sam Sheppard

The Milner Centre for Evolution, Bath, United Kingdom

Abstract

Diarrhoeal disease remains a major cause of child morbidity, growth faltering and mortality in low and middle income countries (LMICs), with *Campylobacter* among the most common causes. Previous work has identified the major sources in the UK and US (e.g. contaminated poultry), however little is known about the risk factors and transmission routes in LMICs, where incidence is extremely high (up to 85% of children infected before 1yr) and suggests a different epidemiology. Risk factors such as household crowding, poor sanitation, consumption of contaminated water and cohabitation with animals, all constitute potential transmission risks but their relative importance is unknown. This is a major concern as frequent or chronic enteric (re)infection is linked to significant morbidity and growth faltering in children. Comparative genomics offers a solution for untangling complex transmission networks. *Campylobacter jejuni* and *C. coli* primarily inhabit the gut of birds and mammals and signatures of adaptation can be detected in their genomes. Therefore, by sequencing the genome of human clinical isolates and faecally contaminated environments it is possible to discern its origin. Pilot genomics studies of strains from humans, animals and food in LMICs have identified genomic variation in strains that may indicate differences in source, survival, transmission or virulence (compared to the UK). In particular we have identified globally distributed strains and lineages; country-specific strains; rapid dissemination of accessory genes in specific regions; evidence of within-household spread and strains associated with asymptomatic infection or high levels of antimicrobial resistance.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B009

Understanding the evolution and metabolic capabilities of the *Butyrivibrio* group

Sara Pidcock[1], Timofey Skvortsov[1], Fernanda Santos[2], Karen Siu Ting[1], Chris Creevey[1], Sharon Huws[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Federal University of Viçosa, Viçosa, Brazil

Abstract

Exploring and understanding the phylogeny of the *Butyrivibrio* group is imperative if we are ever to fully understand the consortium of ruminal microbial enzymes that are responsible for the catalysis of multifaceted reactions, such as biohydrogenation.

At present, taxonomic classification of the *Butyrivibrio* group is based primarily on butyrate production. This approach has become antiquated with the development of sequencing technologies and downstream bioinformatics analysis. This study investigated the taxonomic relatedness and functional capacity of the ruminal *Butyrivibrio* group using 72 genomes. Seventy-one *Butyrivibrio* group genomes were obtained via JGI (the Hungate 1000 project), and one additional bacterial strain was sequenced by ourselves. A 40 marker phylogenetic tree was constructed and visualised with the interactive Tree Of Life (iTOL), and pangenome analysis conducted using Spine/ClustAGE. Orthologous gene affiliations were identified using OrthAgogue, and glycosyl hydrolase families were identified using dbCAN then aligned with Clustal Omega. Data obtained showed that three primary clades were observed, namely the genus *Pseudobutyrivibrio*, *B. fibrisolvens*, and the remaining *Butyrivibrio* species. Pangenome analysis and orthologous gene affiliations revealed greater diversity within *Butyrivibrio* than *Pseudobutyrivibrio. Butyrivibrio* clades consistently showed smaller core genome sizes in comparison to *Pseudobutyrivibrio*, with core genome percentages as low as 4%, indicating high levels of variance. Glycosyl hydrolase alignment shows extensive sequence dissimilarity between genes on a nucleotide and amino acid level These findings suggest that the *Butyrivibrio* group are highly evolved to maintain competitiveness in the rumen and emphasises the need for further research into the biochemical capacity of the *Butyrivibrio* group.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B010

Investigating the pangenomes of microbial eukaryotes.

Charley McCarthy[1,2], David Fitzpatrick[1,2]

[1]Genome Evolution Lab, Dept. of Biology, Maynooth University, Maynooth, Ireland. [2]Human Health Research Institute, Maynooth University, Maynooth, Ireland

Abstract

Pangenomes evolve in prokaryotes as a result of promiscuous horizontal transfer of genetic material and other rapid evolutionary processes. Although eukaryotes are generally under more restrictive evolutionary constraints than prokaryotes (e.g. lower levels of HGT), pangenomic structure has also been identified in plant, algal and fungal species. A number of methodologies for pangenome analysis have been published in recent years, but these methodologies are generally specifically-intended or optimized for prokaryotes. We have developed a software pipeline based around the previously-published PanOCT method of pangenome construction with additional pre- and post-processing methodologies tailored for eukaryote pangenome analysis. We have previously used this pipeline to analyze the pangenomes of model fungal species such as *Saccharomyces cerevisiae*, and we are currently testing our pipeline by constructing and analyzing the pangenome of the industrially-relevant yeast species *Yarrowia lipolytica*. Analysis of fungal pangenomes shows that core and accessory eukaryotic species genomes encompass a variety of phenotypes and suggest that gene duplication events play a larger role in eukaryote pangenome evolution than HGT.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B011

Genome size reduction: design and analysis of a minimal metabolic network for yeast

Giorgio Jansen[1,2], Giuseppe Nicosia[1], Steve Oliver[1]

[1]Cambridge Systems Biology Centre , Cambridge, United Kingdom. [2]University of Catania, Catania, Italy

Abstract

The issue of genome minimization for a living cell has been the subject of intense study, and raises important theoretical questions and practical opportunities. We have developed an *in-silico* methodology, based on genome-scale models, to minimize the number of active genes encoding enzymes and transporters in a cell's metabolic network. In the resultant *minimal metabolic networks,* all the remaining active genes are essential for keeping the network working to achieve the biomass value predicted by Flux Balance Analysis*.* We have tested our approach on a set of genome-scale metabolic models of various eukaryotic and prokaryotic organisms, but have focussed on *Saccharomyces cerevisiae.* The nutrient environments employed have comprised both known and automatically generated sets of nutrients and relative maximum uptake rates. The results generate more than 1000 unique minimal networks for a single condition, demonstrating the complexity of the minimization problem. We then performed a frequency analysis on the affected genes to discover patterns or similarities among the networks and the redundancy of specific pathway-related genes. In addition to this, we also evaluated the networks using a *pathway-oriented robustness analysis*, to see how the networks respond to random variation in the reaction fluxes. Finally, we have done a cross-species comparison of our algorithm's results to highlight some of the homologous genes that are retained in the networks of a majority of species. Thus, our work has produced a tool for *in silico* genome minimization that permits the discovery of *mandatory* genes in the minimal metabolic networks.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B012

Protein Families are the Punchline of the Pangenomes

Lisa Crossman

SequenceAnalysis.co.uk, Norwich, United Kingdom. School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

Abstract

Short-read draft whole genome assemblies can contain many contigs and be impacted on by repeat regions, such as those caused by mobile element activity or inherently repetitive gene structure. Annotating such assemblies for gene content and functional activity can be challenging. This can be especially true if the predicted genes are fragmented across contigs, very large, repetitive or of unusual nucleotide content. Very high and low %GC genomes also come with additional issues. The Pfam domain database[1] is a widely-used large collection of protein families, each represented by multiple sequence alignments and Hidden Markov Models (HMMs). Rather than studying the predicted whole gene content of draft genomes, or presence/absence of specific genes in pan-core genome analyses, we examined predicted protein content by Pfam domain complement. Here we present Punchline, a workflow written in Python 3, to study the genetic content of pangenome assemblies including draft assemblies by looking at the complement of short protein domains. The domains can be used in statistical comparisons of Bacterial groups of interest as provided to the workflow of Punchline. In addition, we show the application of Punchline to specific genomic data.

1 1. The Pfam protein families database in 2019:S. El-Gebali, J. Mistry, A. Bateman, S.R. Eddy, A. Luciani, S.C. Potter, M. Qureshi, L.J. Richardson, G.A. Salazar, A. Smart, E.L.L. Sonnhammer, L. Hirsh, L. Paladin, D. Piovesan, S.C.E. Tosatto, R.D. Finn **Nucleic Acids Research** (2019) doi: 10.1093/nar/gky995

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B013

Oxford Nanopore sequencing elucidates a novel *stx2f* carrying prophage in a Shiga toxin producing *Escherichia coli*(STEC) O63:H6 associated with a case of haemolytic uremic syndrome (HUS).

David Greig[1,2], Timothy Dallman[1], Claire Jenkins[1]

[1]Public Health England, London, United Kingdom. [2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom

Abstract

AIM: The aim of this study was to use Oxford Nanopore sequencing to characterise a Shiga-toxin producing *Escherichia coli* (STEC) O63:H6 responsible for a recent case of haemolytic uremic syndrome (HUS).

METHODS: STEC isolate 377323 and a comparator *stx* negative *E. coli* isolate of the same lineage and serotype (382634) were sequenced on an Oxford Nanopore minION R9.4 flow cell and an Illumina HiSeq 2500. Nanopore FAST5 data were basecalled and demultiplexed by Albacore. Assemblies were generated using Unicycler and polished using Nanopolish, Pilon and Racon before screening for prophages using PHASTER.

RESULTS: The Shiga toxin subtype was confirmed to be *stx2f*, which is rarely associated with the onset of HUS in STEC. Comparing the two genomes, the *stx* negative isolate harboured five prophages compared to eleven prophages in the STEC isolate, with only one in common. Both samples contained an intact locus of enterocyte effacement pathogenicity island. The Shiga toxin encoding prophage was 42.5kb in size and predicted to contain 71 coding sequences. 377323 also encoded an 85kbp IncFIB plasmid, not present in 382634. This plasmid encodes a *tra*conjugation cassette and the virulence *bfp*cassette encoding for a type IV bundle forming pili.

CONCLUSION: Long read sequencing enabled the characterisation of a rare STEC and related *E. coli* in relation to both prophage content and plasmid content and revealed significant differences in the pan-genome in addition to the acquisition of the novel *Stx2f* phage. This data contributes to the understanding of non-O157 STEC associated with HUS.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B014

Comparative pangenomics of *Campylobacter* species

Evangelos Mourkas[1], Sion Bayliss[1], Koji Yahara[2], Jessica Calland[1], Ben Pascoe[1], Samuel Sheppard[1]

[1]The Milner Centre for Evolution, University of Bath, Bath, United Kingdom. [2]Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Abstract

Bacterial genome sequencing has become very popular over the last 15 years in the field of bacterial genomics and evolution with over >1000 genome sequences generated for the zoonotic *Campylobacter jejuni* and *Campylobacter coli* species. The rapid improvement of culture and sequencing techniques has led to isolation and sequencing of new *Campylobacter* species from a variety of sources including birds, mammals, reptiles and the environment. Inter-species recombination has been demonstrated for *C. jejuni* and *C. coli*, but little is known about recombination between other species in the genus. We analysed the population genomic structure of whole genome sequenced isolates from different *Campylobacter* species using pangenome and phylogenetic analyses to investigate core and accessory gene variation and putative gene function. Characterizing the extend of genome segregation among multiple *Campylobacter* species isolated from the same and different hosts improved understanding of how ecology (physical isolation) maintains species and the extent to which intrinsic mechanistic and adaptive barriers are eroded when species cohabit. This broadly defines the limit of interspecies recombination and the non-reducible pangenomically-defined species. This raises important questions about the nature of species specific recombinational barriers, the genes that constitute the inter-species mobilome and the emergence of zoonoses through reticulate evolution in agricultural animals.

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B015

Lineage-specific evolution in *Listeria monocytogenes* detected by analysis of a panel of Swiss isolates from food and human origin

Roxana Zamudio[1], Joseph D Ralph[1], Richard D Haigh[1], Megan De Ste Croix[1], Taurai Tasara[2], Katrin Zurfluh[2], Min Jung Kwun[3], Nicolas J Croucher[3], Stephen D Bentley[4], Roger Stephan[2], Marco R Oggioni[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Zurich, Zurich, Switzerland. [3]Imperial College London, London, United Kingdom. [4]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Abstract

Listeria monocytogenes is the causative agent of Listeriosis, a foodborne infection and characterised by outbreaks with high mortality. In the past years, outbreak analysis has shifted from PFGE to core genome MLST (cgMLST) and a difference of ≤10 cgMLST alleles was identified as the most appropriate cut off. The cgMLST scheme contributes significantly to the analysis of outbreaks but is less suitable to provide insight in the overall population structure. In the present work, we characterize the differences in the evolution of lineage I and II and the impact they have on the identification of outbreak isolates starting from the genome sequences of a panel of 166 Swiss *L. monocytogenes* clinical and food isolates. We included in the analysis recently published genomes from Germany (414 isolates) and Holland (128 isolates). Using data of pairwise cgMLST and SNP differences of these 708 isolates, we can clearly identify the genetic diversity associated to outbreaks (≤10 differences), sublineages (≤150 differences) and lineages. The sublineages identified by SNP and cgMLST match the clustering of the PopPUNK software. When broken down for lineages, data show clearly that lineage II has a lower mutation rate within sublineages but a higher diversity between sublineages. Admixture analysis confirms that the increased diversity between sublineages in lineage II is due to horizontal gene transfer and recombination. While our data show that genome evolution is lineage-specific in *L. monocytogenes*, the cut off for the identification of outbreaks remains identical between both lineages.

B016

Pangenomic analysis of *Staphylococcus pseudintermedius* to better understand antimicrobial resistance profiles

Alison MacFadyen, Gavin Paterson

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Although *Staphylococcus pseudintermedius* are commensal bacteria of dogs, they are also opportunistic pathogens, being the primary cause of canine skin and ear infections. Recently, more attention has been given to this species, due to the emergence of antimicrobial resistance. Further to this a methicillin resistant *S. pseuintermedius* was isolated from a human and was subsequently found to be multidrug resistant. To better understand the frequency of carriage of antimicrobial resistance, as well as the presence of multidrug resistance, we examined the pangenome of over 200 *S. pseudintermedius* isolates from across the globe. Focussing on methicillin resistance we were able to identify staphylococcal cassette chromosome *mec* (SCC*mec*) types unique to specific Bayesian Analysis of Population Structure (BAPS) groups, including groups which carried *mec* resistance genes independent of a known SCC*mec* element. In addition, we identified an SCC*mec* element, within isolates from North America, that shares 99% nucleotide identity with a recently described non-typeable SCC*mec* element carried by *Staphylococcus aureus* isolated from a human in the Netherlands. Beyond methicillin resistance we found over 50% of those strains analysed were putatively multidrug resistant (resistant to 3 or more antimicrobial drug classes). This highlights the diverse resistance determinants present in animal staphylococci and the importance of monitoring *S. pseudintermedius.*

The microbial pangenome

Presentations: Wednesday evening and Thursday lunchtime

B017

Evolution and spread of bacterial transposons

Grace Blackwell[1,2], Zamin Iqbal[1], Nick Thomson[2]

[1]EMBL-EBI, Hinxton, United Kingdom. [2]Wellcome Sanger Institute, Hinxton, United Kingdom

Abstract

Transposons are mobile genetic elements (MGE) that can carry additional genetic cargo nonessential for their own transposition. This cargo can include antibiotic or heavy metal resistance genes or those increasing metabolic plasticity. In addition to transposing across or between the chromosome and other replicons in a single cell, they can transfer between bacteria as passengers on conjugative plasmids that are capable of intercellular transfer.

Transposons can be grouped on mechanisms of movement and by lengths of the bounding inverted repeats or of target site duplication created by transposition. Class II transposons, such as Tn*3* and Tn*501* utilise two-step replicative transposition involving a transposase, *tnpA*, and a resolvase, *tnpR,* gene. The Tn*402*/Tn*5053* family has four genes, *tniABQR,* required for transposition, while Tn*7* and relatives contain five (*tnsABCDE*).

Despite their role in antimicrobial resistance dissemination and a detailed mechanistic view of transposition there have been no studies aimed at revealing the evolutionary history of transposon families by interrogating global genome sequence datasets. This is largely because the ability to search all existent bacterial sequences is non-trivial and only recently realised.

We took a selection of 37 representative variants of the above transposon families and queried their nature and distribution across sequence space using bigsi (http://www.bigsi.io/), a searchable index of the bacterial ENA (455,632 datasets, Dec 2016) and Shovill (https://github.com/tseemann/shovill). Constrained by the sequence data that exists and the biases this may engender, this analysis provides broad insights into the prevalence and spread of important MGE.

B018

Resolving complex mobile genetic elements with nanopore sequencing

Stefan Rooke

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Sequence-based surveillance of antimicrobial resistance (AMR) is becoming increasingly prevalent – with the emergence of low-capital investment long-read technology in the form of Oxford Nanopore Technologies (ONT) MinION enabling such surveillance in infrastructure poor low-middle income countries. In such settings, the surveillance of mobile genetic elements, which can facilitate the rapid spread of beneficial AMR genotypes horizontally throughout bacterial populations, and across species boundaries may provide a relatively low-cost target for surveillance.

In this study we used an ONT MinION to whole genome sequence *E coli* isolates from Nairobi, Kenya for which Illumina data had previously been generated alongside producing whole genome sequences for novel isolates collected for a model surveillance project in Busia, Kenya. The Illumina sequenced isolates had been found to carry a consistently co-occurring set of antibiotic resistance genes (tetA, strAB, sul1, bla-TEM, and dfrA7), conferring resistance to five antibiotic classes. The exact genomic context of which could not be determined with Illumina sequence data alone. Whilst those isolates collected in Busia demonstrated phenotypic resistance to those classes. Initially suspected to be borne on regionally disseminated plasmids; the long-reads generated by the MinION allowed the full resolution of these genes across several closely associated transposable elements, which were further found to be integrated chromosomally, across these geographically independent isolates. This study demonstrates that value of long-read sequencing in the resolution of regionally important mobile genetic elements, simultaneously allowing further value to be added to previously produced long-read data.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B019

Investigating alternative AUG usage in avian Influenza A virus segment 2

Elizabeth Billington[1], Rute Maria Pinto[1], Holly Shelton[2], Paul Digard[1]

[1]Roslin Institute, Edinburgh, United Kingdom. [2]Pirbright Institute, Woking, United Kingdom

Abstract

Influenza A viruses (IAV) have a segmented, negative sense RNA genome. PB1-F2 is an IAV accessory protein encoded by segment 2, in the +1 reading frame. IAVs from avian hosts generally encode full length PB1-F2s, which contrasts with human IAVs which frequently have C-terminal truncations. Many reported activities of PB1-F2, including innate immune antagonism, require motifs in its C-terminal domain. Full length PB1-F2 is translated from AUG 4 of segment two, but one or more of AUGs 7, 8, and 9 may also serve as independent initiation codons for the C-terminal domain.

Products from the AUGs 7-9 are expressed during infection by a vaccine strain IAV, but their presence or absence had no effect on virus growth in vitro. We generated a panel of isogenic viruses, containing segment 2 from an avian H5N1 IAV, which differed in the presence or absence of the various AUG start codons in segment 2. No difference in growth kinetics in vitro or viral polymerase activity, measured using a mini-replicon assay, was observed for any of these mutants.

However a significant difference in mean plaque size on MDCK cells was seen when individual changes were made to any of AUGs 7 - 9, suggesting a subtle effect on virus fitness possibly caused by loss of expression of PB1-F2 C-terminal fragments. In addition structural predictions suggest that the AUG mutations will affect secondary structure of full length PB1-F2. Our works suggests segment 2 protein expression from multiple AUGs could impact of the virus replication cycle.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B020

Optimized CRISPR/Cas9 editing of large viral DNA genomes for the generation of recombinant virus collections

Alberto Domingo López-Muñoz, Alberto Rastrojo, Antonio Alcamí

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

Abstract

The CRISPR/Cas9 genome-editing system is a powerful strategy to generate precisely, efficiently and rapidly recombinant viruses. Nevertheless, although genome editing with CRISPR/Cas9 has been described to make large DNA recombinant viruses, this system has not been optimized yet for a quick and easy use in a variety of viral families, nor cheapened for general and routine use.

We have developed a method which consists of two rounds of transfection-infection, providing each one with a different guide-RNA (gRNA) to target different positions from the same locus of interest. Selection of transiently-transfected cells was performed with puromycin instead of flow cytometry. Drug selection of transfectants in cell culture made this determinant step more accessible and cheaper without the need for flow cytometry within BLS-2 facilities.

We selected the *195P* pseudogene from Ectromelia virus strain Naval as a target locus for eGFP insertion. We strongly increased the percentage of +GFP plaques to 50% of total plaques. This 2-step/2-gRNA method was applied in combination with our "Green to Black" approach, in which a +GFP virus loses its fluorescent signal just in case of proper homologous directed repair (HDR), which significantly increased by 25% and reduced false positive candidates. This method also allowed us to generate a collection of recombinant type 1 and 2 Herpes simplex viruses in the *US4* gene.

This optimized method can be used for accessible, precise and highly efficient generation of recombinant viral collections for a desire locus, facilitating the screening of recombinant candidates and the routine use of this methodology.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B021

Functional and Structural Studies of Chikungunya Virus nsP1 Protein.

Kate Loveday, Emily Levitt, Trang Nguyen, Thomas Edwards, Andrew Tuplin

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a single-stranded, positive-sense RNA virus of the *Alphavirus* genus. CHIKV is an arbovirus, whose spread is mediated by *Aedes* species mosquitos and is associated with debilitating joint pain and febrile symptoms in infected humans. A lack of vaccine or specific antivirals, combined with increasing global spread, has facilitated the re-emergence of CHIKV in recent years. Our research focuses on the CHIKV encoded non-structural protein-1 (nsP1), which has methyltrasnferase activity and is essential for virus genome replication. Through a combination of structural, biochemical and reverse genetic approaches, we aim to investigate the relationship between the molecular structure and both canonical and non-canonical functions of nsP1, at different stages of CHIKV replication.

We demonstrated that substitution of an in-frame methionine (M^{24}), towards the N-terminal of nsP1, severely inhibits CHIKV replication in a host cell-dependent manner. Specifically, we demonstrated that an M^{24}>A substitution had no significant effect on sub-genomic replicon replication but blocks production of infectious CHIKV virions – suggesting a role in later stages of virus replication, such as packaging or egress, rather than genome replication or translation. Utilising such a reverse genetic approach, analysis of a panel of M^{24} substitutions has improved our understanding on non-canonical yet essential functions of nsP1 during CHIKV replication. In order to further elucidate both the structural and biochemical basis for the observed mutant phenotypes, we developed a system for bacterial expression and purification of recombinant CHIKV nsP1 and have established crystallography trails, prior to analysis of the molecular structure by X-ray crystallography.

B022

Antagonism of mosquito innate immunity by the chikungunya virus nsP3 protein

Jinchao Guo, Yanni Gao, Mark Harris

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a re-emerging *Alphavirus* transmitted by Aedes mosquitos and causing fever, rash and chronic arthralgia. There are no vaccines or antiviral agents available for CHIKV therefore it is important to understand the molecular details of virus replication. To address this, we previously conducted a mutagenic analysis of the central alphavirus unique domain (AUD) of the CHIKV non-structural protein 3 (nsP3), testing replication of a subgenomic replicon in a variety of mammalian and mosquito (*Aedes albopictus*) cell lines. One mutant (M219A) exhibited a different phenotype in two *Aedes albopictus* cell lines (U4.4 and C6/36): replicating as wildtype in C6/36 but was blocked in U4.4. As U4.4 cells have an intact RNAi response whereas C6/36 have a frameshift mutation in the Dicer-2 (Dcr2) gene and express an inactive Dcr2 protein, we proposed that the replication of M219A was suppressed by the RNAi antiviral response in U4.4 cells, while wildtype nsP3 was able to counteract this response.

To further investigate this hypothesis we have extended the mutagenic analysis to screen other residues in proximity with M219 within the AUD. All of these mutants retain wildtype levels of replication in mammalian and C6/36 cells, except for W220A which replicates poorly. Evaluation of the replication of these mutants in U4.4 is ongoing. We are also pursuing a CRISPR/Cas9 approach to ablate expression of Dcr2 in U4.4 cells, and generating stable C6/36 cells expressing Dcr2 to confirm our hypothesis. Our studies will shed light on how CHIKV nsP3 can antagonise mosquito innate immunity.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B023

Adapting cell-free systems to understand the replication of foot-and-mouth disease virus.

Danielle M. Pierce, Joseph C. Ward, David J. Rowlands, Morgan R. Herod, Nicola J. Stonehouse

University of Leeds, Leeds, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV), a single stranded RNA virus in the *Picornaviridae* family, is the causative agent of foot-and-mouth disease (FMD). The positive-sense genome acts as viral messenger RNA and contains a single open reading frame that is translated to produce one polyprotein. This polyprotein is subsequently cleaved to produce all the viral structural and non-structural proteins required for viral replication. Production of new viral genomes occurs in specialised virus-induced organelles (also known as 'replication complexes'), where the viral genome and non-structural proteins localise with host factors in membrane vesicles. Many of the viral-host interactions that occur within this complex are still poorly understood and challenging to dissect.

Cell-free systems can be a powerful system for understanding molecular virus-host interactions. Using a variety of cell-types we are establishing an *in vitro* replication system for FMDV in cell-derived lysates. Such systems, previously established for poliovirus, allow viral protein translation, polyprotein processing and replication to be studied *in vitro,* whilst allowing increased control of reaction conditions to identify essential components. Using a FMDV replicon system in cell-free lysate we have demonstrated translation and processing of viral proteins *in vitro*. Polymerase activity assays and varying buffer components are being used to investigate the optimal conditions required for the switch between RNA translation and viral replication to take place within this system. We are now combining these *in vitro* systems with imaging techniques to investigate protein co-localisation and replication complex composition over time throughout virus replication.

B024

Impacts of HIV-1 protease activity on Pol viral incorporation and virus titers

Fu-Hsien Yu[1], Kuo-Jung Huang[2], Chin-Tien Wang[1,2]

[1]Institute of Clinical Medicine,1 National Yang-Ming University School of Medicine, Taipei, Taiwan. [2]Department of Medical Research,2 Taipei Veterans General Hospital , Taipei, Taiwan

Abstract

Background: HIV-1 protease (PR) is encoded by pol which is initially synthesized as a Pr160gag-pol via a ribosomal frameshift mechanism. The frameshift event occurs at a frequency of about 5% during translation of Pr55gag, resulting in Pr160gag-pol expression at a much lower level compared to Pr55gag. Pol was reported to be capable of viral incorporation albeit at a lower level compared to Pr160gag-pol, suggesting the Gag-Pol package largely depends on Gag domain. Cotransfection experiments with Gag and Pol expression vectors indicate enhanced PR activity by increasing Pol expression remarkably reduces both virus yields and virus-associated Pol products. Accordingly, suppression of PR activity may improve Pol viral incorporation. To test this possibility without cotransfecting Gag and Pol expression plasmids, we engineered a construct capable of expressing HIV-1 Gag and Pol.
Methods: A “self-cleaving” 2A peptide was inserted between the Gag and Pol coding sequences. PR activity was attenuated by a single amino acid substitution. Each of the constructs was transiently expressed in 293T cells, and virus assembly and processing were analyzed by Western blot. Virus titers were determined by a single-cycle-infection assay.
Results: Analyses indicate the 2A-containing construct can package HIV-1 Pol and produce infectious virions. Virus titers and virus–associated Pol products were noticeably increased when PR activity was attenuated by a point mutation.
Conclusions: The reading frameshift may be evolutionally selected to express HIV-1 Pol as a Gag-Pol which exhibits stronger PR activity and higher viral incorporation efficiency than Pol despite its low expression level.

B025

Analysis of the novel role of NS5A domain I in the assembly of infectious hepatitis C virus particles

Shucheng Chen, Mark Harris

University of Leeds, Leeds, United Kingdom

Abstract

Hepatitis C virus (HCV) is an enveloped virus with a positive-sense, single-stranded RNA of approximately 9.6 kb, a member of the genus *Hepacivirus* within the family *Flaviviridae.* The genome contains a single large open reading frame encoding a 3000 residue polyprotein. The non-structural 5A protein (NS5A) is a highly phosphorylated protein, which is comprised of three domains (I, II and III).

Previously, we demonstrated that two residues within NS5A domain I (V67 and P145) play critical roles in HCV assembly challenging the dogma that NS5A domain I exclusively participated in genome replication. In this study, we identified 8 surface exposed residues of domain I which were located in close proximity to V67 and P145. The mutants were cloned into a JFH-1 derived subgenomic replicons (mSGR-luc-JFH1) to confirm whether they are required in genome replication. The results of luciferase assay suggested that I52A exhibited the same phenotype as V67A and P145A and is a further candidate for regulating assembly of HCV.

In parallel we sought to investigate whether domain I was involved in the interaction of NS5A with cyclophilin A (CypA), a cellular peptidyl-prolyl isomerase required for HCV replication. CypA can be inhibited by cyclosporin A (CsA), which also inhibits HCV genome replication. Surprisingly, all three mutant replicons (I52A, V67A and P145A) were more sensitive to CsA treatment than wildtype, suggesting that domain I does indeed interact with CypA. Ongoing studies will therefore investigate the roles of both domain I and CypA in genome replication and assembly of infectious HCV particles.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B026

The modulation of autophagy by African swine fever virus

Gareth Shimmon[1], Linda Dixon[1], Tom Wileman[2], Chris Netherton[1]

[1]The Pirbright Institute, Pirbright, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

African swine fever virus (ASFV) causes a lethal haemorrhagic disease of domestic pigs with mortality rates of up to 100%. An outbreak in Russia in 2007 has since spread into Europe. There is currently no vaccine available, however infection with attenuated strains of ASFV can protect against infection with closely related virulent strains. Autophagy is a conserved, essential cell process that regulates multiple pathways that are critical for mounting an effective immune response. Experiments have shown that inhibiting the ability of viruses to regulate autophagy can lead to enhanced immune responses. We have shown that ASFV does not require autophagy for replication and that autophagosome formation is inhibited during infection. In addition, through analysis of key proteins in the upstream autophagy pathway, we describe a novel mechanism of ASFV inhibition of autophagy. This research will expand our understanding of the interaction between ASFV and the autophagy pathway with the potential that a low virulent ASFV strain with an altered ability to modulate autophagy will provide enhanced immunity against virulent isolates.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B027

Ribosome profiling of porcine reproductive and respiratory syndrome virus

Georgia M. Cook[1], Pengcheng Shang[2], Yanhua Li[2], Katherine A Brown[1], Adam Dinan[1], Ying Fang[2], Andrew Firth[1], Ian Brierley[1]

[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, USA

Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus of huge economic importance, infecting the porcine host leading to infertility, morbidity, and mortality. Its genome contains a canonical -1 programmed ribosomal frameshift (PRF) site that facilitates expression of the viral replicase, and a second, non-canonical signal which induces both -1 and -2 PRF to generate alternative forms of a viral non-structural polypeptide, nsp2. In contrast to canonical frameshift sites, the frameshift at the non-canonical site is not stimulated by a downstream secondary RNA structure, but is instead the first known example of protein-directed frameshifting, stimulated by a trans-acting complex of a viral (nsp1β) and a cellular (PCBP) protein.

We investigated frameshifting in PRRSV by ribosome profiling, using the vaccine strain, SD95-21, and a derivative with mutations at the nsp2 site that render it PRF-defective. Highly efficient PRF was observed at both the canonical, RNA pseudoknot-dependent -1 PRF site (efficiency of 43-56%), and the nsp1β/PCBP-dependent site (combined -1 and -2 PRF efficiency of 20-24%). Investigations are underway as to whether the presence of nsp1β during viral infection stimulates non-canonical frameshifting on host mRNAs.

We also carried out RNA-Seq and differential transcription analysis in parallel with ribosome profiling to garner further insight into the functions of the nsp2 transframe proteins, previously shown to be involved in innate immune suppression.

This ribosome profiling analysis has also revealed the presence of a short but highly expressed upstream ORF in the 5'UTR of PRRSV.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B028

Functional RNA structures as targets in emerging arboviruses

Oliver Prosser[1], Andres Merits[2], Nicola Stonehouse[1], Andrew Tuplin[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Tartu, Tartu, Estonia

Abstract

Emerging arthropod-borne viruses, such as Zika virus (ZIKV) and Chikungunya virus (CHIKV), represent a significant and increasing threat to global health. Despite the expanding prevalence of their vectors, the *Aedes sp.* mosquitoes, and the potential for major epidemics, there are currently no specific antiviral compounds or vaccines available for either viral pathogen.

The positive-strand genomes of ZIKV and CHIKV, members of the flavivirus and alphavirus genera respectively, contain functional, structured *cis*-acting RNA elements. We are investigating a range of approaches for targeting these RNA elements and analysing the effect on virus replication at different stages of their life cycle.

Gaining high resolution structural data is essential prior to targeting RNA elements, and consequently, we mapped RNA structural elements within the ZIKV 5' genome region using a combination of biochemical SHAPE probing, thermodynamic predictions and phylogenetic analysis. We are currently validating our structural data by analysis of mutant phenotypes in a reverse genetic system.

Using antisense locked nucleic acid oligonucleotides (antisense-LNA), we demonstrated that functional RNA elements in CHIKV can be specifically targeted - inhibiting replication in both sub-genomic replicon and infectious virus systems. Surface plasmon resonance confirmed that the antisense-LNA binds to a specific stem-loop target with a K_d of 310nM and has an IC_{50} of 35nM in the sub-genomic replicon system. In future work, we aim to investigate selection of RNA-aptamers against CHIKV and target ZIKV genomic stem-loops using antisense-LNAs.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B029

S-earching for mutations: Truncation of the FMDV 5' UTR S-fragment results in second site mutations within the non-structural proteins

Joseph Ward[1], Lidia Lasecka-Dykes[2], Sarah Gold[2], Caroline Wright[2], Morgan Herod[1], Toby Tuthill[2], David Rowlands[1], Nicola Stonehouse[1]

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

Abstract

Foot-and-mouth disease virus (FMDV) is a single stranded RNA virus in the picornavirus family. It is the causative agent of foot-and-mouth disease, globally the most important disease of cloven hoofed animals. The FMDV genome has several features that are not found amongst other viruses within the *Picornaviridae*. These include a large 5' untranslated region (UTR), almost twice the length of that found in enteroviruses, containing highly structured RNA elements unique to FMDV. These unique elements include a 360 nucleotide region located at the extreme 5' end of the genome and predicted to form one large hairpin loop, termed the S-fragment. To date there is no prescribed role to the S-fragment.

Using a combination of FMDV replicons and recombinant viruses, we have shown that both replicon and viral replication can tolerate truncations to the bottom of the S-fragment up to removal of 195 nucleotides (i.e reducing the total size by 50%). Interestingly, one of these mutant viruses, was found to have additional mutations in the non-structural proteins, some of which were in residues found to be conserved in all reference sequences reported. Mutations were discovered in both the 2C and 3D proteins and were enriched in the population over time, suggesting a selective pressure for their maintenance. Using *in vitro* polymerase assays, we suggest that the mutation arisen in the 3D polymerase alters the RNA binding capacity of this protein allowing for increased RNA recombination. This, provides a novel insight into the interactions between the 3D polymerase and the S-fragment.

B030

Visualising influenza virus replication using a click chemistry approach

Olivia Swann, Thomas Peacock, Peter O'Hare, Wendy Barclay

Imperial College London, London, United Kingdom

Abstract

Influenza A viruses (IAVs) have a significant impact on public health through seasonal epidemics. Moreover, the sporadic emergence of zoonotic and pandemic strains represents an additional global threat. Understanding how novel IAV strains adapt to infect and transmit between humans is essential for effective surveillance and may provide rationale for anti-viral therapeutics.

IAVs replicate their RNA genomes in the host cell nucleus. Host proteins, such as the proviral factor ANP32, are co-opted to support viral replication and transcription, whilst other inhibitory factors act to restrict. Ultimately the outcome of infection in different species depends on compatibility with these factors, however the precise nuclear localisation of these interactions is not clearly defined. It is not known whether viral RNA synthesis takes place at discrete sites, or whether the virus modifies the cell to produce viral factories in a similar manner to other viruses.

We are utilising a click chemistry approach to investigate the spatial details of IAV replication and to probe the differences in nuclear localisation between human-adapted vs poorly-adapted avian origin IAVs, to gain insight into mechanisms of host restriction. Influenza genomes labelled with the click reagent 5-ethynyl uridine (5-EU) remain infectious and can be visualised through the cycloaddition of azide-tagged fluorescent dyes. Utilising this tool, the co-localisation of incoming viral particles with nuclear sub-domains, as well as key host factors, are being examined. Moreover, the localisation of human- vs avian-adapted virus in human and avian cells, as well as cell lines lacking key host factors, are being compared.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B031

Structural and Phenotypic Analysis of Chikungunya Virus RNA Structures During Viral Genome Replication and Translation

Catherine Kendall[1], Henna Khalid[1], Marietta Mueller[1], Alain Kohl[2], Andres Merits[3], Nicola J Stonehouse[1], Andrew Tuplin[1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom. [3]University of Tartu, Tartu, Estonia

Abstract

Chikungunya virus (CHIKV) is a pathogenic, positive-sense RNA virus of the *Alphavirus* genus, which causes fever and debilitating joint pain in humans. CHIKV is transmitted by *Aedes* mosquitoes and is currently re-emerging in the absence of approved vaccines or antiviral therapies. One approach to vaccine production is to attenuate CHIKV replication through disruption of genomic RNA secondary structures. We have mapped the RNA structure of the 5' region of the CHIKV genome using selective 2'-hydroxyl acylation analysed by primer extension (SHAPE) to investigate intramolecular base-pairing at single-nucleotide resolution. We have identified five highly-conserved RNA structures within the *nsP1*-coding region of ORF-1 and, using a reverse genetics approach, determined their impact on virus replication in infectious virus and luciferase-reporter subgenomic replicon systems. Our results suggest that RNA structures within the *nsP1*-coding region are required for efficient CHIKV genome replication in mammalian and mosquito cells, potentially via vertebrate/invertebrate host-specific mechanisms. For example, disruption of one of the stem-loops inhibits CHIKV replication in mammalian cell lines, while having no significant effect in mosquito cells. Restoration of the structure via compensatory silent mutations restores replication - indicating RNA structure-dependent enhancement of CHIKV replication. Conversely, disruption of an adjacent stem-loop inhibits CHIKV replication in mosquito cells but not in mammalian cell lines. Our structural data also suggests that higher-order interactions within this region impact CHIKV replication. As arboviruses continue to re-emerge, it is critical that we improve our understanding of their replication cycles. RNA structures may constitute novel targets for vaccine attenuation or drug design.

Virology workshop: Gene expression and replication

Presentations: Wednesday evening and Thursday lunchtime

B032

Application of next generation sequencing for the elucidation of genes and pathways involved in the host response to bovine respiratory syncytial virus

Dayle Johnston[1], Bernadette Earley[1], Matthew McCabe[1], Gordon Blackshields[1], Ken Lemon[2], Catherine Duffy[2], Michael McMenamy[2], Sara Louise Cosby[2], Jaewoo Kim[3], Jerry Taylor[3], Sinead Waters[1]

[1]Teagasc, Animal & Grassland Research and Innovation Centre (AGRIC), Teagasc, Grange, Co. Meath, C15 PW93, Dunsany, Ireland. [2]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [3]University of Missouri, Columbia, USA

Abstract

High rates of calf mortality in the first 12 months of life, results in significant economic losses in Europe and the USA. Bovine respiratory disease (BRD) accounts for the largest proportion of calf mortality. There is a paucity of literature concerning the host response to BRD. In a controlled challenge study in artificially reared dairy calves (155 (S.D. 14) kg), the influence of the host response to bovine respiratory syncytial virus (BRSV) was examined. At AFBI Holstein-Friesian calves were either challenged with BRSV (n=12) or mock challenged with phosphate buffer saline (n=6). Calves were euthanised on day 7 post-challenge. Bronchial lymph nodes were collected and flash-frozen at -80^{o}C. RNA was extracted and sent to the University of Missouri's DNA Core Facility for RNA-Seq library preparation and sequencing. Sequenced reads were adapter trimmed, quality assessed using FastQC and aligned to the bovine genome (UMD 3.1) using STAR. Differential gene expression analysis was performed using EdgeR, and pathway and gene ontology analyses were carried out using g:Profiler and Ingenuity Pathway Analysis (IPA). There was a clear separation between BRSV challenged and control calves based on $\log_2$ fold gene expression changes, despite an observed mild clinical manifestation of the disease. There were 934 differentially expressed genes (DEG) ($p<0.05$, FDR<0.1, fold change >2) between the BRSV challenged and control calves. Over-represented pathways and gene ontology terms among the DEG were associated with immune responses and included: GO:0051607 defense response to virus, the KEGG pathway Influenza A and the IPA pathway Interferon Signaling.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B033

Generation of a recombinant GFP-tagged infectious bronchitis virus (IBV)

Isobel Webb[1,2], Sarah Keep[1,3], Jamie Stuart[1,4], Michael Oade[1,5], Paul Britton[1], Erica Bickertron[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Bristol, Bristol, United Kingdom. [3]University of Edinburgh, Edinburgh, United Kingdom. [4]University of York, York, United Kingdom. [5]University of Glasgow, Glasgow, United Kingdom

Abstract

Infectious Bronchitis Virus (IBV) is a highly contagious *gammacoronavirus* which infects poultry. Using reverse genetics, enhanced green fluorescence protein (eGFP) has been inserted into a pathogenic strain of IBV, M41, in place of a newly identified open reading frame (ORF), ORF7. ORF7 has been identified in several IBV strains including M41 and apathogenic strain Beau-R. ORF7 is located immediately downstream of the N gene, preceding the 3' UTR. This region has been chosen because we are interested in investigating whether a protein can be transcribed from the associated transcription regulatory sequence (TRS-B) . M41 has a deletion at the 3' end of the genome and does not encode all of ORF7, suggesting it is not essential for the viral replication and is not required for a pathogenic phenotype. The TRS-B, TAACA for ORF7 in Beau-R, is non-canonical and is located after the stop codon for the N gene. In M41 only part of the TRS-B is present, TAA, so nucleotides CA were added alongside the eGFP sequence. A number of viruses were successfully rescued. Growth kinetics in primary Chicken Kidney cells (CK) were comparable to the parent virus M41-K. The stability of the eGFP sequence has been assessed in CK cells and in embryonated eggs. The newly constructed TRS-B however was not utilised suggesting that it is not solely the TRS-B that controls the transcription of ORF 7.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B034

Glucose regulated protein 78 (GRP78) interacts with Zika virus envelope and is required for a productive infection

Jamie Royle[1], Carolina Ramírez-Santana[2], Claire Donald[1], Snezhana Akpunarlieva[3], Rommel Gestuveo[1], Juan-Manuel Anaya[2], Richard Birchmore[3], Alain Kohl[1], Margus Varjak[1]

[1]MRC -University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Bogota, Colombia. [3]Glasgow Polyomics Facility, College of Medical, Veterinary and Life Sciences, University of Glasgow , Glasgow, United Kingdom

Abstract

Zika virus (ZIKV) is a member of the *Flaviviridae* family and was until recently a relatively obscure tropical disease. Subsequently, ZIKV has been shown to be the causative agent of fetal abnormalities and Guillain-Barré syndrome in outbreaks across the Americas and so efforts towards delineating important factors in the viral lifecycle have increased. Combining protein pull-down from human A549 cells with mass spectrometry, it was found that ZIKV envelope (Env) interacts with the endoplasmic reticulum (ER) resident chaperone, glucose regulated protein 78 (GRP78). Flaviviruses such as Japanese encephalitis virus and dengue virus are known to co-opt ER resident proteins and members of the unfolded protein response, including GRP78, to enhance viral infectivity and propagation. The role these proteins play during the ZIKV lifecycle has yet to be elucidated.

To determine the importance of this interaction during ZIKV infection, A549 or Huh7 cells were treated with GRP78-specific siRNAs prior to infection with a NanoLuc expressing reporter virus or a wild-type virus. Depletion of GRP78 significantly reduced both virus luciferase readings and viral titres, indicating that GRP78 is necessary for efficient infection of mammalian cell culture. In contrast, inhibition of GRP78 with small molecule inhibitors did not reduce ZIKV infection. Interestingly, immunofluorescence of ZIKV infected cells reveal that GRP78 re-localises following infection and co-localises with Env, potentially at viral replication factories. Further experiments have shown that GRP78 is important for infection post entry and replication, and that putative GRP78 interactions partners are also required during infection.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B035

The Role of Cellular Proteins in Zika Virus Recombination

Michaela Dermendjieva, David Evans

University of St Andrews, St Andrews, United Kingdom

Abstract

Zika Virus (ZIKV) is a mosquito-borne positive-sense RNA virus of the family *Flaviviridae* which recently emerged, causing serious neurological complications such as Guillan-Barre Syndrome in adults and microcephaly in newborns. The drivers of ZIKV emergence and evolution are currently unknown. Positive-sense RNA viruses have the capacity to acquire extensive genetic changes through the process of recombination which contributes to their genetic diversity and capacity to evolve fast. There are two mechanisms of RNA virus recombination: replicative, which occurs when the viral RNA-dependent RNA Polymerase (RdRp) switches template during replication, and non-replicative - when pieces of viral RNA get processed and stitched together by cellular proteins. The aim of this study is to develop a non-replicative recombination assay to evaluate the propensity of ZIKV to recombine in mammalian and mosquito cell culture and to investigate factors of the cellular RNA metabolism pathway which may be involved in this process. Several cellular proteins have been suggested to affect virus recombination such as Exoribonuclease 1 (XRN1). XRN1 is known to interact with the ZIKV genome by degrading it in a 5'-3' direction until it encounters a secondary structure in the 3' untranslated region (UTR) of the virus genome known as XRN1-resistant RNA (xrRNA). With the help of recently developed reverse genetic tools for ZIKV, we want to better understand the mechanistic details of recombination and its role as a driver of virus evolution in the mammalian host and mosquito vector, which would shed light on virus emergence and evolution.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B036

Human interactome of MERS-CoV accessory proteins

I'ah Donovan-Banfield[1], Joshua Lee[1], Lisa Stevens[1], Kate Heesom[2], Andrew D Davidson[1], David A. Matthews[1]

[1]School of Cellular Medicine, University of Bristol, Bristol, United Kingdom. [2]Proteomics Facility, University of Bristol, Bristol, United Kingdom

Abstract

Middle East respiratory syndrome coronavirus (MERS-CoV) is a betacoronavirus that causes severe acute respiratory illness with a reported high mortality rate. Coronaviruses typically encode accessory proteins which are non-essential for viral replication but are involved in innate immune evasion and pathogenesis. In this study, we have identified cellular proteins that interact with the MERS-CoV accessory proteins ORF3, ORF4a, ORF4b and ORF5. We generated tet-inducible HEK293 cell lines expressing FLAG-tagged versions of the MERS-CoV accessory proteins using the Flp-In recombinase system. Expression of the accessory proteins in the stable cell lines were validated using Western blot and immunofluorescence analysis. The FLAG-tagged accessory proteins were then isolated by affinity pulldown and the cellular interactome of the viral proteins analysed by Tandem Mass Tag labelling followed by high-throughput LC-MS/MS. Our results have shown interactions between MERS-CoV accessory proteins and proteins involved in host innate response pathways. Interestingly, ORF4A, ORF4B and ORF5 have many host protein interactors that are enriched in nuclear transport pathways, suggesting their possible role in nucleo-cytoplasmic transport modulation. We have also identified interactions between ORF4a and host proteins that bind to double stranded RNA and others with roles in the innate immune response. Additionally, we have confirmed positive and negative effects of some host proteins on MERS-CoV infection using siRNA knockdown studies and immunofluorescence assays. Our results demonstrate the involvement of MERS-CoV accessory proteins with host proteins involved in innate immunity and with the limited specific treatments currently available, targeting these proteins may serve as a potential therapeutic strategy.

Virology workshop: Gene expression and replication
Presentations: Wednesday evening and Thursday lunchtime

B037

HIV-1 infectivity of cells is enhanced at mitosis: a role for Vpr?

Joseph Gibbons , Wing-yiu Jason Lee, Áine McKnight

Blizard Institute , London , United Kingdom

Abstract

Restriction factors are present in all cells including non-immune cells and protect them from invading viruses. RNA-associated Early-stage Antiviral Factor (REAF) was identified from a whole genome siRNA screen for restriction factors to HIV-1 (*1*). siRNA induced knockdown of REAF increases viral infectivity, while over-expression of the protein limits infection (*2*). Here we show that during mitosis, REAF is specifically excluded from the chromatin region and that mitotic cells have an increased susceptibility to HIV-1. We also demonstrate that in HIV-1 infection of monocyte-derived macrophages, Vpr is responsible for the degradation of nuclear REAF. Furthermore, silencing REAF expression in cycling cells by RNAi causes cells to accumulate in the G2/M phase. This result is consistent with previous observations that Vpr induces cell cycle arrest after infection (*3*).

1. L. Liu *et al.*, A whole genome screen for HIV restriction factors. *Retrovirology* **8**, 94 (2011).

2. K. M. Marno *et al.*, Novel restriction factor RNA-associated early-stage anti-viral factor (REAF) inhibits human and simian immunodeficiency viruses. *Retrovirology* **11**, 3 (2014).

3. J. B. Jowett *et al.*, The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle. *J Virol* **69**, 6304-6313 (1995).

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B038

Identifying the molecular mechanisms of tanshinones, derived from the Chinese herbal remedy Danshen, in an innovative model system

Judith Schaf, Philip Beesley, Robin Williams

Royal Holloway University of London, Egham, United Kingdom

Abstract

Innovative treatment strategies for human diseases are urgently needed in a wide range of debilitating or chronic conditions. One approach to do this is to characterise the molecular mechanisms of traditional Chinese medicines that have been widely implicated in therapeutic function but lack mechanistic insight. Danshen is a good example of a promising medicine that contains a group of lipophilic compounds called tanshinones, proposed as potential treatments for several human diseases including Alzheimer´s disease and cancer. The aim of this project is to identify the molecular targets of the four major tanshinones (tanshinone IIA, cryptotanshinone, tanshinone I and dihydrotanshinone) using *Dictyostelium discoideum* as a model system. All four tanshinones inhibit the growth of *Dictyostelium*, with IC_{50} values ranging from 0.7 - 4.1 µM, consistent with concentrations used in mammalian studies. This suggests that molecular targets for tanshinones are present in *Dictyostelium*. To investigate these targets, a mutant library was screened using various tanshinones to identify resistant cells. In total, 78 independent tanshinone-resistant mutants have been isolated, identifying eight different genes. Interestingly, three of these genes encode proteins associated with the canonical Wnt signalling pathway, with one mutant lacking the *Dictyostelium* FslJ protein, an ortholog of human Frizzled receptors. These data suggest a role for tanshinones in targeting a potential Wnt-like signalling pathway in *Dictyostelium*. Future studies will therefore investigate tanshinones in regulating this Wnt-like signalling pathway in *Dictyostelium*, with subsequent translational experiments seeking to validate this mechanism in the therapeutic treatment of human diseases.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B039

EXPLORING THE METABOLIC POTENTIAL OF OLEAGINOUS ACTINOMYCETES IN BIODIESEL PRODUCTION FROM CASSAVA WASTEWATER

MARCUS AWONIYI

UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, United Kingdom

Abstract

The depletion in fossil fuel reserves has instigated the search for renewable sources such as biofuels. Biofuel production provides a sustainable alternative to fossil fuels. However, the progression of biofuel industry has been largely affected by several uncertainties including the sustainability of production processes. Finding an economically viable process and the right substrates have been a source of constant debate. Biodiesel production is one such area which is gaining momentum in the last few decades. We are currently investigating cassava wastewater as a substrate and also as a source of oleaginous bacteria. The production of tri-acyl glycerol from mycolic acid containing actinomycetes predominantly *Rhodococcus*, has been identified as a potential source. These bacteria which are largely ubiquitous provide a significant amount of triglyceride when cultivated under low cost waste. The growth of oleaginous bacteria for the accumulation of triglycerides on low cost and abundant, cassava waste still remains unexplored, especially in Nigeria which is the largest producer of cassava in the world. We started our initial investigations on the cassava waste water sample following culture dependent and independent approach. We prognosticate that the identification of microflora isolated from different stages in the sample will provide a basis for understanding the nature of the substrate and the potential for the synthesis of biodiesel from cassava waste water.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B040

Comparison of MRSA skin infection models with HaCaT keratinocytes and a 3D-organotypic skin model.

Nilakshi Barua, Nilakshi Barua

Chinese University of Hong Kong, Shatin, Hong Kong

Abstract

Background and Aims: Skin is a major site for *Staphylococcus aureus* colonization and invasion. A 3D-organotypic skin model was generated by co-culturing keratinocytes with fibroblasts at air-liquid interface enabling the proliferation, migration and differentiation of the keratinocyte to mimic terminal differentiation of the epidermis.

Methods: The adherence and internalization of MRSA strain types ST8, ST30, ST59, ST22, ST45 and ST239 were evaluated in the HaCaT keratinocytes and a 3-D organotypic skin model. Acridine orange staining and/or anti-*Staphylococcus aureus* antibody were used for bacterial localization. TUNEL assay was used to evaluate cell death due to apoptosis.

Results: Maximum adherence to HaCaT cells were exhibited by both MRSA ST59 and ST8 strain types ($p=0.129$, Tukey post-hoc test). The maximum percentage of internalization was exhibited by both ST59 and ST30. With ST8, ST30, ST59 and ST239 types, bacteria were present within the cytoplasm whereas localization of ST22 and ST45in the phagosomes of HaCaT keratinocytes were observed. Study of cell death in HaCaT keratinocytes was limited to 24 hours due to dislodgement of the keratinocytes with all six strains. The 3-D skin model proved to be better to study MRSA transmigration and cell death which could be monitored for longer time points. ST59 exhibited maximum adhesion and internalization, $p<0.001$ (Two-way ANOVA) and induced maximum cell death ($p<0.001$) in the 3D skin model. Double-labeling assay showed variation of bacterial transmigration in the skin model with different MRSA types.

Conclusion: The 3-D skin model proves to be a better model to study MRSA skin infections.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B041

Galleria mellonella: a novel infection model for screening potential anti-mycobacterial compounds against members of the *Mycobacterium tuberculosis* complex

Masanori Asai[1], Yanwen Li[1], Jasmeet Singh Khara[1,2], Brian Robertson[1], Paul Langford[1], Sandra Newton[1]

[1]Imperial College London, London, United Kingdom. [2]National University of Singapore, Singapore, Singapore

Abstract

Animal infection models are vital as drug screens for novel therapeutics to tackle the global tuberculosis (TB) epidemic. However, all pre-existing models have limitations which include ethical constraints. Therefore, efforts to reduce and/or replace conventional animal models in TB research are warranted. Previously, we reported the use of *Galleria mellonella* (greater wax moth, GM) as a novel infection model for the *Mycobacterium tuberculosis* (MTB) complex, using *Mycobacterium bovis* BCG *lux*, a bioluminescent mutant which allows for the rapid quantification of bacterial burden *in vivo*.

Here we investigated the drug screening potential of GM infected with a lethal dose of BCG *lux*, treated with first or second-line antimycobacterial drugs over a 96 h period; where drug efficacy was determined every 24 h through bioluminescent measurement of larval homogenates. Improved survival outcome was observed in all larvae treated with antimycobacterials when compared to untreated controls. Furthermore, all drug treatments except pyrazinamide resulted in a significant reduction in bioluminescence of BCG *lux in vivo*. Isoniazid and rifampicin displayed the highest survival outcome and greatest *in vivo* drug efficacy, in line with observations reported in mice. However, combined or multiple dosing of either drug showed little to no difference over single dose mono-therapy.

Our results demonstrate that GM is a promising infection model for members of the MTB complex, with significant potential for its use in the drug development pipeline as a pre-screening model for novel therapeutics, thereby reducing experimental usage of animals in TB research.

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Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B042

Investigating antibiotic tolerance in cystic fibrosis using *ex-vivo* biofilm lung model

Marwa Hassan, Esther Sweeney, Freya Harrison

School of Life Sciences, University of Warwick, Coventry, United Kingdom

Abstract

Bacterial biofilms are known to have high antibiotic tolerance which directly affects clearance of bacterial infections in cystic fibrosis patients. Current antibiotic susceptibility testing methods are either based on planktonic cells or do not reflect the complexity of biofilms *in vivo*. Consequently, inaccurate diagnostics affect the treatment choice, preventing bacterial clearance and developing antibiotic resistance. This leads to prolonged treatment especially in cystic fibrosis. In this study, we are using an *ex-vivo* biofilm lung model to study antibiotic tolerance in cystic fibrosis to develop better diagnostics. Sections of pig bronchiole were prepared as previously described, infected with lab strains and clinical isolates of *Staphylococcus aureus* or *Pseudomonas aeruginosa* and incubated in artificial sputum media for 2 days to form biofilms. Then, lung-associated biofilm and bacterial aggregates in the surrounding artificial sputum were challenged separately with antibiotics and their bacterial load was quantified. All isolates were also tested for antibiotic susceptibility using standard planktonic and biofilm methods. The results showed increased antibiotic tolerance/resistance of both *S. aureus* and *P. aeruginosa*, >100-fold MIC against all tested antibiotics. All sensitive bacterial isolates, according to the standard antibiotic susceptibility testing, demonstrated a resistant phenotype in the *ex-vivo* biofilm lung model. We are investigating mechanisms behind bacterial persistence in this biofilm model to help better predict the *in vivo* efficacy of antibiotics. We demonstrate a realistic model for antibiotic susceptibility testing clinically and in anti-biofilm drug development to help understanding antibiotic resistance and tolerance in biofilms.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B043

Identifying the underlying mechanisms of decanoic acid in health using a simple model organism

Eleanor Warren, Robin Williams

Royal Holloway, Egham, United Kingdom

Abstract

The medium chain triglyceride (MCT) ketogenic diet is a high fat diet used to prevent seizures in patients with drug resistant epilepsy. As well as treating epilepsy, there is emerging evidence that the MCT diet could be useful in other areas of health, with the potential for use in neurodegenerative disorders, metabolic disorders and cancers. Metabolism of the diet involves the breakdown of triglycerides to release decanoic acid, with new evidence attributing the therapeutic effects of the diet to this fatty acid. Elucidating the mechanisms of the diet is important to improve existing epilepsy treatments, and to validate use of the diet in other areas of health. In this study we employ *Dictyostelium* to identify targets of medium chain fatty acids. We show that *Dictyostelium* is sensitive to decanoic acid during growth at concentrations comparable with levels found in the blood of patients on the diet (around 157 μM). Through utilising a mutant library screen, we have identified potential new targets, including multiple proteins known to interact with the ubiquitous AAA ATPase p97 (cdcD). By ablating these targets in *Dictyostelium* and monitoring the effects on known functions of cdcD*,* we have shown that decanoic acid could be influencing both lipid droplet turnover and autophagy, potentially via the inhibition of mTOR signalling. Thus, through using *Dictyostelium,* we propose a new cellular mechanism of decanoic acid, with the potential for improving our understanding of the role of the MCT diet in health and disease.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B044

Modelling Chronic *Pseudomonas aeruginosa* Infection in Ex vivo Porcine Lung Tissue

Niamh Harrington, Esther Sweeney, Marwa Hassan, Freya Harrison

University of Warwick, Coventry, United Kingdom

Abstract

The chronic lung infections associated with cystic fibrosis are characterised by the interactions of polymicrobial biofilm-forming communities, which affect tissue damage and antibiotic resistance. How different microbes interact in the lung, and how this relates to antimicrobial resistance (AMR) and persistence, remain poorly understood. This is partly due to current lab models of lung infection not accurately modelling the environment and ecosystem of chronically infected lungs. Animal models are typically based on short-term infections in hosts that are otherwise considered healthy, thus do not accurately model the chemical environment of tissue damaged by chronic infection. Despite *in vitro* models attempting to model this environment, they still ignore the crucial spatial structure of the host tissue and microbial biofilms. We are using a clinically realistic, high-throughput model of chronic infection designed to accurately mimic the conditions in CF lungs: lung tissue taken from pigs slaughtered for meat, cultured in synthetic mucus. We demonstrate that our previously described *ex vivo* pig lung model of cystic fibrosis infection is able to successfully replicate key features of chronic infection of the dominant pathogen *Pseudomonas aeruginosa*, and has the potential to be a high-throughput approach to further understanding of interspecies interactions in polymicrobial biofilm infections. The model also addresses the need for replacement alternatives, as outlined by the 3 R's, for the use of animals in research, by using lung tissue from pigs that have been slaughtered for meat, thus considered waste product.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B045

The Dictyostelium rhomboid proteases and mitochondrial disease

Elinor Thompson, Mehak Rafiq

University of Greenwich, Chatham Maritime, United Kingdom

Abstract

Rhomboid membrane proteases are integral to pathogenicity of several microbial eukaryotes and the role of mitochondrial rhomboids is important in pathologies beyond infectious disease. By virtue of their ability to cleave tethered proteins, this relatively recently discovered protein family have been found to activate substrates, release mobile signals, and progress cell regulatory pathways. The *Dictyostelium* model microbe allows the study of an evolutionarily ancient set of rhomboids, aberrant expression of which affects phagocytosis, response to chemoattractants, phototaxis, growth and cell size, ATP levels, and mitochondrial ultrastructure. We report the particularly mitochondrial focus of rhomboids in this amoeba and consider the relevance of this beyond the model organism, given the central role of the human mitochondrial rhomboid in mitophagy, ageing and neurodegenerative disease.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B046

Pigs, PAMs and Pathogens: using primary alveolar macrophages from abattoir acquired porcine lungs to model pulmonary infections.

Johanna McNicholl, Illaria Maurizio, David Carrenos Yugueros, Peter Andrew, Marco Oggioni

University of Leicester, Leicester, United Kingdom

Abstract

The similarity between the porcine and human immune system makes pigs an ideal model for studying infectious disease. We have been using alveolar macrophages recovered from abattoir-sourced porcine lungs to create a model to study *Streptococcus pneumoniae* infections in the lung.

Pneumonia is a leading cause of death from infectious disease, with *S. pneumoniae* the most common cause of bacterial pneumonia. Although much studied, there is still a lot we don't know about the early stages of infection. Recent research in our lab has found a previously unknown intracellular phase for *S. pneumoniae* within splenic macrophages

Alveolar macrophages were recovered by bronco-alveolar lavage and used in gentamicin protection assays. Macrophages challenged with *S. pneumoniae* at an MOI of 25 bacteria per cell were found to contain live bacteria up to 5 hours after infection. Analysis of samples collected at 45, 90, 180 and 300 minutes after challenge found less than 10% of the challenge dose present at 45 min, decreasing to 0.02% after 5 hours. The presence of bacteria inside the macrophages was confirmed by confocal microscopy.

Studies are on-going to unpick the processes that allow the bacteria to resist phagocytosis by the macrophages, and to use the model to investigate the interaction of the macrophages with other respiratory pathogens. The use of abattoir-acquired porcine cells contributes to the 3Rs – reducing and replacing laboratory animals, and refinement of method as pigs are more akin to humans than mice.

J. McNicholl is sponsored by the Daphne Jackson Trust.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B047

Using human iPSC derived small intestinal organoids as a model for enteric disease caused by Enterotoxigenic E. coli and Vibrio cholerae

Leanne Kane[1,2], Christine Hale[1], David Goulding[1], Matthew J Dorman[1], Simon Clare[1], Brendan Wren[3], Nicholas Thomson[1,3]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]London School of Hygiene and Tropical Medicine, London , United Kingdom. [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Within the last ten years, iPSC (induced pluripotent stem cells) have been widely shown to have the ability to be re-programmed to produce a wide range of tissues in the presence of certain growth factors.

In this project, we re-direct human stem cells derived from fibroblasts into complex 3D small intestinal structures termed organoids. These organoids have been shown to possess all cell types that are present in small intestinal tissue such as, enterocytes, goblet cells, enteroendocrine cells and Paneth cells, as well as possessing microvilli and crypt structures.

We demonstrate that it is possible to microinject into the lumen of these small intestinal organoids and to manipulate the conditions for infection of non-invasive bacteria such as Enterotoxigenic *E. coli* (ETEC) and *Vibrio cholerae*.

Looking at known bacterial virulence factors, we have shown there are differences in patterns of infection among different strains of entero-pathogenic bacteria. In addition, we have shown that the induced human organoids (iHO) elicit a recognisable and measurable host response to bacterial toxin.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B048

The utilisation of organoids and macrophages derived from Human induced pluripotent stem cells as model systems to investigate host-bacterial interactions

Christine Hale, Leanne Kane, Matthew Dorman, Nicholas Thomson

Sanger Institute, Cambridge, United Kingdom

Abstract

Abstract

Using human induced pluripotent stem cell (hiPSC) technology we are developing methods to examine host-bacterial interactions. Due to the fact that undifferentiated human induced pluripotent stem cells are amenable to genetic engineering, can be cultured indefinitely and can further be differentiated into multiple cell types, we are exploiting both organoid and macrophage systems to investigate the interactions between host cells and diarrhoeal pathogens, including enterotoxigenic Escherichia coli and Vibrio cholerae. Utilising both wild type and relevant knockout hiPSC lines we are probing both initial interactions and subsequent utilisation of pathways for the effects of toxins. The further analysis of genetically engineered bacteria extend the usefulness of this model system, and complement the availability of mutant host cells, towards the simultaneous genetic analysis of both pathogen and host.

Focus on 3Rs: the growing role of organoids and microbial models to understand human and animal diseases

Presentations: Wednesday evening and Thursday lunchtime

B049

Modelling biofilms on infected chronic wounds

Yanyan Cheng, Paul De Bank, Albert Bolhuis

University of Bath, Bath, United Kingdom

Abstract

Chronic wounds, for instance venous, pressure, arterial and diabetic ulcers, are a major health problem throughout the world. Compared with normal wounds, those that take more than four weeks to heal are defined as chronic. Interestingly, the numbers of patients suffering from chronic wounds and the cost for treatment have been increasing during the past two decades. There is increasing evidence that suggests that bacteria infect those chronic wounds and there exist as a biofilm, which affects the wound healing and success of wound treatment. To study biofilms in infected wounds, both *in vitro* and *in vivo* biofilm models have been developed. In this project, the colony biofilm assay was used to determine antibiotics effect on removing biofilm. The results of this study so far indicated that mature Staphylococcus aureus biofilms were resistant to vancomycin treatment, which works effectively on killing planktonic cells. However, other antibiotics used topically for healing infected chronic wounds, for example, gentamicin, tetracycline, fusidic acid and mupirocin, were more effective at killing mature biofilms in the colony biofilm model. These sets of experiments were also done with pig skin based on colony biofilm assay. This project aims to build up a new *ex vivo* dynamic model using pig skin and a 3D print flow chamber to mimic chronic wounds. Hence, the results gathered from the colony biofilm assay will be compared with those obtained for the newly developed model. This model can then be used to study the drug delivery and topical treatment of chronic wounds.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B050

Enhancing protective efficacy of poultry vaccines through targeted delivery of antigens to antigen presenting cells

Angita Shrestha[1,2], Dr Munir Iqbal[1]

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

Abstract

In recent years, poultry production has been under constant threat of infectious diseases such as avian influenza and Newcastle disease resulting in the poultry trade reduction and zoonotic infection risk. Vaccination has become one of the important measures in controlling such diseases. One way of increasing the efficacy of these vaccines is to deliver specific antigens selectively targeting antigen presenting cells (APCs). This could be achieved by coupling APC receptor-specific antibody to the antigen of choice. To achieve this, we are targeting avian influenza hemagglutinin (HA) protein to the chicken dendritic cell (DC) surface receptors. We have recombinantly produced H9HA antigen fused to single chain fragment variable antibodies (scFv) against the chicken DC surface receptors. To assess the HA activity of our recombinantly produced H9HA fused to scFv antibody, hemagglutination assay was adopted. Our constructs were able to agglutinate chicken red blood cells and therefore retained HA activity. Furthermore, the scFv antibodies fused to HA were able to detect their respective antigens via western blot indicating that these scFv antibodies are functional. To further characterise our H9HA fused scFv antibodies *in vitro,* their binding ability to chicken DCs will be assessed using flow cytometry. Therefore, we have created functional H9HA antigen targeting to chicken DCs which we aim to use in developing enhanced vaccines towards avian influenza virus. In future, we will clone our constructs into herpesvirus of turkey to generate a recombinant viral vector vaccine which we hypothesise would enhance the immune response in chickens.

Virology workshop: Antivirals and vaccines
Presentations: Wednesday evening and Thursday lunchtime

B051

Analysis of hepatitis C virus genotype 3 resistance to direct acting antivirals

Guilherme Rodrigues Fernandes Campos[1,2], João Paulo Vilela Rodrigues[3], Cintia Bittar[2], Bruna Forte Aguiar[3], Silvana Gama Florêncio Chacha[4], Fernanda Fernandes Souza[3], Leonardo Régis Leira Pereira[3], Ana de Lourdes Candolo Martinelli[3], Mark Harris[1], Paula Rahal[2]

[1]University of Leeds, Leeds, United Kingdom. [2]São Paulo State University, São José do Rio Preto, Brazil. [3]University of São Paulo, Ribeirão Preto, Brazil. [4]Federal University of São Carlos, São Carlos, Brazil

Abstract

Treatment of hepatitis C virus (HCV) with direct acting antivirals (DAAs), results in a sustained virological response (SVR) that varies according to the viral genotype. Genotype 3 is the second most prevalent in Brazil and presented the lowest response among all genotypes, this was associated with the occurrence of resistance substitutions in the viral genome. The aim of this study was to analyze genotype 3 viruses circulating in Brazilian patients who do not respond to treatment with Daclatasvir to investigate the presence of novel substitutions within NS5A, and to investigate the role of these substitutions in resistance to different NS5A inhibitors *in vitro*. A total of 60 patients have been analyzed, including 4 relapsers. Samples collected before (all patients) and after treatment (relapsing patients) were analysed by RNA extraction, cDNA synthesis, amplification by Nested-PCR and direct Sanger sequencing. Many previously undefined substitutions were indeed observed. These included S98G which, although detected in 15% of pre-treatment samples, substitution appeared in 75% of post-treatment samples from the non-responding patients and only in 10.7% of responding individuals. For post-treatment samples in relapsing patients, this substitution had a prevalence of 50%. Due to the high prevalence, this substitution showed potential to be associated with therapy failure. The phenotypes of this substitution and others identified in the study are being evaluated in the context of a genotype 3a subgenomic replicon and infectious virus to determine their effect on DAA resistance and/or potential fitness cost.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B052

Production and characterization of antibodies neutralizing H9N2 avian influenza virus

Deimante Lukosaityte[1,2], Jean-Remy Sadeyen[1], Munir Iqbal[1], Paul Digard[2]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom

Abstract

Monoclonal antibodies (mAbs) that neutralize influenza A virus have been shown to be potent therapeutic reagents if used pre- or post - exposure to the pathogen. Whilst the majority of this research has focused on human antibody therapeutics, there is an urgent need for generation of antibodies able to neutralize avian influenza viruses (AIV). Several mAbs against H9N2 virus have previously been generated using mouse hybridomas. To facilitate development of passive immunization strategies, the variable chains of these antibodies were characterized in the context of isotype and species specific Fc fragments. Replacement of the IgG2 Fc region with an IgG1 Fc region enhanced neutralizing activity for one of the tested antibodies. Additionally, chicken chimeric antibodies were generated which showed comparable neutralization titers to those generated by the original hybridomas. To identify if antibodies could function as a single chain variable fragment antibodies (scFvs) these were produced in insect S2 cells. This confirmed that H9N2 virus can be neutralized by scFvs. However, an example with high HI titers but lost detectable neutralizing activity was found, possibly due to the loss of bivalent interaction between antigen and antibody. Antibodies showing superior neutralization activity *in vitro* will be subsequently tested for their potency in *in vivo* infection. We propose that passive immunization can reduce the impact of AIV in poultry by inducing immediate protection and bypassing immunocompromised individuals.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B053

A range of antigen capture and production strategies for VLPs based on hepatitis B virus core protein

Jehad Alzahrani[1,2], Kaniz Fatema[1], Lee Sherry[1], Keith Grehan[1], Martin Stacey[1], David Rowlands[1], Nicola Stonehouse[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Shaqra University, Riyadh , Saudi Arabia

Abstract

Vaccines are effective medical interventions and have an enormous impact on human and animal health. Although the most widely used vaccine strategies against viral pathogens, including live attenuated vaccines and inactivated vaccines have provided effective tools against a variety of diseases such as polio, measles and smallpox, they also have some limitations. These traditional methods of generating vaccines are unsuitable for some major human pathogens such as Ebola or HIV. Furthermore, some current vaccines are not ideal for individuals with weakened immune systems.

Virus-like particles (VLPs) are self-assembling proteins that mimic the structure of the origin virus capsid and are highly immunogenic, yet lack the genetic information required to cause an infection, therefore making them a safer alternative vaccine. Additionally, VLPs can be manipulated to present a number of different antigens.

We have a novel and flexible VLP vaccine presentation system based on hepatitis B core (HBc) antigen, termed 'tandem core'. We are studying a modified form of 'tandem core' VLPs which presents a scaffold protein known as an Affimer at the surface of the assembled particle. The Affimer can recognise a sequence tag which is fused to a 'target antigen', so creating complexed particles which display multiple copies of the 'target antigen' at their surfaces. The multimeric display of antigens proteins at the surface of recombinant VLPs can significantly improve their immunogenicity. Here, we have produced HBc VLPs using both *E. coli* and *Pichia pastoris* expression systems and determined their capability for antigen capture via the Affimer scaffold sequence.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B054

Characterising the Cell-Mediated Immune Response to Lumpy Skin Disease Virus

Najith Wijesiriwardana[1,2], Ismar Haga[1], Beatriz Sanz-Bernardo[1], Simon Graham[1,2], Pip Beard[1]

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

Abstract

Lumpy skin disease (LSD), a high-impact disease of cattle and water buffalo, is a direct threat to the European cattle industry. The causative agent is the lumpy skin disease virus (LSDV), an enveloped dsDNA virus which belongs to the family Poxviridae. Affected cattle present multiple cutaneous nodules that are characteristic of a LSDV infection. The case fatality rate of LSD is low, however affected animals suffer substantial production losses including weight loss and reduced milk production. On a wider scale, LSD is a high consequence transboundary disease with mandated export restrictions imposed on affected countries leading to substantial economic costs.

Currently, there is no cure for LSDV infected cattle. Hence, mass vaccination is an important component in minimizing the spread of LSDV. There are only a handful of commercially available live attenuated vaccines in the current market. These vaccines vary in efficacy, quality, and safety, which leaves some scope for further development. This improvement is hampered by a poor understanding of the immunology of LSDV, particularly the protective immune mechanisms.

In this project, we are investigating the immune response to LSDV with a focus on characterising the cell-mediated immune response. Peripheral blood mononuclear cells from LSDV immunised/infected cattle and flow cytometry assays are used to detect the production of IFN-γ by CD4+ T-helper cells and CD8+ cytotoxic T cells in response to live LSDV stimulation. Our goal is to improve understanding of the immune response to LSDV in order to develop effective vaccines and control programs in the future.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B055

Selection of thermally-resistant mutants of enterovirus A 71 for future application as a genome-free VLP vaccine.

Mona Shegdar [1,2], Natalie Kingston[1], Oluwapelumi Adeyemi[1], David Rowlands[1], Elisabetta Groppelli[1], Nicola Stonehouse[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Saudi Ministry of Health , Riyadh , Saudi Arabia

Abstract

Enterovirus A 71 (EVA71), a member of the *Picornaviridae*, is an important causative agent of hand, foot and mouth disease (HFMD). It has been associated with severe neurological complications and mortality among children.

Vaccination remains the only answer to reduce the burden of EVA71 in the absence of antiviral therapies. Leading vaccine candidates are currently inactivated virus, utilising virus-like particle (VLP) technology may be considered to produce a safer and cheaper vaccine alternative. VLPs are assembled from viral structural proteins similar to the naturally produced empty capsid of the virus, and capable of inducing potent adaptive immune responses while lacking the ability to replicate. However, an efficacious EVA71 VLP vaccine requires the maintenance of VLP structure in a native conformation and therefore the appropriate display of epitopes. This study aims to build thermally stable and immunogenic EVA71 VLPs as the basis of future genome-free vaccine candidates.

As an RNA virus, EVA71 has high mutation rate due to error-prone replication resulting from the absence of proofreading, thus viruses exist as populations with high degree of genetic and phenotypic heterogeneity, termed a quasispecies. Based on our established protocols with poliovirus, EVA71 mutants with enhanced stability were selected through cycles of increasing thermal stressing.

Capsid stabilising mutations were identified, engineered into an EVA71 infectious clone, expressed in mammalian cells and further characterised for stability and antigenicity. The mutant virus candidates resulted in the production of thermally stable EVA71, and initial studies have indicated that some of the mutants retained native antigenicity.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B056

A synthetic biology approach for African horse sickness vaccine platforms

Meredith Stewart[1], Alejandro Lopez[2], Vanessa Herder[1], Javier Ortego[2], Massimo Palmerini[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]INIA-CISA, Madrid, Spain

Abstract

African horse sickness virus (AHSV) is the causative agent of African horse sickness (AHS), a highly fatal disease of equids. Currently, live attenuated vaccines are used to control AHS especially in South Africa, but they are in general subject to restrictions. We took advantage of previously published AHSV structural and antigenic data to simplify AHSV vaccine development using an AHSV-4 reverse genetics approach. We systematically substituted the tip and central domains of AHSV-4 outer capsid protein VP2 with the corresponding region from other serotypes to generate a chimeric protein. AHSV S2 chimeric and mono-reassortant viruses for all 9 serotypes were recovered by reverse genetic using AHSV-4 as backbone and used to immunise guinea pigs. Presence of neutralising antibody titres were determined using a fluorescence-based neutralisation assay. The exchange of the tip and central domain of VP2 switched the serotype specificity of the rescued chimeric viruses, however, sera from AHSV-4VP2DTip only neutralised the homologous AHSV-4 reference virus. Mono-reassortants, but not recombinant viruses containing chimeric VP2, induced antibodies with low levels of cross-neutralisation between phylogenetically related serotypes. Interestingly, most of the sera raised were able to neutralise AHSV-4, indicating the presence of other neutralising epitopes within the virus. These results raise the possibly of generating a single virus that affords protection against multiple serotypes. Our research highlights the ability to manipulate the AHSV genome to rapidly generate 'synthetic' viruses using a single platform approach for vaccine development.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B057

Investigating specific mutations in the PB2 gene of H5N1 Influenza conferring host adaptations in mice

Heather James, Carolyn Nicolson, Kate Guilfoyle, Othmar Engelhardt, Ruth Harvey

NIBSC, South Mimms, United Kingdom

Abstract

Highly pathogenic H5N1 influenza viruses can cause potentially lethal infection in humans. The influenza challenge model in mice is well-established and important in preclinical studies. However, as infection in mice does not always present with the same clinical symptoms as human infections it is often necessary to mouse adapt the chosen influenza strain to produce a suitable challenge virus. Two mouse adapted (ma) H5N1 challenge strains, NIBRG-14ma and NIBRG-23ma, were produced by serial passage in mice followed by a single passage in mammalian cells and were shown to have increased infectivity and lethality compared to their parent viruses. Previous work investigated the genetic molecular markers associated with the host adaptation of these H5N1 viruses using Next Generation Sequencing (NGS) and showed a single nucleotide change in the PB2 gene compared to the parent virus. This resulted in a non-synonymous amino acid change of both ma viruses (Guilfoyle et al, Poster 159 Edinburgh, April 2017).

It was hypothesised that this change increased the PB2 activity *in vivo* leading to increased pathogenicity. To help further understand the mechanisms of host adaptation, we investigated if the ma viruses showed increased RNP complex processivity (measured via protein output in cell culture) compared to their parent virus. Additionally, we sought to confirm whether the single nucleotide change occurred during the single passage in mammalian cells or was a result of serial passage in mice and was therefore a true host adaptation.

Virology workshop: Antivirals and vaccines

Presentations: Wednesday evening and Thursday lunchtime

B058

Application of Next Generation Phage Display technology to study cross reactivity in closely-related flavivirus species

Anitha Varghese[1,2], Janet Daly[1], Kevin Gough[1], Mara Rocchi[2]

[1]University of Nottingham, Sutton Bonnington, United Kingdom. [2]Moredun Research Institute, Penicuik, United Kingdom

Abstract

Flaviviruses are a large family of viruses, some members of which cause human and veterinary disease and pose a potential risk of death. The transmission route is typically through the bite of arthropods such as ticks or mosquitoes and. They are a major cause of emerging and re-emerging viral infections. One of the major issues faced when carrying out serology diagnostics is the risk of cross reactive antibodies among closely related species.

Next Generation Phage Display is a molecular biology technique combines phage display with next generation sequencing. A phage library is created by inserting peptide sequences into a phagemid vector. Each phage in the resultant library displays a particular peptide on its external surface and the resultant phagemid is packaged within the phage particle. The main advantage of phage display is linking the phenotype (peptide binding properties) with genotype (the peptide gene within the phagemid)

Serum antibodies from flavivirus infected species are immobilised on a solid support and incubated with the phage library. Following washing steps to remove non-specific binding, the phage are rescued and propagated in bacteria. This process is called biopanning and is repeated up to 4 times. The phage genomes are sequenced using Ion Torrent sequencing and through analysing the sequences, the antigenic peptide regions can be identified.

The most antigenically potent sites can then be used to create a diagnostic assay such an ELISA.

Biobased circular economy and bioremediation
Presentations: Wednesday evening and Thursday lunchtime

B059

A preliminary investigations of novel dehalogenase producing bacteria from Antarctic Psychrotropic Bacillus sp.Ih1

Ismail Haruna

Bauchi State University Gadau, Gadau, Nigeria

Abstract

A preliminary investigations of novel dehalogenase producing bacteria from Antarctic Psychrotropic *Bacillus sp.Ih1*

Ismail Haruna

[1]Department of Microbiology, Bauchi State University Gadau, PMB 65 Gadau Bauchi State, Nigeria
(E-mail: ismailharuna68@yahoo.com)

2, 2 dichloropropionic acids (Dalapon) like most halogenated compounds are commonly used as herbicides and employed in agricultural areas and industries.Toxicity of these xenobiotic compounds causes serious environmental problems. Bacillus sp ih1 was isolated from top cliff soil collected from Antarctica.The bacteria was first grown on Antarctic bacterial medium and later transferred to a minimal medium containing 2,2,dichloropropionic acid as carbon source. It grew slowly in the minimal media in different concentrations of10mM, 20mM, 30mM and 40mM of 2,2 DCP. The best growth was observed in 20mM of 2,2-DCP with 32 hours as doubling time.To monitor the degradative activity of the bacteria, halide ion assay was carried out to check the release of chloride ion. The best release of chloride was 0.657mMol/L in 20mM of 2,2-DCP.The bacteria was identified using 16SrRNA, genomic DNA extraction and PCR amplification of 16SrRNA was performed using universal primers 27F and1492R. Nucleotide blast (BLASTn) showed 97% similarity with bacillus sp. Results from biochemical tests further confirm the bacteria as bacillus sp.Using phylogeny.fr, sequences from nucleotide blast result were used to build a phylogeny tree based on neighbor to neighbor joining.

Key words: 2,2 DCP , dehalogenase, degradation

Biobased circular economy and bioremediation

Presentations: Wednesday evening and Thursday lunchtime

B060

Utilization of Hydrocarbons and Lignin-like compounds by Alcaligenes sp. isolated from Ilorin alfisol loam

david Adetitun[1], Babu Fathepure[2], H Hugh[2], Olatunji Kolawole[1], Albert Olayemi[1], Adetomiwa Bonioly[1]

[1]University of Ilorin, Ilorin, Nigeria. [2]Oklahoma State University, Stillwater, USA

Abstract

Alcaligenes sp. was isolated from kerosene polluted soil in Ilorin. Ability of *Alcaligenes* to thrive on kerosene, other hydrocarbons and lignin-like compounds was tested in vitro. The ultimate goal was to use *Alcaligenes* sp. to degrade hydrocarbons in contaminated niches and lignin in plant biomass for the generation of biofuel. *Alcaligenes* sp. was originally grown on mineral salts medium with kerosene as sole energy and carbon source. The capacity of *Alcaligenes* sp. to degrade both aromatic and aliphatic hydrocarbons including hexane, hexadecane, cyclohexane, phenol, benzoate, benzene, toluene, ethylbenzene, xylene (BTEX) and eleven lignin-like compounds (anisoin, benzylvanillin, cellobiose, cinnamic, lignin, methylvanillin, syringic acid, vanillic acid, veratryl alcohol, veratric acid and. xylan) was tested. Degradation of BTEX was monitored using gas chromatograph, use of benzoate and phenol was examined using spectrophotometer while utilization of hexadecane, hexane, cyclohexane and the lignin-like compounds was measured by standard plate (colony) counts. The isolate utilized ($p<0.05$) kerosene significantly. The organism utilized ($p<0.05$) hexadecane, cyclohexane, phenol and benzoate, but not hexane, benzene, toluene, ethylbenzene and xylene. *Alcaligenes* sp degraded 2mM benzoate to 0.2mM in 2 days. Hence, natural populations of *Alcaligenes* sp. could be readily used at different rates to degrade hexadecane, cyclohexane, benzoate, phenol, anisoin, benzylvanillin, cellobiose, cinnamic, lignin, methylvanillin, syringic acid, vanillic acid, veratryl alcohol, veratric acid and. xylan,. The utilization of cinnamic acid, vanilic acid and vatic acid was insignificant in week 0 and 2 but peaked in week 4. *Alcaligenes* sp capable of degrading hydrocarbons and lignin compounds could be sourced from oil polluted sites.

B061

Applying transposon-directed insertion site sequencing to industrially important, solventogenic species *Clostridium saccharoperbutylacetonicum*

Laurence de Lussy-Kubisa, Roy Chaudhuri, Robert Fagan

The University of Sheffield, Sheffield, United Kingdom

Abstract

Solventogenic *Clostridium spp* have significant potential as a source of renewable biochemicals. Production of solvents such as butanol and acetone from members of this genus is already being commercialised by industry. However, the scale of biological knowledge of these *Clostridia* lags behind the scale of their application. With the advent of next generation sequencing methods, transposon mutagenesis now provides a large-scale, high-throughput forward approach to understanding the genetics of these species. We are applying transposon directed insertion-site sequencing (TraDIS) to the industrially-relevant solventogenic species *Clostridium saccharoperbutylacetonicum*. We are utilising this robust TraDIS pipeline to uncover the essential genome of the species under laboratory conditions. Furthermore, we are investigating the species' tolerance to butanol, a key limiting step in the fermentation process. In collaboration with Green Biologics, we are also examining the essential genome of the speices under industrially important fermentaion conditions. Understanding the genes that are conditionally essential in these contexts will be key in advancing the biological knowledge of the speices as well as providing information that can improve the fermentation process.

Biobased circular economy and bioremediation
Presentations: Wednesday evening and Thursday lunchtime

B062

Identification and quantification of Antibiotic Resistant Bacteria & Genes in an aquaculture facility which uses a novel bioactive filtering system.

Aidan O'Flaherty, Fiona Walsh

Maynooth University, Maynooth, Ireland

Abstract

The Advanced Biotechnology for Intensive-Freshwater Aquaculture Wastewater Reuse (ABAWARE) project, which is part of the European Commission's Water Joint Programming Initiative 2016 Joint Call, aims to increase the efficiency and resilience of water use in aquaculture and minimise its negative impact on the environment and human health. .This research, which forms one part of the total ABAWARE project, aimed to ascertain the impact of using microbiota and certain plant species, in conjuncture with a more traditional Recirculated Aquaculture System (RAS), as a filtering system had on the ARB & G abundance in various samples taken from an aquaculture facility. Sediment and water samples were taken from the inflow, the main fish basin, after the bioactive ponds before filtration, and after filtration. The resistance genes present in these samples were detected using the Wafergen smartchip real-time qPCR system. This system allows for the simultaneous quantification of 348 distinct Antibiotic Resistance Genes for each sample. The samples also underwent microbiome analysis via 16S rRNA metagenomic sequencing. Mothur was used to analyse the sequencing data. This data informs us of the changes in the microbial population changes that are enacted by the various stages within the aquaculture facility.

Biobased circular economy and bioremediation

Presentations: Wednesday evening and Thursday lunchtime

B063

Meeting the rare earth expanding circle

Megan Barnett[1], Simon Gregory[1], Eimear Deady[2], Barbara Palumbo-Roe[1]

[1]British Geological Survey, Nottingham, United Kingdom. [2]British Geological Survey, Edinburgh, United Kingdom

Abstract

The increasing demand for rare earth elements (REE) is fuelled by their importance in a green energy future, with the demand for dysprosium predicted to increase by 5% annually by 2026. Bioleaching approaches are being investigated for the recovery of REE and other precious metals from waste materials, however even 100% recovery will not be able to meet increasing demand. Therefore, extraction from primary sources will be required. REE do not form high concentration ores, so their extraction can require processing large volumes of material, however REE are frequently associated with other raw materials and REE are sold as by-products of iron mining from Chinese deposits. Bioleaching offers the potential to produce valuable by-products from existing mining operations or to remediate historical mine waste. The diverse nature of REE-bearing minerals means that a variety of established and emerging bioleaching approaches could be applied: organic acid leaching, oxidative leaching of sulphidic ores, and reductive leaching of oxidised ores. We have applied these processes to three bauxites, demonstrating varied responses to bioleaching with each bauxite. The combination of low concentration ores and varied sources provide a challenge to recovery: however, it is one that microbes could take on.

Biobased circular economy and bioremediation

Presentations: Wednesday evening and Thursday lunchtime

B064

Formation of selenium- and tellurium-containing nanoparticles during the growth of filamentous fungi

Xinjin Liang[1,2], Magali Aude Marie-Jeanne Perez[3], Joerg Feldmann[3], Laszlo Csetenyi[1], Geoffrey Gadd[1]

[1]University of Dundee, Dundee, United Kingdom. [2]University of South Wales, Treforest, United Kingdom.
[3]University of Aberdeen, Aberdeen, United Kingdom

Abstract

Microbial Se or Te reduction offers a potential route to biorecovery of these elements from solution. Reduction is often efficient and large amounts of these metalloids can be removed from solution, resulting in extensive precipitation around biomass. This is more effective than biomethylation, which can result in only small amounts of removal, and would necessitate a further trapping step to recover volatilized methylated derivatives. In this research, the fungi *Aureobasidium pullulans, Mortierella humilis, Trichoderma harzianum, and Phoma glomerata* were used to investigate the formation of selenium- and tellurium-containing nanoparticles during growth on selenium- and tellurium-containing media. Most organisms were able to grow on both selenium- and tellurium-containing media at concentrations of 1 mM and this resulted in extensive precipitation of elemental selenium and tellurium on fungal surfaces observed by the bright red and black colour changes. Red or black deposits were confirmed as elemental selenium and tellurium, respectively, by X-ray powder diffraction. Apart from elemental selenium and tellurium, selenium oxide and tellurium oxide were also found after growth of *Trichoderma harzianum* in the presence of 1 mM selenite and tellurite together with the formation of elemental selenium and tellurium. The hyphal matrix provided nucleation sites for metalloid deposition with extracellular protein and extracellular polymeric substances serving to localize the resultant Se or Te nanoparticles. These findings are relevant to remedial treatments for selenium and tellurium contamination, and possible novel approaches for selenium and tellurium biorecovery from liquid matrices.

Biobased circular economy and bioremediation

Presentations: Wednesday evening and Thursday lunchtime

B065

From trash to treasure - turning plastic waste into biodegradable polymers using bacteria.

Brian Johnston[1], Marek Kowalczuk[1,2], David Hill[1], Fideline Tchuenbou-Magaia[1], Itohowo Jonah[1], Iza Radecka[1]

[1]University of Wolverhampton, Wolverhampton, United Kingdom. [2]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland

Abstract

Mountains of plastic waste consisting of carrier bags and medical packaging are buried in landfill sites dumped in rivers around the world annually. Unfortunately plastics generated by the petrochemical industry are not biodegradable and therefore accumulate in the environment at a rate of over 25 million tones year^{-1}. Therefore there is a huge demand for biodegradable plastics. Polystyrene (PS), polyethylene (PE) and polypropylene (PP) are problematic materials, used for appliance housings, disposable cutlery and general packaging. This study investigates the utilisation of waste PS, PE and PP as a potential additional carbon sources using bacteria to synthesise polyhydroxyalkanoates (PHAs); a value-added material, able to replace some conventional fossil-fuel plastics while being non-toxic, fully biodegradable and biocompatible. Prodegraded waste PS and PP, and thermally treated PE were used as supplementary carbon sources to tryptone soya broth (TSB and BSM) for 48 hour fermentations [1,2,3]. The bacterial strain *Cupriavidus necator* H16 was selected as it is non-pathogenic, genetically stable, robust and one of the best natural producers of PHA. The accumulation of PHAs varied from 17% (wt / wt) of dry biomass in TSB controls to 39-66% for PS, PE and PP thermally treated samples. The polymers obtained were analysed with nuclear magnetic resonance (NMR) and electrospray ionisation tandem mass spectrometry (ESI-MS/MS) to characterise their chemical structure. In conclusion, certain thermal treatment protocols of the waste plastics were shown to be viable for PHA production; with 3-hydroxybutyrate and up to 12 mol % of 3-hydroxyvalerate and 3-hydroxyhexanoate co-monomeric units formed.

Biobased circular economy and bioremediation
Presentations: Wednesday evening and Thursday lunchtime

B066

Dairy processing wastewater as a feedstock for microbial bioplastic production

Arno Fricke[1], Jerry D. Murphy[2], Niall D. O'Leary[1]

[1]University College Cork, Cork, Ireland. [2]Environmental Research Institute, Cork, Ireland

Abstract

The end of the European milk quotas in 2015 resulted in a steep increase of Irish milk production from 5 to over 7.2 billion litres annually. Dairy processing ads value, but generates up to 10 litres wastewater (WW) per litre processed. Organic pollutants in the WW need to be removed before discharge. Our approach aims to turn this waste into a resource for polyhydroxyalkanoates (PHA), bioplastic production. Bioplastics are promising materials to reduce our dependency on fossil fuels and waste production. The first production step includes an adaption of the dairy processing WW in an anaerobic, hydrolytic reactor, where acidogenic bacteria metabolise the organic fraction of the WW and form volatile fatty acids (VFAs) the building blocks of PHA. The second step comprises the adaption of biomass towards PHA accumulation. The adaption is driven by an aerobic dynamic feeding strategy to increase the formation of the storage molecule PHA. Addition of the WW as substrate is followed by a starvation period, where bacteria capable of PHA storage have a selection advantage. Over time the mixed microbial system is therefore optimised for PHA accumulation. In a final production step the adapted WW and biomass is combined in a fed-batch reactor to produce the end product PHA. The laboratory scale system could be established and is currently optimised. A later scale-up will help to assess the full economic potential of this waste to value approach.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B067

Identifying the role of complement receptor 2 (CR2) on follicular dendritic cells (FDCs) in the persistence of foot and mouth disease virus (FMDV).

Lucy Gordon[1,2], Neil Mabbott[3], Bryan Charleston[1], Eva Perez[1]

[1]The Pirbirght Institute, Surrey, United Kingdom. [2]The University of Edinburgh, Edinburgh, United Kingdom. [3]The Roslin Institute, Edinburgh, United Kingdom

Abstract

More than 50% of cattle (regardless of foot and mouth disease (FMD) vaccination) become persistently infected for long periods of time (years) after exposure to FMD virus (FMDV). The mechanisms associated with establishment of persistent infections are still poorly understood. I am testing the hypothesis that complement receptor 2 (CR2) on follicular dendritic cells (FDCs) located in the germinal centers of the lymphoid tissue, are involved in the trapping and long-term persistence of FMDV and that this persistence is essential for the maintenance of long-term antibody responses to FMDV.

The aim of this study was to assess FMDV antigen retention and the generation of the specific immune response *in vivo,* in a mouse model. Groups of mice were treated with an anti-CR2 monoclonal antibody, named 4B2, which has been described previously to block CR2 long-term *in vivo*, blocking for 6 weeks after a single injection of 2mg (Kulik et al., 2015). After treatment with 4B2, animals were infected with FMDV and lymphoid tissues and serum samples evaluated for the presence of antigen and the humoral immune response, respectively.

The ability of 4B2 to block the binding of FMDV and PAP immune complexes (ICs) to FDCs *in vitro* as well as results about the role of FDC in trapping FMDV via CR2 *in vivo* is currently being investigated and will be discussed.

B068

Investigating the interaction between Influenza A virus and the chicken microbiome

Klaudia Chrzastek, Khalid Zakaria, Dagmara Bialy, Jean-Remy Sadeyen, Holly Shelton

The Pirbright Institute, Pirbright, Woking, United Kingdom

Abstract

Low pathogenicity avian influenza virus (LPAIV) of the H9N2 subtype has been endemic in poultry populations throughout Asia, Middle East, Europe, and Africa since it emerged in China during 1994. These viruses have caused severe economic losses for the poultry industry as they result in reduced egg production and moderate to high mortality in broiler chickens. Control of H9N2infection of poultry is difficult and even a long-term vaccination programmes have not prevented the spread and endemic establishment of this disease. In chickens, LPAIVs have tropism for both the respiratory and gastrointestinal tract. The commensal microbiome is known to play a critical role in shaping host defences against pathogens and infection by influenza has been shown to alter this population in mammals and birds. We hypothesis that modulation of the commensal microbiome populations at sites of influenza replication can modulate host responses to influenza infection and this can have a resultant impact on infection kinetics. We used groups of chickens that were fed a commercially available probiotic supplemented diet from day 1 post hatch or a non-supplemented diet and at 2 weeks of age challenged them with a H9N2 virus. We monitored viral shedding, changes in microbiome and followed innate responses prior and during infection of the chickens. Our results are presented here.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B069

Studying the Mammalian adaptation potential of Influenza A viruses in a single infection cycle

Mohammad Khalid Zakaria[1], Rebecca Frise[2], Thomas Peacock[2], Wendy S Barclay[2], Holly Shelton[1]

[1]The Pirbright Institute, Surrey, United Kingdom. [2]Imperial College, London, United Kingdom

Abstract

Various influenza A viruses (AIVs) have infected humans in the last decade. These infections result from spill over into humans at times of AIV poultry outbreaks with severe clinical consequences for those infected. These AIVs do not have the ability to be efficiently spread among humans via respiratory droplets or aerosols and subsequently cause a pandemic. However, adaptation of these avian influenza viruses to humans by mutation or reassortment may change this. We know that AIVs require adaptation of several critical characteristics in order to effectively spread between humans. Therefore, in order for human pandemic emergence, AIV that crosses into a human host must already be sufficiently able, or must rapidly adapt to overcome these constraints to transmit to a subsequent human host before infection is cleared by an immune response. In this study, we used mice and analysed the mutations acquired by avian origin H7N9 and H9N2 isolates in a temporal fashion during a single infection cycle in individual hosts. Viruses recovered from infected mice lungs were subjected to Next Generation sequencing (NGS) to identify mammalian adaptation enhancing mutations. Moreover, the timing of these mutations was correlated to the host response, with particular emphasis on cytokine profile and innate immune responses during the time prior to and following viral sequence changes to help understand the virus-host dynamics and the host environment favouring adaptation. Furthermore,understanding of the determinants and mechanisms of adaptation and transmission may aid in assessing the risks posed by avian influenza A viruses to human health.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B070

Human immunodeficiency virus type 2 (HIV-2) dynamics and whole genome deep sequence analysis in Mauritian-origin cynomolgus macaques (*Macaca fascicularis*)

Claire Ham, Debbie Ferguson, Adrian Jenkins, Jo Hall, Neil Almond, Neil Berry

NIBSC, Potters Bar, United Kingdom

Abstract

Human immunodeficiency virus type 2 (HIV-2) is a pathogenic human retrovirus with a distinct natural history and lineage derivation from pandemic HIV-1. HIV-2 infections in humans are the result of zoonotic transmission from sooty mangabey monkeys naturally infected with SIVsm. Unlike HIV-1, HIV-2 infects other non-human primate species, including baboons and macaques. We determined the infectivity and infection kinetics of a Gambian-origin HIV-2 isolate HIV-2SBL_{6669} strain in Mauritian-derived cynomolgus macaques (MCM) in the context of a heterologous superinfection resistance study. The ability of HIV-2 to replicate in unvaccinated MCM with limited host genetic MHC and TRIM5 spectrum was determined where the kinetics of plasma HIV-2 RNA mimic the phenotypic response typically observed in HIV-2-infected humans. HIV-2_{SBL669} replication is not completely restricted in this species and may establish a persistent infection. This is determined by moderate peak viral loads (10^{5-6} log_{10} HIV-2 RNA copies/ml) controlling to a low level determined by qRT-PCR and detectable in-situ hybridisation signals in lymphoid tissue 20 weeks after challenge. In MCM vaccinated for 20 weeks with an attenuated SIV, we observed high levels of superinfection resistance as determined by virus-specific PCR analysis of tissues and low total plasma viral RNA levels in vaccinates compared to unvaccinated HIV-2 controls. Recovery of whole viral genome and next generation sequence analysis of challenge controls, including low-level breakthrough variants characterises viral challenge and vaccine-escape viruses. HIV-2 in cynomolgus macaques recapitulates many of the features of HIV-2 infections in humans.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B071

Unravelling a co-nsP-iracy: The role of chikungunya virus non-structural protein 3 in replication and pathogenesis

Siu Yi Lee, Nicola Stonehouse, Mark Harris

University of Leeds, Leeds , United Kingdom

Abstract

Chikungunya virus (CHIKV) is a member of the Alphavirus genus, transmitted to humans by mosquitoes of the *Aedes* genera. Infection with CHIKV causes chikungunya fever, which in many cases can lead to chronic joint disease, leaving patients with reduced ambulation. Despite its rising potential as a threat to global health, no effective vaccine or antiviral agent for protection or treatment are available. The CHIKV non-structural protein 3 (nsP3) is essential to the virus lifecycle and is believed to be a component of the genome replication complex. However, to date, the exact role of this protein has yet been determined. Although a conserved polyproline motif in the C-terminal hypervariable domain of nsP3 has been reported to interact with cellular SH3 domains, the function of this motif remains enigmatic. To address this question we generated a panel of mutations in this motif and tested the phenotype in the context of both a subgenomic replicon and full-length infectious virus, in both mammalian and mosquito-derived cell lines. Most of the mutations were well tolerated in the sub-genomic replicon, however, a subset either attenuated or completely abolished production of infectious CHIKV. These results suggest that as well as its role in genome replication, nsP3 also functions during assembly and release of infectious virus particles and that the C-terminal polyproline motif is a critical determinant of this function.

B072

Compartmentalised Culture and Differentiation of a Human Neuroblastoma Cell Line to Study Herpes Simplex Virus Latency

Michael Nicoll[1], Amy Jacobs[1], Stacey Efstathiou[2]

[1]NIBSC, Potters Bar, United Kingdom. [2]Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Abstract

Herpes simplex viruses (HSV) establish life-long latent infection in sensory neurons, punctuated by periodic reactivations into full virus replication. Whilst studies using small animals and their primary tissues continue to teach us much about these processes, to discern (and therapeutically interfere with) the molecular mechanisms governing HSV latency in humans, it is necessary to develop a human neuronal model of latency.

In our laboratory, we have begun to use neurons differentiated from the SH-SY5Y human neuroblastoma cell line to study HSV infection. As these neurons are fully permissive to HSV, establishing a quiescent infection akin to latency requires artificial blocks to lytic replication, such as the use of replication-defective viruses or antiviral treatment prior and during wildtype virus infection.

In the past few years, it has been shown using *in vitro* cultures of primary animal neurons that restricting wildtype neurotropic herpesvirus infection to axon termini can induce the establishment of latency, whereas cell body infection of the same cells favours full lytic replication. These elaborate studies have focused upon culturing primary animal neurons within compartmentalised chambers that allow cell bodies and axon termini to be fed with physically separated reservoirs of growth medium.

In this report, we describe the differentiation and outgrowth of SH-SY5Y cells in two different compartmentalised culture systems, and are beginning to characterise infection following the addition of HSV-1 to cell bodies or axon termini.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B073

Detection of influenza D virus in respiratory disease samples from Northern Irish cattle

Hannah Dane[1], Catherine Duffy[2], Maria Guelbenzu[3], Ben Hause[4], Sean Fee[2], Shirley McConnell[2], Fiona Forster[2], Michael McMenamy[2], Ken Lemon[2]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Agri-Food & Biosciences Institute, Belfast, United Kingdom. [3]Animal Health Ireland, Belfast, United Kingdom. [4]Kansas State University , Manhatten, USA

Abstract

Influenza D virus (IDV) is a newly described member of the *Orthomyxoviridae* family, initially identified during a 2011 outbreak of respiratory disease in North American pigs. Cattle were subsequently shown to be the main reservoir of the virus and accumulating evidence suggests a role for IDV in bovine respiratory disease complex. During the winter of 2017/2018, cattle submitted to the Agri-Food & Biosciences Institute for post-mortem with confirmed respiratory disease were tested for the presence of IDV by real-time RT-PCR. Virus isolation was performed in Swine Testes cells and full-genome sequence determined. Of 104 cattle with confirmed respiratory disease, 9 tested positive for IDV (8.7% prevalence). Virus was detected in both the upper and lower respiratory tract. Lung tissues from IDV positive samples were negative for the presence of bovine herpesvirus 1, bovine respiratory syncytial virus, bovine viral diarrhea virus and parainfluenza virus 3. Of the 9 cattle which tested positive for IDV, 3 tested positive for coronavirus. Histological analysis of lungs from IDV positive samples revealed pathological features including necrosis, neutrophil infiltration of alveolar spaces, fibrosis, congestion, oedema and haemorrhage. Sequenced isolates were shown to cluster with European isolates of the D/swine/Oklahoma/1334/2011 clade. To date, IDV has been detected in North America, Mexico, Japan, China, France, Italy and the Republic of Ireland. This study is the first to identify IDV in UK cattle herds. The presence of IDV in respiratory disease samples supports a role for this virus in bovine respiratory disease complex.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B074

Evolutionary Evidence for Multi-host Transmission of Cetacean Morbillivirus

Wendy K. Jo[1], Jochen Kruppa[1], Andre Habierski[1], Marco van der Bildt[2], Sandro Mazzariol[3], Giovani Di Guardo[4], Ursula Siebert[1], Thijs Kuiken[2], Klaus Jung[1], Albert Osterhaus[1], Martin Ludlow[1]

[1]University of Veterinary Medicine Hannover Foundation, Hannover, Germany. [2]Erasmus Medical Center, Rotterdam, Netherlands. [3]University of Padova, Padua, Italy. [4]University of Teramo, Teramo, Italy

Abstract

In recent decades, outbreaks of cetacean morbillivirus (CeMV) in dolphins and whales have caused several mass mortality events (MME). This virus was initially recognized in 1990, when dolphin morbillivirus (DMV), a strain of CeMV, led to a MME in striped dolphins from the Mediterranean Sea. Furthermore, it has been documented to cross the species barrier, causing an epidemic in Mediterranean monk seals in 1997. In this study, we have deep sequenced a number of European CeMV strains from original cetacean tissue samples from different species and geographical locations circulating at different time points (1988-2016) to investigate CeMV evolutionary history and assess viral molecular signatures of host adaptation. We generated 14 new full-length sequences of CeMV (8 wild-types and 6 isolates). Using Bayesian phylogenetic analysis, we estimated a substitution rate of 2.81×10^{-4} substitution/site/year. The analysis also indicated that DMV and porpoise morbillivirus (PMV) diverged approximately five hundred years ago. Additional phylogeographic analysis of partial phosphoprotein sequences indicated that evolutionary dynamics of CeMV is neither host- nor location-restricted. Moreover, DMV appears to have undergone strong purifying selection without any evidence of positive selection. In addition, patterns of amino acid changes were distinguished within each clade, but no amino acid substitution was identified suggestive of DMV adaptation to dolphin or whale species. Furthermore, cell-to-cell fusion and growth kinetics assays indicate that CeMV can use seal CD150 as a cellular receptor. Thus, it appears that CeMV can readily spread among multiple cetacean species and poses a spillover risk to isolated phocid species.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B075

BTV-GLUE: A new bioinformatic resource for genomic studies of Bluetongue virus

Joshua B. Singer[1], Kyriaki Nomikou[1,2], Joseph Hughes[1], Peter Mertens[3,4], Robert Gifford[1], Massimo Palmarini[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, Glasgow, Scotland, United Kingdom. [2]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire, UK, Nottingham, United Kingdom. [3]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Leicestershire, UK, Nottingham, United Kingdom. [4]The Pirbright Institute, Pirbright, Woking, United Kingdom

Abstract

Bluetongue virus (BTV) is an arbovirus transmitted by biting midges (Culicoides pp.). BTV causes a severe disease (bluetongue) in domestic and wild ruminant species with high levels of morbidity and mortality. Bluetongue has emerged as an important disease in sheep and cattle worldwide. The BTV genome is composed by ten linear dsRNA segments, packaged within a triple-layered icosahedral protein-capsid, and encode 7 structural and 4/5 non-structural proteins.

To date, there are at least 27 BTV serotypes (mainly determined by the VP2 outer capsid protein) circulating worldwide. In addition, high rates of reassortment involving all genome segments have been documented, complicating epidemiological studies and vaccination programmes.

We have developed BTV-GLUE (http://btv.glue.cvr.ac.uk), a new bioinformatics sequence data resource for bluetongue virus. Sequences from the NCBI nucleotide database are curated along with complementary sequence metadata and are integrated together inside GLUE (http://tools.glue.cvr.ac.uk), a data-centric software package for capturing virus sequence data and organising it along evolutionary lines. The dataset also contains reference sequences with genome feature annotations, multiple sequence alignments, defined clades and phylogenetic trees, for each BTV segment and clade. A new automated genotyping tool for all segments has been developed. The resource may also be used as an offline bioinformatics toolkit. BTV-GLUE will help the BTV community to study varying aspects of BTV biology and evolution and will facilitate the adoption of a nomenclature that more easily distinguishes the properties of BTV strains circulating worldwide.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B076

DEVELOPING A PSEUDOTYPING ASSAY FOR ZIKA VIRUS GLYCOPROTEIN.

Fernando Ruiz Jimenez[1], Humberto Perez Olais[2], Barnabas King[1], Patrick McClure[1], Jonathan Ball[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]CINVESTAV, Mexico city, Mexico

Abstract

Zika virus (ZIKV) was originally described during 1947 in Uganda when it was isolated from a sentinel macaque in the forest of Zika. After its discovery, discrete outbreaks were reported in Africa and Asia, but it was until 2007 when an outbreak in the Yap Islands where it was identified as a pathogen capable of causing epidemics and became associated with microcephaly.

We analysed 10 ZIKV sequences from key locations, tracking the virus' spread through the Americas, following the Yap Island outbreak. We identified 8 mutations within the M (prM) and E proteins which may alter the tropism or entry kinetics of the virus.

To test the influence of these glycoprotein mutants on virus entry we have explored lentivirus pseudotypes with a luciferase reporter to measure infectivity. So far, the model involving the use of PNL 4.3 HIV based retroviral backbone has not given favourable results so in the aim to try to improve the conditions and favour the process we tested different conditions including a matrix of concentrations between the retroviral backbone and the viral glycoprotein. The addition of other viral proteins was also tested in an effort to produce particles capable to infect the target cells.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B077

Exploring the molecular inter-relationship of SRPK1 and SRSFs during Human Rhinovirus (HRV)-infection and their subsequent effects on viral replication .

Christoforos Rozario[1], Sheila Graham[1], Andrew Stevenson[1], Rose Maciewicz[2,3]

[1]MRC-UoG Centre for Virus research, Glasgow, United Kingdom. [2]Respiratory, Inflammation, Autoimmunity IMED, AstraZeneca, Gothenburg, Sweden. [3]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom

Abstract

INTRODUCTION: HRV infections cause asthma exacerbations, while HRV-induced wheezing in pre-school children is associated with asthma development in later life. Molecular mechanisms underlying this pathophysiology remain elusive, while HRV-C is increasingly associated with disease severity. Transcriptomic analysis of human respiratory (nasal) epithelia from an *in vivo* HRV-C infection model revealed changes in expression of cellular Serine/Arginine Protein Kinase 1 (SRPK1) and its substrates, the Serine/Arginine Splicing Factors (SRSFs) suggesting that HRV infection targets pre-mRNA splicing and/or its regulating factors.

HYPOTHESIS: 1) HRV splicing modulation results in host transcriptome changes, leading to impaired anti-viral responses and/or immunoregulation; **2)** HRV-induced changes to SR proteins result in enhanced HRV-translation and replication.

METHODS: HRV-A RNA was synthesised by in-vitro transcription from pRV16.11. HRV replication was measured by RT-qPCR and titration in HeLa cells. Protein expression was monitored by western blotting and confocal microscopy. SPRIN340 was used as a specific SRPK1 inhibitor. Alternatively, SRPK1 was depleted via siRNA interference.

RESULTS: 1) SRPK1, SRSF1-6 and SRSF9 are expressed in both A549 and HeLa cells; levels were greater in the latter.

2) SRPIN340 and siSRPK1 treatments decrease the levels of SRPK1 leading to decreased expression of SRSFs in HeLa and A549 cells.

3) HRV16 infection decreased SRPK1, SRSF1-6 and SRSF9 expression in HeLa and A549 cells.

CONCLUSION: Our initial data suggest that HRV-infection specifically alters molecules involved in pre-mRNA splicing, providing us with insights into the molecular mechanisms underlying the distinct pathology of HRV infection, as well as the aetiology of HRV-associated asthma.

Virology workshop: Pathogenesis
Presentations: Wednesday evening and Thursday lunchtime

B078

Molecular evolution of Zika viruses

Alanna Gallagher, Marcus Dorner, Michael Skinner, Efstathios Giotis, Carolina Herrera

Imperial College London, London, United Kingdom

Abstract

Zika virus (ZIKV) is a member of the Flaviviridae family that has garnered recent worldwide attention due to the teratogenic effects associated with its infection and its ability to cause explosive epidemics. Due to the lack of testable phenotypes as well as the availability of technically feasible reverse genetics systems, there has been a relative absence of studies into the inter-strain sequence differences between the recent Pacific, South American and Caribbean epidemic strains.

Previously, we have observed that *ex vivo* phenotypic differences exist between two particular strains of ZIKV - the Pacific strain, H/PF/2013 & the Caribbean strain, PRVABC59/2015. Of note, these two isolates differ by just a handful of coding and UTR sequence changes. Therefore, using a reverse genetics system based on an infectious cDNA clone of the Brazil/Paraiba_01/2015 strain, we aim to uncover the genetic determinants responsible for the observed phenotypic differences, by creating intra-species ZIKV chimaeras. Taken together these experiments will investigate the evolution of ZIKV after the 2013-14 French Polynesian outbreak, which may aid in the prediction of, and protection from, future epidemics.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B079

Aberrant RNA replication products of highly pathogenic avian influenza viruses and its impact in the mammalian associated cytokine storm

Rebecca Penn, Raul-Yusef David-Sanchez, Jason Long, Wendy Barclay

Imperial College London, London, United Kingdom

Abstract

Highly pathogenic avian influenza viruses (HPAIVs), can sporadically cross the species barrier and cause zoonotic infections in mammalian hosts (including humans), with often fatal consequences. Severe disease has been associated with an overexuberant host innate immune response known as hypercytokinema (cytokine storm) where excessive levels of pro-inflammatory cytokines are produced. Previous work in our laboratory shows that high levels of viral replication by HPAIV in murine myeloid immune cells correlated with high cytokine levels and mapped this phenotype to the polymerase genes. Innate sensing of IAV is performed by the cytoplasmic helicase RIG-I, which recognises blunt ended double stranded RNA with 5'-triphosphate extremities, a pattern present in the viral genome. Defective viral genomes (DVGs) can be produced by aberrant RNA replication and these are also recognised by RIG-I and could play a role in hypercytokinemia.

Our studies aim to probe for the presence of DVGs in influenza infected cells *in vitro*, and in lung samples from infected mice *in vivo* as well as in murine immune cells *ex vivo*, using RT-PCR and sequencing. We will establish whether DVGs are correlated to pathogenicity and high pro-inflammatory cytokine levels. Preliminary data utilising minigenome assays show that the polymerase genes from an HPAIV H5N1 strain do generate DVGs, whereas DVGs could not be detected from a H3N2 seasonal polymerase. Ultimately, we aim to identify mutations within the polymerase genes that contribute to virulence and by using reverse genetics, create mutant viruses that test the hypothesis that aberrant polymerase activity drives hypercytokinemia.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B080

Infectious bursal disease virus (IBDV) replicates in the gut associated lymphoid tissue and alters the gut microbiome of chickens

Rebecca Daines[1], Joy Leng[1], Dan Horton[1], Roberto La Ragione[1], Andrew Broadbent[2]

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

Abstract

Infectious bursal disease virus (IBDV) is a member of the *Birnaviridae* family that infects B cells in chickens, leading to immunosuppression and mortality. Immunosuppression is known to exacerbate the colonisation and shedding of zoonotic gut bacteria, for example *Campylobacter jejuni*, *Salmonella* Enteritidis and *Escherichia coli*, for reasons that are poorly understood. In order to address this, we infected groups of chickens (n= 6) with either a classical strain (F52-70) or a very virulent strain (UK661) of IBDV. At 3 days post-infection, both strains were found to replicate in the gut-associated lymphoid tissue of the caecal tonsils. 16s rRNA sequencing revealed that in birds infected with IBDV, regardless of strain, there was a decrease in bacterial diversity in the caecal tonsils, but an increase in diversity of bacteria shed from the cloaca. Secretary IgA binding to commensal bacteria is known to influence the composition of the microbiome, and we speculate that IBDV alters the repertoire of sIgA thereby altering the microbiome composition. Interestingly, we found the number of clostridial species was reduced following IBDV infection. Clostridial species have been shown to induce Treg populations in mice and we speculate that IBDV-mediated changes in the microbiome affect the population of different immune cells in the mucosa. Taken together, we hypothesise that IBDV infection directly affects the B cell population and indirectly affects other immune cell populations in the gut and alters the gut microbiome, which leads to a more favourable environment for zoonotic bacterial infections to colonise.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B081

High through-put proteomic and secretomic analysis of Dengue virus infected Huh-7 liver cells reveals dysregulation of acute-phase proteins.

Viravarn Luvira[1,2], Phillip Lewis[1], Kate Heesom[1], Andrew Davidson[1]

[1]University of Bristol, Bristol, United Kingdom. [2]Mahidol University, Bangkok, Thailand

Abstract

Acute-phase proteins (APPs) are host plasma proteins that are mainly secreted by the liver, in response to acute injury such as infection and inflammation. A number of clinical studies have revealed changes in the level of APPs in the serum/plasma of dengue infected patients. However, the effect of dengue virus (DENV) infection on APP synthesis by the liver, an important target organ, is not well understood. In this study, proteins in lysates and secretomes derived from Huh-7 liver cells infected with DENV-2, heat-inactivated DENV-2 or mock infected were analysed by 10-plex tandem mass tag (TMT) labelling of proteins, in combination with quantitative mass spectrometry. The results showed 6317 and 2818 proteins were reliably identified and quantified in the cellular proteomes and secretomes respectively. There were 124 and 29 proteins significantly increased and 147 and 75 proteins significantly decreased ≥1.5 fold in the cellular proteome and secretome of DENV-2 infected Huh-7 cells respectively, compared with mock infected cells. Both positively and negatively regulated APPs including albumin, fibrinogen, complement proteins, alpha-1 antitrypsin and antithrombin III were significantly decreased ≥1.5 fold in dengue infected Huh-7 cells and in the secretome. The changes in the amounts of selected APPs were validated by western blotting. The mechanism/s underlying the alteration in APP amounts in liver cells during DENV infection is currently being investigated.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B082

Zika Virus: Out of sight but not out of the mind. Viral persistence and host responses within the central and peripheral nervous systems.

Debbie Ferguson, Jo Hall, Claire Ham, Adrian Jenkins, Elaine Giles, Sarah Kempster, Neil Berry, Neil Almond

NIBSC, South Mimms, United Kingdom

Abstract

Following the rapid spread of Zika virus infection through the Caribbean and Americas, model systems of Zika virus infection have been established to determine disease pathology and potential health impacts for those infected with this neurotropic flavivirus.

NIBSC has established both Old World (rhesus and cynomolgus macaques) and New World (red-bellied tamarins) non-human primate disease models of human Zika virus infection. Animals were infected sub-cutaneously with the Caribbean Zika isolate PRVABC59 and pairs terminated during primary viremia (3dpc) or post clearance of plasma viremia (42 and 101dpc). FFPE sections from multiple tissues, including brain and peripheral nerves were analysed for Zika virus RNA (RNAscope), viral proteins and host responses (immunohistochemistry).

All animals became infected and rapidly cleared high levels of peripheral viremia.

Zika virus RNA was detected in multiple tissues from 3dpc with low levels of viral RNA and viral NS1 protein remaining detectable through to 101dpc. Viral RNA was associated with both glial and neuronal cells throughout the brain and schwann cell nuclei in peripheral nerves. Neuroinflammation (astrogliosis, microgliosis, peri-vascular cuffing) and increased levels of CD3+, CD8+ or CD45+ cells were present from 3dpc through to 101dpc. Disruption of MAP2 neuronal dendrite staining and peripheral nerve myelin basic protein staining was also observed.

The continued detection of Zika virus within tissues 3 months post infection and in the absence of a detectable peripheral viremia raises questions regarding potential long term effects of this virus and associated inflammatory responses within the nervous systems.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B083

Zika Virus: Persisting Tissue Reservoirs and their Potential for Clinically Relevant Pathology

Debbie Ferguson, Jo Hall, Claire Ham, Adrian Jenkins, Elaine Giles, Sarah Kempster, Neil Berry, Neil Almond

NIBSC, South Mimms, United Kingdom

Abstract

The rapid spread of Zika virus through the Caribbean and Americas appears associated with greater levels of congenital zika and Guillain-Barre syndromes than previously seen. In addition, new disease pathologies such as persisting sexual transmission and severe liver injury have been identified. With zika vaccines years away from licensing an understanding of potential long-term health consequences following adult infection needs to be gained, especially where organs from infected people may be used for transplantation.

To model the pathology of human Zika virus infection Old World and New World non-human primates were sub-cutaneously infected with a Caribbean Zika virus isolate. Following termination during primary viremia or later time points post peripheral viral clearance FFPE tissue sections were analysed for Zika virus RNA (RNAscope) and host responses (immunohistochemistry).

Zika virus RNA was detected in multiple tissues from 3dpc with low levels of viral RNA detectable by RNAscope through to 101dpc. New World non-human primates retained higher levels of persisting virus within tissues, notably within spleen, small intestine, kidney, liver and genital tissues. Differing pathologies were observed with Zika virus detected clustered within kidney glomeruli and associated with liver inflammatory infiltrates within New World species.

Such pathologies are being documented in adults with resolved primary infection symptoms. This includes clinical complications following solid organ transplantation where immune suppression may allow viral reactivation either from the patient or transplanted organ. In the absence of a protective vaccine the potential risks from persisting viral reservoirs needs to be understood to ensure appropriate clinical management.

B084

Investigating the requirement for host cell chloride ion channels during human respiratory syncytial virus infection

Hayley Pearson, Samantha Hover, Eleanor Todd, Jonathan Lipiatt, John Barr, Jamel Mankouri

University of Leeds, Leeds, United Kingdom

Abstract

Ion channels are a diverse class of transmembrane proteins, which selectively allow ions across cellular membranes, influencing a multitude of cellular processes. Modulation of these channels by viruses is emerging as an important host-pathogen interaction, and has been demonstrated to regulate critical stages of the virus multiplication cycle including entry, replication and egress.

Human respiratory syncytial virus (HRSV) causes severe respiratory tract infections (RTIs) globally and is one of the most lethal respiratory pathogens for infants in developing countries, with many cases leading to severe lower respiratory tract infections, and the development of bronchiolitis. Evidence also suggests that childhood HRSV infection contributes towards the increased incidence of adult asthma. There is no HRSV vaccine, and the only treatment is immunoprophylaxis that is prohibitively expensive and only moderately effective; thus new treatment options are required.

In this study, by infecting human lung epithelial cells with HRSV in the presence of various broad-range channel modulators, Cl^- channels were identified to play an important role during HRSV infection. Time of addition assays using these broad-acting Cl^- channel blockers identified the stages within the HRSV lifecycle that were dependant on Cl^- channel activity, and the use of family-specific Cl^- channel blocking drugs identified a small sub-family of Cl^- channels which, when inhibited, resulted in significantly reduced HRSV multiplication. We are now identifying the specific Cl^- channel(s) facilitating the multiplication of HRSV using genetic means, and well as assessing the importance of Cl^- channels in replication cycles of other negative sense RNA viruses.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B085

Unravelling the features of Influenza As entry mechanism

Oliver Desbki-Antoniak [1], Samantha Hover[2], Christian Tiede[3], Darren Tomlinson[2], Jamel Mankouri[2], Juan Fontana [1]

[1]University of Leeds, Leeds, United Kingdom. [2]University of Leeds , Leeds, United Kingdom. [3]University of Leeds , Leeds , United Kingdom

Abstract

Influenza virus is the causative agent of the 'flu'. According to the World Health Organisation, Influenza causes up to 5 million cases of severe flu and 500,000 deaths annually. To release its genome inside the cell and start an infection, Influenza virus must fuse its envelope with the endosomal membrane of the host, making this process an excellent drug target. In order to attempt to control, or better yet, eradicate this pathogen, a greater understanding of its entry mechanism, a fundamental aspect of the viral life cycle, is required. It is well established that Influenza A virus (IAV) fusion is driven by the viral glycoprotein hemagglutinin (HA), which when exposed to low pH transitions from a meta-stable pre-fusion to post-fusion state. However, it is becoming increasingly apparent that the ionic balance of the endosomes also has a significant role in the entry of enveloped viruses. Specifically, our preliminary studies on Influenza virus suggest that K^+concentrations within the endocytic pathway play a significant role in Influenza A fusion events. Through the use of broad-spectrum K^+channel inhibitors, a dose-dependant reduction in Influenza infectivity in tissue culture can be observed. Further to these studies, we have isolated Affimers (novel antibody-like proteins that can be produced in large quantities in *E.coli*) that recognise HAs from different Influenza subtypes (H3N2 and H1N1). Using these Affimers, we aim to develop an early detection method to distinguish between bacterial respiratory infections and 'the flu' to alleviate mounting pressures on an already diminishing antibiotic treatment system.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B086

Effect of antibacterial lipids on biofilm formation by Streptococcus mutans

Veritia Harvey, Damien Brady, Carmel Kealey

Athlone Institute of Technology, Athlone, Ireland

Abstract

Streptococcus mutans is the major cariogenic organism associated with Dental Caries, a widespread chronic disease of the oral cavity (Muras, et al. (2018). It is associated with oral biofilm formation, production of organic acids, and has the capacity to out-compete non-cariogenic commensal species (Lemos, et al., 2013). Recent studies carried out on fatty acids demonstrated effective antimicrobial activity against *S. mutans* (Hughes, 2014). This study will evaluate the activity of lipids on *S. mutans* biofilm formation and proposes that dietary constituents may be used as a natural therapy to maintain oral hygiene.

Clinical isolate S. mutans 3014 D5929 was exposed to various concentrations of lipid for 24 hours. Crystal violet assay was performed for quantification of biofilm biomass. Fluorescent microscopy using SYTO® 9 and Alexa Fluor® 647-labelled dextran conjugate was performed to visualise biofilm formation pre- and post-exposure to MCO.

Biofilm biomass was reduced for all lipid concentrations. Fluorescent microscopy indicated a significant reduction in bacterial cell number and a lack of structural biofilm upon exposure to fatty acid mixtures when compared to control.

The research demonstrated that lipid does have *S. mutans* based antimicrobial and antibiofilm capabilities.

Virology workshop: Pathogenesis

Presentations: Wednesday evening and Thursday lunchtime

B087

D-Amino acids do no inhibit biofilm formation in Staphylococcus sp

Tomasz Szank, Carmel Kealey, Damien Brady

Athlone Institute of Technology, Athlone, Ireland

Abstract

D-amino acids are responsible for cell wall re-modelling in staphylococcus and are capable of inhibition and mature biofilm disassembly. *Staphylococcus aureus* and *Staphylococcus epidermidis* are recognised as recurrent nosocomial pathogens and a common cause of biofilm-associated infections. The combination of amino acids used in the study consisted of D- and L- isomers of tyrosine, methionine, tryptophan and phenylalanine. A semiquantitative microplate crystal violet assay was used to assess the effect of amino acids on biofilm development. Biofilm viability staining using fluorescent microscopy was performed to assess the effect of the amino acid mixtures on biofilm development on submerged surfaces. None of the amino acids when tested individually or as a mixture could reduce biofilm formation. However, at the highest concentration tested 25 mmol 1-1 equimolar D-amino acid mixture of tryptophan, phenylalanine, tyrosine and methionine caused a considerable biofilm inhibition in three staphylococcus strains. Microscopy analysis showed that initial surface attachment remained unaffected at 25 mmol 1-1 mixture of D-amino acids but bacteria did not proceed to form mature biofilms. This suggests inhibition of protein synthesis or a lack of polysaccharide extracellular adhesin formation as no aggregates were observed.

The reported bioactivity of D-amino acid on biofilm development and disassembly has been conflictual. It has been established that D-amino acids are incorporated in the bacterial cell wall suggesting they play a role in the complexity of biofilm lifecycle. However, our study indicates that they play no direct role in the inhibition of biofilm formation in staphylococcus.

Virology workshop: Pathogenesis
Presentations: Wednesday evening and Thursday lunchtime

B088

St. Abb's Head phlebovirus – a separate virus species or a strain of Uukuniemi phlebovirus?

Agnieszka M Szemiel, Brian J Willett

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

Abstract

St. Abb's Head virus (SAHV), a member of the genus *Phlebovirus* (family *Phenuiviridae*, order *Bunyavirales*), belongs to the largest group of negative strand RNA viruses. All phleboviruses share a genome structure that comprises three segments of negative-sense or ambi-sense RNA. The viral genome is composed of the small (S), medium (M) and large (L) RNA segments. The S segment encodes the nucleocapsid (N) protein, the M segment encodes the precursor for the viral glycoproteins (Gn and Gc) and the L segment encodes the viral RNA-dependent RNA polymerase (RdRp). Some viruses within the genus also encode non-structural proteins within their S or M segments.

SAHV was isolated from a pool of seabird ticks (*Ixodes uriae*) collected at a seabird colony in St. Abb's Head National Nature Reserve, Berwickshire, Scotland in 1979. Antigenically, SAHV appeared to be related to the Uukuniemi serogroup of phleboviruses. Similarly, the proteins of SAHV shared similar biochemical properties to Uukuniemi phlebovirus. Here, we describe an in depth molecular characterisation of SAHV. Using next generation sequencing technology, we demonstrate that SAHV is very closely related to the Uukuniemi phlebovirus (UUKV). We examine the growth of SAHV in mammalian, avian and tick celllines and define its target cell tropism.

The biological and chemical tales of the antibiotic makers
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B089

Biosynthesis and Antimicrobial Activities of *Allium Cepa* Silver Nanoparticles against Some Clinical Isolates

Tosin Senbadejo, Muhammad Abba Bunu

Fountain university oshogbo, Osun state, Nigeria

Abstract

ABSTRACT
The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. This study revealed the convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (*Allium cepa*) extract. In this study preparation of silver nanoparticles by using plants extract of onion (*Allium cepa*) has been investigated. Characterization of different properties of the prepared nanoparticles by techniques such as, UV spectroscopy and FTIR are carried out. The antimicrobial activity of the prepared silver nanoparticles on different microorganisms such as *Staphylococcus aureus* and *Escherichia coli* was carried out. The silver nanoparticles showed antimicrobial activity against Gram positive and Gram negative bacteria. From the experiment it was found that the synthesized nanoparticles have a significant antimicrobial activity.
Keywords: Silver nanoparticle, *Allium cepa*, *Staphylococcus aureus*, and *Escherichia coli*

The biological and chemical tales of the antibiotic makers
Presentations: Wednesday evening and Thursday lunchtime

B090

In vitro screening of antibacterial and antioxidant properties of stem of *Sphaeranthus indicus* Linn

DHANANJAY TANDON, ASHWINI KUMAR GUPTA

SCHOOL OF STUDIES IN LIFE SCIENCE, PT RAVISHANKAR SHUKLA UNIVERISTY, RAIPUR, India

Abstract

This study aimed to identify the bioactive compound (s) possessing both the antimicrobial and antioxidant activity. The antibacterial activity of hexane, ethyl acetate, methanol polar fraction and aqueous extract of stem of *Sphaeranthus indicus* was evaluated against MTCC bacterial strains *Bacillus cereus*-430*, B. subtilis*-441*, Staphylococcus aureus*-96*, S. epidermidis*-435*, Escherichia coli*-1687*, Klebsiella pneumoniae*-3384*, Pseudomonas aeruginosa*-741 and *Proteus vulgaris*-744 with their corresponding clinical isolates. The results revealed inhibitory activity of hexane extract against most of the bacterial pathogens except MTCC *K. pneumonia* and clinical *B. cereus*. The ethyl acetate extract inhibited growth of MTCC *B. cereus*, *B. subtilis*, *S. epidermidis*, *P. aeruginosa* and clinical isolates of *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumonia*. The methanol polar fraction exhibited activity against clinically isolated *S. aureus*, *S. epidermidis*, *P. aureginosa*, *P. vulgaris* while aqueous extracts had no activity against any of the organisms. Among all the extracts showing antioxidant activity, aqueous extract was found to possess highest activity when tested by reducing power, DMPD and DPPH assay. Phytochemical analysis revealed that methanol polar fraction had highest quantity of terpenoids while aqueous extract was rich in phenols and flavonoids. The active compound from hexane extract was isolated by TLC and the active band was detected via bioautography. DPPH was used for detecting antioxidant (s) in TLC plate. A total of 13 bands were obtained after separation in which two bands showed both the antibacterial as well as antioxidant activities. Hence it is speculated that bioactive compound showing antibacterial activity may also possesses antioxidant activity.

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B091

Investigating the efficacy and improved stability against *Staphylococcus aureus* of Lynronne-1D, a modified rumen microbiome derived antimicrobial peptide.

Katie Lawther, Linda Oyama, Sharon Huws

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistant infections are at a crisis point, posing a massive threat to human health with an anticipated annual mortality of 10million people by 2050. Antimicrobial peptides (AMPs) are a promising solution to treat drug resistant infections, with the highly competitive ecosystem of the rumen microbiome being a fruitful resource for mining AMPs.

In this study, we aimed to improve the stability of a known rumen AMP with great therapeutic potential, Lynronne-1. This AMP is efficacious against topical skin infections of Methicillin resistant *Staphylococcus aureus* but has no activity in systemic infections when administered intravenously due to degradation by peptidases. To overcome this hurdle and improve its use intravenously, we substituted the L isoforms N and C terminal amino acid residues to D-isoforms, thereby increasing the stability of the peptide in the presence of trypsin by three-fold.

The activity of the modified peptide, named Lynronne-1D against *S. aureus* was subsequently investigated. Lynronne-1D retained its antimicrobial activity with an MIC of 8 μg/ml against *S. aureus* and improved MICs (>4-fold) in Gram-negative bacteria strains. The peptide had rapid and potent bactericidal activity causing a ≥ 6log CFU/ml reduction in viable *S. aureus* cells within 30 minutes of treatment. It induced membrane permeabilization within 5 minutes and successfully prevented biofilm formation by *S. aureus* cells. Lynronne-1D was also non-cytotoxic to mammalian blood cells. The improved properties of Lynronne-1D over the original peptide makes it a promising therapeutic agent for the treatment of systemic infections of *S. aureus*.

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B092

The Emergent Properties of Streptomyces Observed During Co-Culture and the Genomic and Morphological Characterisation of a Streptomyces lydicus Strain

Jordan Murray[1], Talal Salih[2], Nicholas Tucker[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Mosul, Mosul, Iraq

Abstract

Antimicrobial resistance is one of the biggest global threats to human health in the modern day. No new classes of antibiotics have been approved for clinical use in over 20 years, and so called resistant "superbugs" – such as *Pseudomonas aeruginosa* are becoming more resistant to even last resort antibiotics. *Streptomyces* spp are the most valuable source of antibiotics to date, and genetic analyses has suggested that each strain is capable of producing upwards of thirty secondary metabolites. However, these genes are not all expressed in laboratory monoculture. In this study, four strains of *Streptomyces*were co-cultured in pairs on five different agar media. It was found that out of 108 conditions, 17 were capable of producing antimicrobial compounds that were bioactive against the ATCC ESKAPE pathogens that neither strain was capable of producing alone. Interestingly, instances of loss of phenotype were also observed, where isolates capable of bioactivity had this activity reversed when in co-culture with another streptomycete. A wild isolate of *Streptomyces lydicus* was also the subject of genomic and morphological characterisation in this study. Next-Generation Sequencing (NGS) of this strain was carried out using Ion Torrent, and after assembly was composed of 380 scaffolds. AntiSMASH analysis of this strain predicted 27 secondary metabolite gene clusters within the genome. Furthermore, CARD predicted 23 genes associated with antimicrobial resistance. Also presented here is a novel approach to liquid co-culture, in which modified glassware allows for the chemical interactions of two strains across a membrane, while the bacteria themselves are segregated.

The biological and chemical tales of the antibiotic makers
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B093

Utilising Novel Antimicrobial Peptides Identified from the Rumen Microbiome as a Potential Treatment for Multiple *A. baumannii* Strains

Peter Alexander, Linda Oyama, Sharon Huws

Queen's University, Belfast, United Kingdom

Abstract

Antibiotic and antimicrobial resistance is a global issue, not only for medical and veterinary treatment, but also for economic development. One of the critical bacterial pathogens known to develop resistance to antimicrobial agents is *Acinetobacter baumannii*, one of the ESKAPE pathogens. *A. baumannii* is an aerobic Gram-negative coccobacillus bacterium associated with bacteraemia, urinary tract infections and ventilator-associated pneumonia, and is typically viewed as an opportunistic pathogen. Previous research has shown that clinical strains of *A. baumannii* have susceptibility to novel antimicrobial peptides, specifically those identified from a rumen metagenomic dataset.

Using standardised 96 well MIC testing, 3 antimicrobial peptides (Lynronne-1, Lynronne-2, Lynronne-3, identified as part of a previous research project by the Huws Lab) were compared against multiple strains of *A. baumannii*, in order to show efficacy of treatment against a wider variety of strains. Of the strains tested, a number were clinical isolates with demonstrated resistance (imipenem resistant, OXO-23/OXO-50).

The results show that the antimicrobial peptides had noticeable inhibitory effects on the bacterial growth. There was also variation between the 3 peptides utilised, with Lynronne-1 appearing to have the lowest MIC over the majority of the strains tested.

Further research as part of this project will utilise other identified antimicrobial peptides, including rationally designed peptides, as well as potentially using another gastrointestinal microbiome metagenomic dataset to identify a greater number of novel antimicrobial peptides.

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B094

Production of Fluorinated Fengycins in *Bacillus* spp.

Aoife Phelan [1], Cormac Murphy[1], Steven Cobb[2], Diana Gimenez-Ibanez [2]

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

Abstract

Bacillus spp. produces lipopeptides that are of pharmaceutical and agricultural interest and include iturins, surfactins and fengycins. Their antimicrobial mechanism has been well studied but the role of the lipid chain and its biosynthesis is relatively unknown, especially in fengycin. In this project, we aim to fully understand the mechanisms of fengycin's antimicrobial activity and generate new fengycins with fluorine in the lipid chain. We have already produced fluorinated lipopeptides via precursor-directed biosynthesis using fluorinated amino acids.

The *Bacillus* genome was sequenced by MicrobesNG and a total of 34 gene clusters were identified, of which 19 were fully characterised using antiSMASH. Genes within the fengycin cluster were cleaved during the sequencing process and iturin biosynthetic genes were also present. Four fatty acyl-CoA ligases within the genome were identified using Artemis in an aim to heterologously express them, and carry out activity assays with fluorinated lipid tails. Lipopeptide production was optimised by altering culture conditions (pH, aeration and temperature) and it was found that 37°C is the optimum temperature for biosynthesis.

The biological and chemical tales of the antibiotic makers
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B095

Understanding the adaptive response of *Streptomyces coelicolor* to the glycopeptide antibiotic teicoplanin

Samuel Connelly, Hee-Jeon Hong

Oxford Brookes University, Oxford, United Kingdom

Abstract

Glycopeptide antibiotics are clinically important as a second-line therapy for the treatment for nosocomial infections caused by Gram-positive pathogens. A universal mode of action for glycopeptide antibiotics is to target the terminal residues, D-Alanyl-D-Alanine on the cell wall peptidoglycan intermediate lipid II, arresting the later stages of peptidoglycan biosynthesis. This weakens the cell wall, making it susceptible to rupture. A general resistance mechanism for glycopeptide antibiotics requires the core genes, *vanRSHAX*, that detect the presence of a glycopeptide (VanS) and upregulate genes (VanR) which orchestrate the remodelling of D-Ala-D-Ala on lipid II to D-Ala-D-Lactate (VanHAX). Glycopeptide affinity for lipid II is reduced by 1000-fold and biological activity impaired.

Our previous study has shown that altering the termini of peptidoglycan precursors by VanHAX action was not sufficient for the resistance of the glycopeptide teicoplanin in *S. coelicolor*, which is instead mediated mainly by VanJ. Using RNA-seq, this study is designed to understand how the adaptive response in an *S. coelicolor* wild type (M600) and a *vanJ* mutant strain differ after exposure to teicoplanin. We have also compared these data with available data on the cell wall targeting antibiotics vancomycin, bacitracin and moenoymycin. By doing so, we aim to gain insight into the molecular basis of the improved activity of teicoplanin over vancomycin as well as identify novel cellular targets of teicoplanin which can help inform the design of future glycopeptides with desirable therapeutic properties.

The biological and chemical tales of the antibiotic makers
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B096

Characterising the inhibition profile of an antimicrobial produced by *Enterococcus*

Nathaniel Cole, Fiona Walsh

Maynooth University, Maynooth, Ireland

Abstract

Introduction: Antibiotic resistance is one of the greatest problems facing the 21st century with few new classes of antibiotics being discovered. The forefront of antibiotic discovery has been the soil microbiome and it is still a valuable resource for identifying microbes with possible antibiotic producing capabilities leading to novel classes of antibiotics. This justifies continuing investigation into the soil microbiome for antibiotic producing bacteria, to help tackle the growing trend in antibiotic resistance. A bacterial soil isolate was found to inhibit *Enterococcus faecalis* ATCC 29212 and the aim of this work was to further characterise the inhibition profile of the antibacterial.

Methods: Bacterial plug and supernatant assays were used to access the inhibition ability of a soil bacterial isolate against the WHO "priority pathogens". Identification of the bacteria was carried out using 16S rRNA and whole genome sequencing. Synergy tests were carried out using broth dilution assays.

Results: The soil isolate (N5) was identified as *Enterococcus* and showed antibacterial activity against *Staphylococcus aureus* MRSA and *E. faecalis* VanA. This antibacterial is secreted into the supernatant which still showed inhibitory activity against MRSA and VRE. Synergy with N5 and ciprofloxacin (0.2 µg/ml) against *E. faecalis* ATCC 29212 was also observed.

Conclusion: Both VRE and MRSA are important players in nosocomial infections and are displaying high levels of resistance. Further study of this antibacterial could lead to the development of a new compound to help overcome resistance mechanisms or a novel antimicrobial.

The biological and chemical tales of the antibiotic makers

Presentations: Wednesday evening and Thursday lunchtime

B097

Genome Mining of Ant-Associated Bacteria to Identify Novel Secondary Metabolites

Jacob Hamilton[1], Neil Holmes[1], Sarah Worsley[1], Elaine Patrick[1], Naomi Pierce[2], Ulrich Mueller[3], Jacobus Boomsma[4], Barrie Wilkinson[5], Matthew Hutchings[1]

[1]University of East Anglia, Norwich, United Kingdom. [2]Harvard University, Cambridge, USA. [3]University of Copenhagen, Copenhagen, Denmark. [4]University of Texas at Austin, Austin, USA. [5]John Innes Centre, Norwich, United Kingdom

Abstract

Antibiotic discovery has stagnated since the end of the 1960s, despite an increasing level of antimicrobial resistance in the clinic. However, recently there has been a renewed interest in searching for novel antibiotics, particularly in under-explored niches. Symbiotic relationships between bacteria and eukaryotes, for example plants and insects, can prove to be a fruitful source of novel secondary metabolites. Many ant species form mutualistic relationships with antibiotic-producing bacteria to protect their colonies against parasitic invasion. However, the antibiotic-producing potential of bacteria associated with different ant species has rarely been explored in detail. We isolated 20 bacteria from the colonies of ant species in the genera *Acromrymex, Cyphomyrmex,* and *Mycocepurus*, which are all fungus-farming ants, as well as *Allomerus* and *Tetraponera* genera which predate on other insects. High quality genome sequences were generated for each bacterial strain using PacBio sequencing. AntiSMASH analysis showed that many of the strains had the potential to produce many secondary metabolites, including a modified candicidin-like compound and several other clusters with low percentage homology to known antimicrobial compounds. Under standard lab conditions less than 20% of strains show bioactivity against bacteria *Bacillus subtillis* and fungi *Candida albicans* however their genomes suggest a much higher potential. Pleiotropic methods such as growth media variations and co-culture with other microorganisms are now being used to switch on cryptic clusters in these novel isolates. Genome mining of symbiotic strains could make a valuable contribution to antibiotic discovery since such strains are constantly having to evolve in response to their host.

The biological and chemical tales of the antibiotic makers
Presentations: Wednesday evening and Thursday lunchtime

B098

The antimicrobial activity of *Micromonospora sp.*

David Mark, Jan DeWald, Nicholas Tucker, Paul Herron

University of Strathclyde, Glasgow, United Kingdom

Abstract

With antimicrobial resistance becoming an increasingly severe issue in both the developed and developing regions of the world, new strategies need to be employed to identify and characterise novel antimicrobial activity. *Pseudomonas aeruginosa* is a Gram negative pathogen and a major cause of opportunistic infections in burn victims and cystic fibrosis patients. Due to a wide repertoire of antibiotic resistance mechanisms, these infections are difficult to cure and thus there is a need to develop novel antimicrobial treatments. Members of the Actinobacterial genus *Micromonospora* produce a broad range of bioactive secondary metabolites, a number of which possess antimicrobial properties. The goal of this research is to examine the antimicrobial properties of an actinomycete strain – identified by 16S sequencing as *Micromonospora* – originally isolated from the Atacama desert, Chile, with a focus on identifying and characterising anti-pseudomonad activity. Bioactivity was screened for against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Enterococcus faecium*. AntiSMASH analysis of assembled Illumina sequencing data was used to identify putative biosynthetic gene clusters. Antimicrobial bioactivity was observed, and potential antimicrobial biosynthetic gene clusters were identified through genome mining. This work has identified antimicrobial activity from a region of underexplored ecology, and highlights the importance of sampling and examining areas which have previously been considered too hostile to support life.

The biological and chemical tales of the antibiotic makers
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B099

Streptomyces coelicolor M145 MIP-like proteins: to identify their role in a non-pathogenic organism

Amy Thomas, Lorena Fernández-Martínez

Edge Hill University, Liverpool, United Kingdom

Abstract

Understanding the role of virulence loci within pathogenic organisms can be vital in exploring the evolution of disease. *Streptomyces* species are generally non-pathogenic soil saprophytes, yet within their genome we can find macrophage infectivity potentiator-like proteins (MIPs) (Clark *et al*., 2013). MIPs are a subset of immunophilins associated with virulence in a range of micro-organisms (Norville *et al*., 2011). It is unknown the role they possess in non-pathogenic strains such as *Streptomyces coelicolor* M145. This project will identify the role of MIPs in a non-pathogenic strain through cloning, overexpression and knock out of three genes encoding putative MIP-like proteins (*SCO1638*, *SCO1639* and *SCO2620*). The phenotypes will then be characterised by growth under contrasting conditions. Antibiotic production will also be measured and compared to the wild-type M145 strain. The overexpression and mutant strains will also be tested for the ability to infect amoeba using amoeba infection assays compared to a wild-type control. The results from this study will contribute to the understanding of the role of MIPs and therefore the evolution of virulence within *Streptomyces* species.

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B100

Antimicrobial Activity of Naphthalene Lysine Conjugated Peptide Hydrogels

Marina Ellie Afami[1], Ikhlas El Karim[1], Garry Laverty[2], Fionnuala Lundy[1]

[1]Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom. [2]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

Abstract

The synthesis of hydrogel scaffolds with inherent antimicrobial activity has advantages for their use in tissue engineering. An ultra-short naphthalene lysine conjugated peptide, NapFFK'K', containing naphthalene (Nap) as a molecule of high aromaticity for gel strength, phenylalanine (F) and epsilon variant lysine (K') has previously been shown by us to self-assemble forming hydrogels with inherent antimicrobial properties against a limited number of pathogens tested.

The aim of this work was to extend the antimicrobial activity studies on NapFFK'K' including pathogenic bacteria associated with dental infections.

NapFFK'K' was synthesised using the 9-fluorenylmethoxucarbonyl Solid Phase Peptide Synthesis. Peptide purity was analysed by mass spectrometry. Hydrogel formulation was achieved by suspending the peptide in sterile deionized water followed by addition of NaOH and HCl. Hydrogels were tested at peptide concentrations of 1%, 1.5% and 2% w/v against the Gram positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus*, and the Gram negative bacterium *Fusobacterium nucleatum*. Bacteria inoculumns were exposed on hydrogel surface for 24 hours. Bacterial susceptibility assay, employing the Miles and Misra method, was used to determine antimicrobial activity of hydrogels after 24 hour incubation.

Our results show that peptide hydrogels exhibit antimicrobial properties against both groups of bacteria but at different peptide concentrations. The 1% peptide hydrogels was most effective against Gram positive bacteria whereas the 2% peptide hydrogel was effective against *Fusobacterium nucleatum*.

Given the efficacy of the self-assembling NapFFK'K' peptide hydrogels against oral pathogens, they may have potential use in tissue engineering approaches for regenerative endodontic treatments.

The biological and chemical tales of the antibiotic makers

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B101

Development of bio-inspired protein-based materials as novel transparent adhesives for glass laminates

Gethin Allen[1], Sholeem Griffin[1], Ben Clifford[2], Chris Phillips[2], Geertje Van Keulen[1]

[1]Swansea University Medical School, Swansea, United Kingdom. [2]Swansea University College of Engineering, Swansea, United Kingdom

Abstract

This project aims to develop Chaplins, functional amyloid proteins from *Streptomyces* sp., into a novel nano-thin adhesive material for the defence industry through combining existing protein-based technology with a composite partner. Chaplin proteins were extracted from a range of wild-type strains, while a synthetic promoter system was developed to express and secrete chaplins, which are typically difficult to express and purify.

Chaplin proteins were found to have potentially useful adhesive properties and we have been investigating their application in optically transparent adhesives, since adhesives currently used to bond laminated transparent materials are prone to water ingress resulting in degradation of optical clarity and delamination. These adhesives could also be lighter, thinner, and have a lower environmental impact.

To determine the potential of this material as a novel synbio-adhesive for bonding glass and polycarbonate we have investigated the bonding ability and transparency of natural and engineered amyloid proteins, both alone and with partner biopolymers and bulking materials. We are now exploring ways to improve the properties of the proteins by modifying the amino acid sequences, incorporating binding domains to the composite partner and via chemical modifications. Our projects has provided proof-of-concept for the use of bio-inspired amyloid protein-based materialscompounds as both adhesives and corrosion resistant metal coatings.

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B102

Assessing and optimising culturing methods for the associated-bacteria of two species of deep-sea sponges (class *Hexactinellida*) for antimicrobial bioprospecting.

Poppy Hesketh Best, Kerry Howell, Mathew Upton, Alistair Bishop, Philip Warburton, Nicola Foster, Matthew Koch

The University of Plymouth, Plymouth, United Kingdom

Abstract

There is a need for novel classes of antimicrobials to be discovered in order to tackle the growing challenges of antimicrobial resistance. Deep-sea sponges are drawing much attention due to the phylogenetically diverse and dense communities of microbes that live within their tissues. Bioprospecting these sponges offers the possibility of exploring a niche environment that could contain novel classes of antimicrobials. To assess the suitability of *Pheronema carpenteri* (class Hexactinellida, order Amphidiscosida) and *Rhabdodictyum* sp. (class Hexactinellida, order Lyssacin osida) as a source of antimicrobials, cultivation-dependent strategies were employed.

We assess the culturability of sponge-associated bacterial from P. carpenteri (n = 3) and Rhabdodictyum sp. (n = 2) using 8 treatments; 4 temperature incubation treatments (4, 15, 22-25 and 28°C), nutritional additives (Sponge spicule extract and a low nutrient heterotrophic media additive), and finally a 24 h enrichment stage. Recovered isolates were screen recovered sponge associated-bacteria isolates for bioactivity against *Escherichia coli* and *Micrococcus luteus*. Isolates demonstrating high activity were then tested against 7 clinically relevant pathogens; *Staphylococcus aurerus* 6571, *Streptococcus pyogenes*, *E. coli* 1077, *Salmonella enterica* Serovar Typhimurium LT2, *Klebsiella pneumonia* 681, *Mycoccoccus phlei*, and *Candida albicans*.

More isolates were recovered from *Rhabdodictyum* sp. than *P. carpenteri* ($p < 0.005$). Isolates recovered from *P. carpenteri* demonstrate high antibacterial activity against both Gram-positive and Gram-negative strains. 112 isolates in total were found to be bioactive against *M. luteus*, 55 of which were active against both *M. luteus* and *E. coli*. The highest potion of bioactive compounds derived from a 15°C treatment and from the inclusion of Sponge Spicule Extract as a nutritional additive. This research presents the first attempts of bioprospecting these two species of deep-sea sponges and thus far has shown promise in their suitability.

The biological and chemical tales of the antibiotic makers
Presentations: Wednesday evening and Thursday lunchtime

B103
Identifying novel antimicrobials from anaerobic rumen fungi

Rebeca Evans[1], Gareth Griffith[1], Tony Callaghan[1], Ben Thomas[1], Sharon Huws[2]

[1]Aberystwyth University, Aberystwyth, United Kingdom. [2]Queen's University, Belfast, United Kingdom

Abstract

Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of several herbivorous animals, and by using their powerful hydrolytic enzymes and mechanical forces they degrade plant material in the rumen, essential for rumen efficiency. The rumen microbiome represents an underexplored resource for the discovery of novel microbial enzymes and metabolites, including antimicrobial peptides (AMPs). AMPs are promising drug candidates, and are necessary for targeting the worldwide issue of antimicrobial resistance.

Rumen fluid and faecal samples were collected from various large herbivores, and fungal cultures were grown and maintained under anaerobic conditions. After roll tube culture to isolate single-zoospore cultures, sequencing of LSU was undertaken to identify the fungi to species level. Analysis of genomic data from these cultures, alongside published data was undertaken to explore the diversity of AMPs within these fungal genomes. Using functional and computational screening, potentially novel AMPs have been discovered, with isolates showing encouraging activity against some strains of bacteria. Findings indicate that the rumen microbiome may provide alternative antimicrobials for future therapeutic application.

The biological and chemical tales of the antibiotic makers
Presentations: Wednesday evening and Thursday lunchtime

B104

Antibacterial activity of traditional herbal medicine

Qiqi He

IGFS, Queens university Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistance (AMR) is becoming the biggest substantial threat for public health and societal implications worldwide. Coincidentally, the development of new antibiotics has decreased and downsized by the pharmaceutical companies for the last 40 years. The past decade has witnessed an increasing effort worldwide on exploration of plant-based natural products for new antimicrobial agents. The purpose of this study is to investigate the potential antibacterial activity of traditional herbal medicinal plant against resistant pathogens of public health and economic importance such as *methicillin-resistant Staphylococcus aureus (MRSA)* and *Acinetobacter Baumannii*. The broth microdilution method was used to determine the susceptibility of resistant pathogenic strains selected from WHO priority list. A total of 57 plants were chosen based on traditional knowledge and current scientific information that has been claimed to have antimicrobial activity. Preliminary results showed 75% of screened plants inhibited the growth *MRSA* (NCTC 12493) some of which exhibited similar antibacterial efficiency compared to the vancomycin (positive antibiotic control), while 21% shows an inhibitory effect against *Acinetobacter Baumannii* (NCTC 12156). In addition, significant synergy was observed between some of the plant extracts and vancomycin against MRSA. Further studies are needed for the warrant of these plant candidates to be further developed into therapeutic antimicrobial agents of required efficacy and safety.

The biological and chemical tales of the antibiotic makers

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B105

Unmasking the Potential as Antibiotic Makers of Three *Streptomyces* Strains Isolated in a High-Altitude Ecosystem in Colombia

Linamaria Pintor Escobar[1], María Mercedes Zambrano [2], Lorena Fernández Martínez[1]

[1]Edge Hill University, Ormskirk, United Kingdom. [2]Corporación Corpogen, Bogotá, D.C., Colombia

Abstract

The current threat of antimicrobial resistance, the surge in antimicrobial compounds rendered obsolete and the slow emergence of new classes of antibiotics have triggered an urgent call for novel alternatives to treat infectious diseases. The vast microbial diversity of unexplored environments and the chemical and structural variety of specialised metabolites within it stand as one of the central points to tackle this challenge. This work focuses on the exploration of the potential for antimicrobial compounds in three new *Streptomyces* strains -*Streptomyces* sp. CG885, *Streptomyces* sp. CG893 and *Streptomyces* sp. CG926- isolated in the Natural National Park Los Nevados (Colombia) and with proved production of active metabolites against several ESKAPE pathogens. To this purpose, we constructed a 16S rRNA phylogenetic tree and studied the specialised metabolite potential of these isolates using a genome mining approach [1] to predict the presence of putative Biosynthetic Gene Clusters (BGCs). Findings from this bioinformatic prediction were linked with analysis from active extracts obtained through agar plate extraction to inform prioritization of clusters. So far, the computational analysis has predicted between 68 to 93 specialised metabolite BGCs for each strain. Interestingly, most of these compounds show less than 15% similarity to any known compound in the database and around 69 clusters seem related to previously uncharacterised RiPPs, NRPS and PKS. Further work will focus on the validation and study of those clusters linked to compounds with potential in clinical development.

The biological and chemical tales of the antibiotic makers
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B106

Biosynthesis of Silver Nanoparticles using Baker's Yeast, *Saccharomyces cerevisiae* and its Antibacterial Activities

Ifeyomi Olobayotan , Bukola Akin-Osanaiye

University of Abuja, Gwagwalada, FCT, Nigeria , Abuja, Nigeria

Abstract

The biosynthesis of silver nanoparticles extracellularly using baker's yeast, *Saccharomyces cerevisiae* and its antibacterial activity was investigated in this study. Biosynthesised silver nanoparticles were chaterized by using UV-Visible spectroscopy, which showed a distinct observed absorption peak at 429.00 nm that is attributed to the plasmon resonance of silver nanoparticles; X-ray diffraction, which determined the average size of the silver nanoparticles to be approximately 16.07 nm; presence of oval shaped silver nanoparticles determined by scanning electron microscopy; and Fourier-transform infrared, which revealed notable peaks at 3332.2, 2903.6, and 1636.3 cm^{-1} corresponding to the binding of the silver nanoparticles to active biomolecules, alcohols and phenols, carboxylic acids and aromatic amines respectively. The silver nanoparticles were also found to be stable for ninety days. Antibacterial activity of the silver nanoparticles was also studied. The silver nanoparticles was significantly active ($p > 0.05$) against the test organisms at an extract concentration of 75µg/ml. Concentrations less than or equal to 50 µg/ml were not as effective as the colony forming units at this concentration, 1.61×10^6 for methicillin-resistant *Staphylococcus aureus* and 1.45×10^6 for *Pseudomonas aeruginosa* respectively were about the same range a small the colony forming units of the controls. The silver nanoparticles inhibited methicillin-resistant *S. aureus* more than they inhibited *P. aeruginosa*. The LD_{50} of the synthesized silver nanoparticles after oral administration was seen to be greater than 5000 mg/kg body weight and is therefore thought to be safe. This study supports the use of silver nanoparticles as therapeutic agents.

The biological and chemical tales of the antibiotic makers
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B107

Deciphering the regulatory mechanisms of formicamycin biosynthesis in *Streptomyces formicae*, a novel antibiotic against MRSA

Katherine Noble, Rebecca Devine, Nicolle Som, Matthew Hutchings

The University of East Anglia, Norwich, United Kingdom

Abstract

Derivatives of the secondary metabolites of *Streptomyces* bacteria account for over half of the antibiotics currently used in the clinic. A crucial part of overcoming antimicrobial resistance (AMR) is in the development of new antibiotics that function with a novel mechanism of action, to avoid the rapid acquisition of microbial resistance. We previously reported the isolation of the new species *Streptomyces formicae*, from African *Tetraponera penzigi* plant-ants, shown to have potent activity against several strains on the World Health Organisation's AMR watch list including MRSA. Genetic analysis of this strain revealed one of its 45 biosynthetic gene clusters, a type 2 polyketide synthase, produced all 13 formicamycins, the natural products responsible for *S. formicae's* antimicrobial activity. Several regulators of the formicamycin biosynthetic pathway have been identified, including the major repressor (ForJ). Having purified ForJ, DNA binding assays were performed based on previous cappable-RNA and ChIP sequencing experiments to confirm the interaction of ForJ with different putative promoters in the cluster. By optimising gene-reporter fusion assays in this novel strain, it has been possible to characterise some of the promoters in relation to ForJ to determine the effect of its binding upon activity of the gene cluster. Such work is essential in the progression of novel antimicrobials from the laboratory into a clinic as it must be thoroughly understood how a compound is synthesised and how its biosynthetic pathway is regulated before it can be exploited for overproduction.

The biological and chemical tales of the antibiotic makers

Presentations: Wednesday evening and Thursday lunchtime

B108

A novel actinobacterium species from a mangrove ecosystem- antibacterial activity and chemical characterization

Kannan Asha, Punyasloke Bhadury

Indian Institute of Science Education and Research Kolkata, Mohanpur, India

Abstract

The discovery of streptomycin by Selman Waksman (1943) brought into focus a new avenue of drugs from natural products, i.e., actinobacterial secondary metabolites. It forms more than 60% of total bacterial secondary metabolites (mostly from *Streptomyces*). Interestingly, rare Actinobacteria can produce novel secondary metabolites with unique chemical structures. With the rise of drug resistant microbes, focus on actinobacterial research has shifted towards exploring unusual niches such as those located along land-ocean boundary where freshwater mixes with saltwater. One such ecosystem is the Sundarbans mangrove, located at the apex of Bay of Bengal. The present study aims to identify novel actinobacterial species from Sundarbans which has the ability to produce unique secondary metabolites. Mangrove sediments were collected, overall bacterial diversity and secondary metabolites producing bacterial diversity were elucidated by 16S rRNA and polyketide synthase (PKS) clone library approaches respectively. Fifteen bacterial strains were isolated from the sediment using cultured approach, among which, an isolate I2 was identified based on polyphasic taxonomy. The I2 represents a new species, *Myceligenerans indicum* sp nov. This new species also possess PKS genes which indicate ability to produce secondary metabolite(s). Promising antibacterial activity of this new species was found against *Escherchia coli* XL10, *Bacillus subtilis* and *Vibrio chemaguriensis* Iso1 especially for fraction prepared using acetone and dichloromethane (1:1). Spectroscopic approaches have revealed the presence of functional groups such as amide, allene, isothiocyanate and ketenimine groups.

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B109

Assessing Metabolite Biogeography of *Micrococcus* spp. and *Pseudonocardia* spp. Isolated from Marine Environments

Jonathan Parra, Katherine R. Duncan

University Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Abstract

The study of biogeography enables an understanding of the distribution patterns of biodiversity across space and time[1]. Therefore, by using a trait-based approach, such as antibiotic production, it is possible to assess the evolutionary, geographic and ecological variables that affect the Actinobacteria specialized metabolism[2,3]. This is particularly important as Actinobacteria isolated from marine ecosystems have been shown to be a promising source of new drugs[4,5]. In this study, a comparative metabolomics approach using molecular networking was applied to understand the role of biogeography on the specialized metabolism of *Micrococcus* spp. and *Pseudonocardia* spp. isolated from Arctic and Antarctic marine sediments. The LC-MS/MS analysis showed differences in the specialized metabolism of phylogenetically related strains isolated from different geographic regions. These preliminary results suggest an influence on the microbial chemical space through assessing biogeographic impact. Future work on further marine ecosystems will expand our knowledge on the relationship between the chemistry and ecology of rare Actinobacteria.

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B110

Development of advanced corrosion-resistant coatings with synthetic biology-inspired protein technologies

Craig Allan[1], Geertje Van Keulen[2], Dave Penney[3], Gethin Allan[2], Alex Harold[2], Sholeem Griffin[2]

[1]Swansea University, Swansea, United Kingdom. [2]School of Medicine, Swansea University, Swansea, United Kingdom. [3]School of Engineering, Swansea University, Swansea, United Kingdom

Abstract

The purpose of this research is to further develop existing technology using engineered functional amyloid proteins for corrosion resistance by developing a coating which provides both adhesion to substrate and corrosion resistance. Pathogenic amyloid proteins have been attributed a role in certain diseases such as Alzheimer's Disease. However, the research undertaken here involves non-pathogenic functional bacterial amyloids, using coelicolor hydrophobic aerial proteins (chaplins or Chp) produced by *Streptomyces* for improving corrosion resistance. Chaplins have been proven conceptually to provide corrosion resistance properties on metals and, also provide strong adhesive properties in a multi-composite systems. The composition, application and curing of the better bonding chaplin composite will now be optimised for development of a better performing functional coating, which includes inclusion of small metabolites or natural products acting as corrosion inhibitors. This would then allow for benchmarking against existing corrosion-resistant coatings. Upon determining the most relevant formulae, samples will be analysed by using microscopic techniques such as Scanning Electron Microscopy and Atomic Force Microscopy, while corrosion will be tested using immersion tests, weathering via salt-spray and in-situ scanning vibrating electrode.

The biological and chemical tales of the antibiotic makers
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B111

Antimicrobial and antibiofilm potential of biosurfactants as novel combination therapy against bacterium that cause skin infections

Colleen Dempster, Ibrahim Banat, Roger Mrrchant

ulster University, Coleraine, United Kingdom

Abstract

Biosurfactants (BS) are amphiphilic molecules produced as a secondary metabolite by various bacteria and yeast species and are secreted extracellularly. BS have shown to work in synergy with antibiotics and also demonstrate strong antimicrobial and anti-adhesive characteristics. This coupled with their low toxicity makes them suitable candidates as combination therapies to combat skin infections. In this study, the aim was to investigate the *in-vitro* antimicrobial and anti-biofilm properties of mannosylerythritol lipids (MELs) produced by *Moesziomyces aphidis* against *Staphylococcus aureus* DSM- 20231, *Streptococcus pyogenes* ATCC-19615, *Staphylococcus epidermidis* DSM – 28319, *Pseudomonas aeruginosa* DSM-3227, *Escherichia coli* ATCC – 25922 and *Propionibacerium acnes* DSM-1897. MELs are most predominantly used in skin creams, thus, a rationale was developed to investigate antibiotics used to treat bacterial skin infections, namely, Polymyxin B Sulphate, Neomycin, Mupirocin and Bacitracin.

Minimum inhibitory concentration (MIC) values where determined for each antibiotic and BS per bacterium using the broth dilution technique based on CLSI guidelines. BS where extracted by solvent extraction and characterised using Mass Spectrometry – High Performance Liquid Chromatography, standards were quality assured using MALDI-TOF. Flow cytometry determined percentage dead versus alive for each antibiotic, BS and combination of antibiotic and BS. Scanning Electron Microscopy determined the effect of the BS on the bacterial cell walls. This study proves that BS work synergistically with antibiotics to increase the MIC of the antibiotics resulting in a substantial decrease in antibiotic use and at lower concentration. The use of BS combination therapy has the potential to reduce resistant rates and also lengthen the time taken for resistance to develop.

Fighting fire with fire: deploying microbes in the battle against disease
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B112

Identifying and inhibiting co-aggregation occurring between bacterial respiratory pathogens and commensal species associated with the altered microbiota of chronic pulmonary disease to identify the effect on the development of multispecies biofilms in vitro

Samuel Petrucci, Patrick Kimmitt

University of Westminster, London, United Kingdom

Abstract

Comparisons of pulmonary microbiotas associated with healthy individuals and cystic fibrosis (CF) patients identified a less diverse microbiota among CF patients comprising numerous bacterial species not found among healthy controls. This research aimed to identify if coadhesion occurs between respiratory pathogens *P.aeruginosa* and *S.aureus* and some commensal species associated with the microbiota of CF. This was conducted *in vitro* by spectrophotometric co-aggregation analysis and multi-species biofilm assays in the presence and absence of an inhibitor of lectin-mediated coaggregation, testing the ability of eleven commensal representative species of the CF pulmonary microbiota. Results show that all commensal species selected to represent genera that are associated with the CF pulmonary microbiota are capable of coaggregation *in vitro* with respiratory pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus. S.aureus* co-aggregated with a higher affinity to commensal species than *P.aeruginosa* with a median coaggregation percentage of 62.9% after 24 hours incubation, compared to 41.9% in *P .aeruginosa*. When commensal and pathogenic species were cultured in the presence of lactose, biofilm inhibition was observed in 5 of 12 cultures with *P.aeruginosa* and 7 of 12 with *S.aureus*. These results demonstrate that some commensals associated with CF participate in coaggregation with respiratory pathogens in vitro which has the ability to alter biofilm production. Identifying coadhesion between commensal and pathogenic species in *vitro* suggests that coadhesion could be occurring *in vivo* potentially causing increased susceptibility of the host by enhancing adhesion to the epithelial surface of the respiratory tract, aiding colonization and therefore increased susceptibility to infections.

B113

Arabian Sea associated bacterial strains a source of novel antibiotics against superbugs

Bushra Uzair

International Islamic University, Islamabad, Pakistan

Abstract

One of the most severe health threats to both humans and animals is Antimicrobial resistance development in microorganisms AMR is a significant and growing challenge and to combat this issue new antimicrobials are urgently needed Fifteen microbial strains isolated from Arabian Sea were screened for their capacity to produce antimicrobial metabolites of pharmaceutical interest. These strains were associated to the brown seaweed *Pelvetia canaliculata* (Linnaeus) attached to the rocks of Sonmiani Beach (Karachi, Pakistan).bacterial strains were isolated from sea weeds attached to rocks of Baluchistan coast line using marine agar 2216 and screened for antibacterial activity by agar well diffusion method and crude extract was made and antimicrobial metabolites were purified using silica gel column and structure of pure compound was elucidated using spectroscopic techniques.

Crude extract filtrates from CMG 2180 strain, grew on ZMA medium, showed the most remarkable antimicrobial activity, and thus was chosen for further examination. The identification of CMG 2180 as a probable new type strain of the *Actinobacterium Kocuria marina* was based on phenotypic aspects and biochemical characteristics (e.g. halotolerant Gram-positive micrococcoid) as well as on the nucleotide sequence analysis of its full-length 16S rRNA gene showing the highest similarity with the type strain KMM 3905 (GenBank accession number EU073966). Interestingly, a unique UV-bioactive compound, for which the name of kocumarin was proposed, was isolated and purified from CMG 2180 strain's crude extracts by flash silica gel column chromatography and TLC/HPTLC. Using routine methods, kocumarin demonstrated prominent and rapid activities against all tested fungi and pathogenic bacteria including MRSA. Its chemical structure was unraveled by 1D and 2D-NMR spectroscopy as 4-[(Z)-2 phenyl ethenyl] benzoic acid.

Fighting fire with fire: deploying microbes in the battle against disease
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B114

Arabian Sea associated bacterial strains a source of novel antibiotics against superbugs

Bushra Uzair

International Islamic University , Islamabad, Pakistan

Abstract

One of the most severe health threats to both humans and animals is Antimicrobial resistance development in microorganisms AMR is a significant and growing challenge and to combat this issue new antimicrobials are urgently needed Fifteen microbial strains isolated from Arabian Sea were screened for their capacity to produce antimicrobial metabolites of pharmaceutical interest. These strains were associated to the brown seaweed *Pelvetia canaliculata* attached to the rocks of Sonmiani Beach (Karachi, Pakistan). Isolated bacterial strains were screened for antibacterial activity by agar well diffusion method and crude extract was made and antimicrobial metabolites were purified using silica gel column and structure of pure compound was elucidated using spectroscopic techniques. Crude extract filtrates from CMG strain, grew on ZMA medium, showed the most remarkable antimicrobial activity, and thus was chosen for further examination. The identification of CMG as a probable new type strain of the *Actinobacterium Kocuria* was based on phenotypic aspects and biochemical characteristics (e.g. halotolerant Gram-positive micrococcoid) as well as on the nucleotide sequence analysis of its full-length 16S rRNA gene showing the highest similarity with the type strain KMM 3905. Interestingly, a unique UV-bioactive compound, kocumarin was isolated and purified from CMG strain crude extracts by flash silica gel column chromatography and TLC/HPTLC. kocumarin demonstrated prominent and rapid activities against all tested fungi and pathogenic bacteria including MRSA. Its chemical structure was unraveled by 1D and 2D-NMR spectroscopy as 4-[(Z)-2 phenyl ethenyl] benzoic acid.

Fighting fire with fire: deploying microbes in the battle against disease

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B115

Exploiting inter-species competition to kill medically relevant Clostridia

Stephen Garrett[1], Heather Hulme[2], Richard Goodwin[3], Daniel Walker[1], Richard Burchmore[1,4], Gillian Douce[1], Dónal Wall[1]

[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom. [2]Upppsala Universitet, Uppsala, Sweden. [3]AstraZeneca, Cambridge, United Kingdom. [4]Glasgow Polyomics, University of Glasgow, Glasgow, United Kingdom

Abstract

The human microbiome has been linked to increasing numbers of diseases, with the gut-brain-microbiome axis becoming the focus of more research in recent years. Previous work has identified two novel bacterial metabolites that can cross the blood-brain barrier, with their effects on the brain being undefined. The aim of this study was to identify bacteria isolated from the environment that were capable of inhibiting the growth of strains that produce these metabolites, and subsequently identify the source of inhibition. Environmental samples were collected, grown anaerobically, and isolated colonies tested for inhibition of metabolite-producing strains. One strain was identified as inhibiting the growth of these bacteria. Tests were also carried out to determine the effects of these metabolites on macrophages. When both metabolites were present, higher concentrations appeared to decrease macrophage activity. These metabolites are similar in structure to carnitine, the molecular facilitator of fatty acid uptake into mitochondria. It is possible that the decrease in macrophage activity was due to inhibition of this process, although further testing is required. Research suggests that a member of the *Lachnospiraceae f*amily, closely related to the bacteria of interest, is able to decrease the effects of colitis in mice, (potentially due to the production of these metabolites). However, the possible negative effects on cellular respiration, particularly in the brain, suggests the future direction of this work must be to determine a way to prevent the presence of these metabolites in humans, until more is known about the extent of the effects they may have.

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B116

AMR gene removal by conjugative delivery of CRISPR-Cas9

David Sünderhauf, Elizabeth Pursey, Uli Klümper, Edze Westra, William Gaze, Stineke van Houte

University of Exeter, Penryn, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a growing threat to healthcare. Many AMR-encoding genes are carried on accessory genetic elements like plasmids, which spread between phylogenetically distant members of bacterial communities. CRISPR-Cas based technologies may help combat the spread of AMR genes by removal of such plasmids. To optimise this technology, implementation of a delivery method which can reach a diverse range of bacteria is needed.

Therefore, we engineered broad host-range conjugative plasmid pKJK5 to express Cas9. pKJK5::Cas[GmR] encodes a guide RNA which targets Gentamicin resistance gene *aacC1*, while pKJK5::Cas[nt] encodes a non-targeting guide RNA.

After confirming its Ca9 activity by electroporation of targeted and untargeted plasmids, we set up a proof-of-concept experiment to conjugatively deliver pKJK5::Cas from a donor *Escherichia coli* strain DH5α to unrelated recipient *E. coli* K12, and to remove Gentamicin-resistance encoding target plasmid pHERD30T. For these means, we mixed donors and recipients in liquid media and incubated them overnight; proportions of the total population were enumerated by differential plating.

We found that Gentamicin-resistant recipients were reduced by 33% when treated with pKJK5::Cas[GmR] compared to treatment with pKJK5::Cas[nt], indicating that pKJK5::Cas[GmR] has the ability to remove AMR plasmids from recipient cells.

This proof-of-concept experiment shows how an engineered broad host-range conjugative plasmid is an effective means of removing AMR-encoding plasmids and may be a viable approach to remove resistance genes from complex bacterial communities. To make this method more effective, community experiments as well as optimisation of Cas9 activity are needed.

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B117

Community assembly in the microbiome: ecological insights into infant microbiome development

Katharine Coyte[1,2], Chitong Rao[2], Seth Rakoff-Nahoum[2], Kevin Foster[1]

[1]University of Oxford, Oxford, United Kingdom. [2]Harvard Medical School, Boston, USA

Abstract

In healthy adults, the gastrointestinal tract harbours a diverse community of microbes that play critical roles in health and wellbeing. However, we are not born with this microbiome. Over the first months and years of life the gut microbiome gradually develops, undergoing a process akin to classic primary succession. We here develop ecological theory in order to study the factors that drive these assembly processes. We find that interactions between species can enforce order on microbiome development, with interspecies dependencies driving the predictability of succession. We combine this theory with novel sample processing techniques to interrogate both bacterial and fungal microbiome development in premature infants. We utilize machine learning to infer how members of the microbiome are affected by both one another and clinical interventions. Preliminary results identify specific inter-microbial interactions that may in part drive community dynamics, and identify the impact of antibiotic interventions in perturbing healthy microbiome development. Taken together, these results highlight the importance of disentangling microbial interactions if we are to understand and ultimately manipulate our microbiome communities.

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B118

Isolation of novel bacteriocins active against clinically relevant, pathogenic *Clostridioides difficile.*

Yasmin McLaughlin

University of Nottingham, Nottingham, United Kingdom

Abstract

The emergence of antibiotic resistant, hypervirulent strains of the nosocomial pathogen *Clostridioides difficile*, coupled with increased concerns over the physiological impact of traditional broad-spectrum antibiotics on human health has led to an increased demand for alternative therapies. Bacteriocins are proteinaceous toxins produced by bacteria that kill other bacteria and their antimicrobial activity makes them an attractive potential alternative therapy to antibiotics. This research aims to isolate environmental bacteria from porcine faeces that produce novel bacteriocins with inhibitory activity against *C. difficile*. At present in this study two distinct organisms with protein mediated inhibitory activity against *C. difficile* have been isolated and it has been shown that these strains encode various bacteriocins. The current focus of this research aims to attribute the inhibitory activity to one of the encoded bacteriocins or potentially a novel antimicrobial peptide, which could then be exploited as an alternative therapy against *C. difficile.*

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B119

Moonlighting in myxobacteria: measuring the covariation in GAPDH

Allison Zwarycz, David Whitworth

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Myxobacteria are an order of well-known social predators that able to prey upon a wide range of microbes (including fungi and bacteria). It is currently unknown how they are able to consume such a wide range of prey, although moonlighting proteins, those possessing more than one function, appear to be involved. One moonlighter, is the virtually ubiquitous enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Typically present in the cytoplasm, it has also been found on the outer surface of cells, in the extracellular matrix, and secreted through membrane vesicles. In various pathogenic organisms, GAPDH has been shown to have adhesive properties, binding to several types of human connective tissues. Myxobacterial predation can be viewed as analogous to pathogenic infection, and there is evidence that GAPDH might be involved in attacking prey cells.

To complement experimental analyses of the predatory role of GAPDH in myxobacteria, we undertook sequence analysis including multiple sequence alignment (MSA) and covariation analyses. Covariation calculations, using a variety of metrics and matrices, have been used to identify areas and individual residues where structural, functional or phylogenetic correlations occur within the sequence. The MSA data highlighted key conserved residues, likely involved in the catalytic reaction and potentially involved in adhesion. The covariation scores suggest compensatory mutations have taken place in discrete locations along the sequence. These results can be used to selectively mutate residues in order to determine their effect in predation.

Fighting fire with fire: deploying microbes in the battle against disease

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B120

Antimicrobial properties of macrofungi to *Mycobacterium abscessus* isolated from patients with Cystic Fibrosis (CF)

Cherie Millar[1,2], David Nelson[3], Rachel Moore[2], JR Rao[3], John Moore[1,2]

[1]Northern Ireland Public Health Laboratory, Belfast City Hospital, Belfast, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom. [3]Agri-Food and Biosciences Institute (AFBI), Belfast, United Kingdom

Abstract

Antimicrobial resistance (AMR) has now emerged as a global public health crisis. Of particular concern is AMR associated with the genus *Mycobacterium*, including *M. tuberculosis* and the non-tuberculous mycobacteria (NTM). Emergence of the NTM, in particular *M. abscessus*, in patients with cystic fibrosis (CF) represents both a diagnostic and a treatment dilemma. Such resistance drives the need to investigate novel sources of antimicrobials. Medicinal fungi have a well-documented history of use in traditional oriental therapies. Not only is this an ancient practice, but still today, a medical practice in Japan, China, Korea and other Asian countries which continue to rely on fungal-derived antibiotics. A study was therefore undertaken to examine antimicrobial activity of 23 native macrofungal (mushrooms/toadstools) taxa, collected from woodlands in Northern Ireland against six clinical (CF) isolates of *M. abscessus*, as well as *M. abscessus* Reference strain (NCTC 13031). Free-growing saprophytic and mycorrhizal macrofungi (n=23) belonging to the phylum *Basidiomycota* were collected and were definitively identified employing PCR/ITS DNA sequencing. Macrofungal tissues were freeze-dried and reconstituted prior to employment in antibiotic susceptibility testing. All macrofungi examined showed varying inhibition of the *M. abscessus* isolates examined with the exception *Russula nigricans*. The macrofungi displaying maximum activity against the clinical isolates were (in descending order) *Phlebia radiata*, *Hygrocybe nigrescens* and *Hypholoma fasciculare*. Further work is now required to identify the constituents and mode of inhibitory action of these against the NTMs. Macrofungi may represent a source of novel antimicrobials which have not yet been fully explored nor exploited clinically.

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B121

Penicillium spp. strains as a possible weapon to fight microbial infections

Abigail Broomfield, Marta Filipa Simões

GEMM-Group for Extreme and Marine Microbiology, Biology Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

Bacteria are becoming increasingly resistant to antibiotics, leading to untreatable infections and constituting a major public health hazard (Lewis, 2013). This problem is further increased by the reduction in the number of effective antibiotics to tackle resistant strains (Penesyan *et al*, 2015), so the search for new compounds is seen as a vital priority.

Our study consisted in the analysis of four different environmental strains of *Penicillium* spp., with screening for their extracellular metabolites for potential antimicrobial activity. Several methods were combined and tested for metabolite production and extraction. The strains were grown on: solid media (**I -** potato dextrose agar and **II -** malt extract agar), broth (malt extract broth with **III -** 0 and **IV -** 5% NaCl), and **V –** tap water; extractions were performed using three different solvents: **A -** methanol, **B -** butanol and **C -** ethyl acetate.

All extracts were tested on three different model organisms: *Micrococcus luteus, Escherichia coli and Mycobacterium smegmatis.* These are models for Gram-positive and Gram-negative bacteria, and for Tuberculosis. The extracts were compared and analysed in order to determine minimal inhibitory concentrations for each of the microorganisms tested.

This research presents preliminary results on the development of potential new chemical compounds to help us circumvent the problem of drug resistance.

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Fighting fire with fire: deploying microbes in the battle against disease
Presentations: Wednesday evening and Thursday lunchtime

B122

Identification and characterization of bioengineered nisin derivatives that inhibit the opportunistic pathogen *Staphylococcus epidermidis*.

Clíona O' Shea[1,2], Ellen Twomey[1], Des Field[2], Máire Begley[1]

[1]Department of Biological Sciences, Cork Institute of Technology, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland

Abstract

Staphylococcus epidermidis is a major cause of hospital-acquired infections particularly on indwelling medical devices and implants. Infections caused by this pathogen are difficult to treat with standard antimicrobial agents mainly as the bacterium can establish biofilms on artificial surfaces. Novel control methods are needed and one such alternative may be bacteriocins; these are antimicrobial peptides produced by some bacteria that inhibit other specific bacteria. They are potent, safe, and stable and are easy to produce using biotechnological based strategies. Additionally, as they are gene encoded they can be easily modified or engineered to enhance their activity. In the present study, we demonstrate the ability of the bacteriocin nisin to inhibit a collection of clinical *S. epidermidis* strains under standard laboratory conditions. In addition, a bank of bioengineered nisin derivatives was screened using agar-based deferred antagonism assays and derivatives with enhanced antimicrobial activity compared to the wild-type nisin were identified. These derivative peptides are currently being purified using a combination of chromatography-based approaches and their potency and stability are being examined. Future experiments will focus on examining the ability of the nisin derivatives in inhibiting *S. epidermidis* biofilms on medical device materials (e.g. stainless steel and polyvinyl chloride) both alone and in combination with conventional antibiotics. It is hoped that one of the nisin derivatives analysed in this study may ultimately be used to control or prevent infections caused by *S. epidermidis.*

Fighting fire with fire: deploying microbes in the battle against disease

Presentations: Wednesday evening and Thursday lunchtime

B123

Combating Necrotising Enterocolitis in Preterm infants with Probiotic bacteria

Elizabeth Clements[1], Caroline Orr[1], Gillian Taylor[1], Andrew Nelson[2], Christopher Stewart[3], Stephen Cummings[1], Janet Berrington[4], Nicholas Embleton[4]

[1]Teesside University, Middlesbrough, United Kingdom. [2]Northumbria University, Newcastle upon Tyne, United Kingdom. [3]Newcastle University, Newcastle upon Tyne, United Kingdom. [4]Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Abstract

Necrotising enterocolitis (NEC) causes 21% of deaths in the preterm infant population (< 32 weeks gestational age.) Previous examination of the causes of the NEC have been inconclusive. Subsequently the focus has transferred to interpreting the metabolomic pathways involved in the interaction between the gut and disease-causing factors. *Bifidobacterium* utilise human milk oligosaccharides (HMOs), otherwise indigestible by preterm infants. Specifically, the *Bifidobacteria* strains *infantis*, *breve* and *longum* are required for HMO digestion via genome encoding enzymes for breast milk fed infants. The aim of this investigation was to determine the role of *Bifidobacteria* and the use of probiotics in preventing NEC by examining the stool samples of 51 preterm infants (n = 188). The samples were collated into 26 preterm infants which contracted NEC (n = 64) and 25 preterm infants without NEC (n = 112). The latter no-NEC samples aligned to the NEC samples by date of birth, day of life and gestational age. The stool samples were subjected to 16s rRNA targeted DNA extraction and Next Generation Sequencing (NGS) analysis to identify bacterial abundance, diversity and community structure. Statistical analysis was used to infer metabolomic functionality of the bacteria present, with the aim of reducing the susceptibility to and mortality rate of NEC.

Fighting fire with fire: deploying microbes in the battle against disease
Presentations: Wednesday evening and Thursday lunchtime

B124

Endophytic actinobacteria of herbal rhizomes and their pharmaceutical potential to form L-asparaginase

Nareeluk Nakaew[1], Jatuporn Ngoenkam[1], Monthakan Donphraiyon[1], Thiphakon Yot-an[1], Chatchawan Jompaeng[1], Boonrat Ano-thai[1], Rungroch Sungthong[2]

[1]Naresuan University, Phitsanulok, Thailand. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Pharmaceutical treasure of herbs is well known and some of which have long been served as natural medicines or nourishments to enrich human health. It is conceivable that herbs offer unique habitats for endophytic microbes. Interestingly, the metabolic exchange between endophytes and their herbal hosts does not only allow the microbial subsistence but also enable them to form analog bioactive substances to those produced by their hosts. These challenge us to focus on searching for beneficial microbes from herbs and industrialize them as the producers of pharmaceutical products. In this work, we isolated 37 actinobacteria from rhizomes of fingerroot (*Boesenbergia rotunda*), ginger (*Zingiber officinale*), galangal (*Alpinia galanga*), and turmeric (*Curcuma longa*). These actinobacteria were classified preliminarily based on their morphology to the genus *Streptomyces* (86%) and other unknown genera (14%). To screen for anticancer activity, we found that 68% of all isolated actinobacteria produced L-asparaginase, which is a hydrolytic enzyme that acts as an inhibitor of leukemia. *Streptomyces* sp. ALP03 was among the active isolates exhibited the highest enzyme activity of L-asparaginase and showed a maximum 97.93% similarity of its 16S rRNA gene sequence to *Streptomyces spongiae* Sp080513SC-24^T. The cytotoxicity assays against diverse types of cancer cell lines will be carried out to assess the anticancer potential of isolate ALP03 and the others showing distinct L-asparaginase activity. With these findings, we conclude that herbal rhizomes have yet been a promising source for the discovery of useful microbes and their pharmaceutical products.

Fighting fire with fire: deploying microbes in the battle against disease
Presentations: Wednesday evening and Thursday lunchtime

B125

Screening bacteriophage activity against *E. coli* O157:H7 attached to host cells.

Alison Low, David Gally

University Of Edinburgh, Edinburgh, United Kingdom

Abstract

Bacteriophage (phage) therapy, the use of a specific virus to kill infecting bacteria, is often cited as an alternative to antibiotic therapy. But phage treatment could also play an important role in bio-security against the foodborne pathogen *E. coli* O157:H7. Phage can be relatively easily isolated and shown to have activity against bacterial strains of interest under laboratory conditions. However, to be used successfully for treatment the phage must be active in vivo where the bacteria may be attached to host cells. To overcome this potential hurdle we are using a screen to test phage activity in conditions more realistic to the host environment than standard lab media. As part of this work we have been isolating new phage that are active against *E. coli* O157:H7 strains. We are currently testing these newly isolated phage against different bacterial strains and under a variety conditions looking for characteristics that will make them good candidates for phage therapy. We will then test the activity of promising candidates against *E. coli* O157:H7 attached to bovine epithelial (EBL) cells. The aim of this work is to put together a panel of around 20 phage that not only have the appropriate standard characteristics for phage therapy but have also been shown to have activity on attached bacterial cells. The longer term aim of this work is to use these 'in vivo' active phage as an intervention on cattle colonized with *E. coli* O157:H7 thereby reducing the potential of this pathogen entering the food chain.

Fighting fire with fire: deploying microbes in the battle against disease
Presentations: Wednesday evening and Thursday lunchtime

B126

Creation and characterisation of probiotic libraries for use in poultry and pigs

Helen Brown, Isabella Pursley, Daniel Horton, Roberto La Ragione

University of Surrey, Guildford, United Kingdom

Abstract

Probiotics are defined as live micro-organisms which provide the host with a growth or health advantage. In recent year's with the emergence of antimicrobial resistance, probiotics, and in particular *Lactobacillus*, have become increasingly popular as an alternative control strategies for bacterial pathogens. Recent studies have demonstrated that *Lactobacillus* is not only able to increase the growth rate of animals, an important consideration for commercial farming entities, but is also able to inhibit the colonisation of animals with pathogenic bacteria species such as *Salmonella*, *Campylobacter* and *Escherichia*.

This study aimed to isolate and characterise lactobacilli isolates from commercially reared chickens and pigs. Particular attention was paid to the probiotic potential of the isolates. Our approach combined molecular techniques with *in vitro* screening to rapidly identify this potential. To date 80 and 105 isolates have been collected from pigs and chickens, respectively. All isolates have been confirmed to belong to the *Lactobacillus* genus by PCR, speciated by 16S sequencing and determined to be clonally unique using RAPD PCR. Isolates were subsequently tested for their ability to tolerate low pH, bile, aerobic and anaerobic conditions before assessing their AMR profile and determine their ability to inhibit a panel of clinical and type strains of pathogenic bacteria. Isolates identified as potential probiotics are currently undergoing whole genome sequenced as per EFSA guidelines.

This study has demonstrated that lactobacilli isolates with suitable probiotic properties can be isolated from commercial poultry and pigs. Furthermore, these isolates may prove useful as control strategies for zoonotic bacterial pathogens.

Fighting fire with fire: deploying microbes in the battle against disease
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B127

Targeting Antimicrobial Resistance Genes in Clinical Isolates from Healthcare-Associated Infections Using CRISPR-Cas9

Elizabeth Pursey[1], David Sünderhauf[1,2], Fernanda Paganelli[3], William Gaze[2], Edze Westra[1], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter Medical School, Truro, United Kingdom. [3]University Medical Center Utrecht, Utrecht, Netherlands

Abstract

Of the numerous approaches to counteracting the spread of antimicrobial resistance (AMR), one that is receiving increased attention is the use of CRISPR-Cas-based gene editing technology to remove resistance-conferring sequences from bacteria. Proof-of-principle studies have shown that CRISPR-Cas is able to successfully remove AMR genes from monocultures or very simple bacterial communities in the laboratory, but these constructs and their delivery must be adapted to be used in real-world medical settings.

One area where such a technology may be implemented is tackling healthcare-associated infections. Development of a CRISPR-Cas9 cassette that is able to target particular resistance genes would be a powerful tool in addressing these infections. CRISPR-Cas9 will therefore be implemented to target AMR genes in clinical isolates of vancomycin-resistant *Enterococcus faecium*, and extended-spectrum β-lactamase (CTX-M) or carbapenemase (Oxa-48)-producing *Klebsiella pneumoniae* and *Escherichia coli*, by utilising a broad host range conjugative plasmid to deliver this construct via conjugation. Preliminary work using *E. coli* MG1655 as plasmid donor have shown that all strains can receive the plasmid, and there is significant variation in conjugation frequency between *E. coli* recipients.

Fighting fire with fire: deploying microbes in the battle against disease

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B128

Investigating soil bacteria for novel antimicrobials which inhibit the opportunistic pathogen, Staphylococcus epidermidis

Ellen Twomey[1], Des Fields[2], Maire Begley[1]

[1]Department of Biological Sciences, Cork Institute of Technology, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland

Abstract

According to the European Center of Disease Control and Prevention, *Staphylococcus epidermidis* is rapidly becoming a serious concern in hospitals as a cause of resistant infections, particularly in the case of in dwelling and prosthetic medical devices. To overcome this issue, new, potent antimicrobials with novel target pathways need to be uncovered to both reduce morbidity and mortality, and the spread of resistant microbes. 51 bacterial strains with observable antimicrobial activity against *S. epidermidis* were isolated from samples of soil, collected from various locations around Ireland. The activity was reconfirmed, and the most effective strains were shortlisted using deferred antagonism assays. Characterisation tests, (Gram stains, oxidase and catalase testing and 16S sequencing), were carried out to determine the identities of the bacteria at hand. Presently studies are underway to elucidate the nature of the antimicrobial activity and to govern if the findings are novel. HPLC is being used to purify the compounds, with proteinase K assays evaluating if the antimicrobials are proteinaceous in nature. The future of this study will include the sequencing of any uncovered antimicrobial peptides, MIC determination of antimicrobial compounds, observing the impact on biofilm formation and looking at potential efficacy against other relevant pathogens. With the identification of a novel compounds, this study aims to present an opening into potential new treatment options to help address the current struggle against antimicrobial resistance.

B129

Detection of SHV, CTX-M and TEM Genes In extended spectrum Beta Lactamase producing multi-drug resistant *Escherichia Col*i from clinical isolates in Calabar, Nigeria

OFONIME OGBA[1], MIRACLE ESIERE[1], ROSEMARY ESIERE[2]

[1]UNIVERSITY OF CALABAR, CALABAR, Nigeria. [2]UNIVERSITY OF CALABAR TEACHING HOSPITAL, CALABAR, Nigeria

Abstract

Background: The emergence of multi-drug resistant strains of *Escherichia coli* complicates the treatment of infections. The detection of ESBL genes in bacteria and their antimicrobial resistance patterns can provide information about their epidemiology and transmission. The study aimed to detect ESBL genes that encode; CTX-M, TEM and SHV in *E. coli isolates* in our locality. **Methods:** Clinical *E. coli* isolates were obtained from public and private clinics within Calabar metropolis. Biochemical method was used to re-identify the isolates. Antibiotics susceptibility testing was done using Kirby-Bauer disc diffusion method. Phenotypic detection of ESBL in isolates was done by double disc synergy test (DDST). The ESBL genes were detected using conventional PCR method. **Results:** The ESBL phenotypic positive isolates was (56.6%). The most prevalent gene in the study was CTX-M gene. Antibiotic susceptibility of *E. coli* isolates to commonly used antibiotics was low. Isolates were most susceptible to quinolones (54.7%) and fluoroquinolones (34.0%). The ESBL producing isolates were more susceptible to quinolones but less susceptible to the third generation cephalosporins. There was significant association between gene expression by isolates and antibiotic resistance ($p \leq 0.05$). Isolates with the SHV and TEM genes showed 100% resistance to some tested antibiotics. Isolates with CTX-M genes were also highly resistant. **Conclusion:** The SHV, CTX-M and TEM genes were detected in *Escherichia coli* isolates in our locality. These may have resulted in the high resistance of isolates to commonly used antibiotics which may pose challenges to patient's management.

B130

Environmental smoke exposure may predispose to bacterial-induced periodontal tissue destruction.

Gülcan Danaci[1], Gwyneth J. Lamont[2], Burcu Kanmaz[1], Himabindu Gogeneni[3], David A. Scott[2]

[1]Ege University, Izmir, Turkey. [2]University of Louisville, Louisville, USA. [3]Indiana University, Indianapolis, USA

Abstract

Cigarette users are more susceptible than non-smokers to chronic periodontitis, a bacterial-induced, inflammation-driven, destructive disease of the supporting tissues of the teeth. Limited evidence suggests that environmental smoke may similarly predispose to periodontal diseases. We hypothesized that levels of microbiological mediators and/or inflammatory markers of chronic periodontitis would be intermediate in those exposed to environmental tobacco smoke compared to active and non-smokers. Self-reported non-smokers, current smokers and environmentally-exposed individuals were recruited from a University periodontal clinic. Clinical periodontal measurements, comprising plaque index, probing depth, clinical attachment level and bleeding on probing, were recorded at four sites per tooth. Whole saliva samples were collected and cotinine levels determined by EIA. *Treponema denticola* and *Porphyromonas gingivalis* infection was determined by PCR, while matrix metalloproteinase-8 (MMP-8) and interleukin-8 (IL-8) concentrations were determined by ELISA. Smoking groups were reassigned in accordance with the cotinine data. *P. gingivalis* infection was noted in most subjects, irrespective of smoking status while *T. denticola* infection was noted in 17% of smokers, 0% of environmentally-exposed recruits and in 10% of non-smokers. IL-8 and MMP-8 burdens were each increased in environmental smokers, relative to the other smoking groups. Finally, environmental smokers exhibited mean probing depths and clinical attachment levels intermediate to active smokers and non-smokers, key periodontal diseases parameters which also correlated with cotinine concentrations. Within the limits of this clinical study, environmental smoke exposure may alter the host response to the oral microbiome in a manner associated with increased susceptibility to periodontal tissue destruction.

B131

Efficacy of Extract and Fractions of Abutilon hirtum in Treatment of Multidrug Resistant Salmonellae (MDR) Infections.

Noel Dawang

Plateau State Polytechnic,Barkin-Ladi, Jos, Nigeria

Abstract

The continuous emergence of some *Salmonella* species to even third generation antibiotics is a threat to health care delivery. Thus, this study aimed at screening multidrug resistant (MDR) *Salmonella* isolates and subjecting them to *Abutilon hirtum* extract and fractions as a means of possible discovery of new antimicrobial of plant nature in treatment of MDR Salmonellae infections. Ten serotyped clinical *Salmonella* isolates were obtained from Jos University Teaching Hospital (JUTH), Nigeria. Fraction extracts were obtained by open column chromatography. Phytochemical screening was done using Standard Qualitative method. The bioassay of the crude and fraction extracts (AF9) against MDR *Salmonella* isolates was by Well Agar diffusion and broth micro dilution method respectively. All *Salmonella* isolates exhibited resistance against more than 3 antimicrobials (MDR). However, all the isolates (100%) were susceptible to ciprofloxacin and 60% sensitive to ceftriaxone but showed 100% resistance against amoxicillin, erythromycin, tetracycline and amoxicillin +clavulanic acid. The *A.hirtum* extract showed the presence of alkaloids, flavonoides, tannins, saponins, cardial glycosides, terpenes and steroids, phenol and resins. 80% of the isolates were susceptible to the crude extract at concentration of 200mg/ml but susceptibility decreased as concentration decreased. The MIC and MBC of the plant fraction (AF9) against the isolates ranged from 150µg/ml–300µg/ml and 300µg/ml-600µg/ml respectively. The AF9 showed 90% bactericidal effect on the isolates. *A. hirtum* fractions with further processing may probably be a new antimicrobial for treating MDR *Salmonella* infections.

Key words: Multidrug Resistant Salmonellae, *Abutilon hirtum* extracts, bioactive molecules, Jos, Nigeria

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B132

Investigating the effect of tobramycin dry powder inhaler on the eradication of Pseudomonas aeruginosa biofilms.

Reham Aljalamdeh, Robert Price, Matthew D Jones, Albert Bolhuis

University of Bath, Bath BA2 7AY, United Kingdom

Abstract

Biofilms are sessile communities of microorganisms embedded within a self-generated extracellular polymeric matrix. Such biofilms are found for instance in adults with cystic fibrosis, with pulmonary infections with the Gram-negative bacterium Pseudomonas aeruginosa being particularly common. This infection in CF patients is commonly managed with antibiotic dry powder inhalers, one of which is the aminoglycoside tobramycin. The activity of tobramycin has been well characterized in vitro, but current models that have been used are not very representative for lung infections, and better models would provide a significant advantage as these could be used, for instance, to improve the formulation of dry powder inhalers.

For instance, one question that has not been addressed with current models is whether the size of drug particles emitted from a dry powder inhaler influences the efficacy of the anti-biofilm activity of the antibiotic. In this project, we utilized the Next Generation Impactor (NGI), which is a pharmaceutical instrument used to separate particles into size fractions. We used the NGI to separate tobramycin particles into different sizes and tested the influence of these particles on eradication of P. aeruginosa biofilms, which were grown using as colony biofilms that closely mimics conditions in the lung where biofilms are grown on a substrate-air interface. Preliminary evidence indicated smaller tobramycin particles are better in eradication of P. aeruginosa biofilms as compared to larger particles. Our results may represent a step towards improving the formulation of tobramycin dry powder inhalers to be effective in eradicating P. aeruginosa biofilms.

B133

ATHLETE'S FOOT: ASSOCIATED MICROBES AND RISK FACTORS OF INFECTION TRANSMISSION AMONG FOOTBALL PLAYERS

OFONIME OGBA, BARILEE IDOO-BUBE

UNIVERSITY OF CALABAR, CALABAR, Nigeria

Abstract

Background: Tinea pedis is one of the most common superficial skin infections and represents a major public health problem globally. It is common among athletes especially soccer players. This cross sectional prospective study was carried out to determine the degree of occurrence of tinea pedis and the associated risk factors among soccer players. **Methods:** Eighty subjects with visible lesion of tinea pedis were enrolled for the study after obtaining informed consent and Ethical clearance from the subjects and relevant authorities respectively. A structured questionnaire was administered to the subjects for data on risk factors for infection and demography. The AFSI was used to assess the lesions. Skin scrapings were obtained from lesions for analysis. Samples were subjected to microscopy, culture and physiologic testing. **Results:** A total of 52/80(65.0%) athlete's foot infection rate was recorded in the study. Dermatophytes recovery rate was 29/52(55.8%) while yeasts and non-dermatophytes moulds' recovery rate was 23/52(44.2%). Subjects with AFSI>1 had (38.5%) infection rates but there was no significant association between AFSI and athletes' foot ($\chi^2 = 5.4$; $p \geq 0.05$). Fungal and bacterial co-infection rate was 42.5%. *Trichophyton meantagrophyte* 8(15.5%) was the most common dermatophyte while *Aspergillus niger* 6(11.5%) was the most common non-dermatophyte. The highest risk factor of infection transmission among subjects was the use of public gym 28(35.0%). **Conclusion:** Dermatophytes and non-dermatophytes were associated with the athlete's foot. The name tinea pedis should be reconsidered. The use of public sports facility may foster infection transmission.

B134

Evaluation of Potential Anti-Infective Coatings for Urinary Catheters using a Bladder Infection Model

Emma L Henly, Melissa M Lacey, Thomas J Smith, Sarah Forbes

Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Background: Catheter associated urinary tract infection (CAUTI) exhibits low antimicrobial susceptibility partially due to the formation of bacterial biofilms on the catheter surface. Uropathogenic *Escherichia coli* (UPEC) are the most frequent causative agent of CAUTI. To reduce the incidence of CAUTI there is a need to develop anti-infective catheter coatings that are refractory to biofilm formation and that demonstrate long-term antimicrobial activity but success to date has been limited. **Aims:** To determine changes in biofilm formation, biofilm viability and cell invasion capability in 8 UPEC isolates after long-term exposure to the biocides triclosan, polyhexamethylene biguanide (PHMB), benzalkonium chloride (BAC) and silver nitrate or the quorum sensing inhibitors cinnamaldehyde, furanone and F-DPD **Methods**: Bacterial isolates were exposed to test biocides and QSI's using an antimicrobial gradient plating system. A high-throughput catheter biofilm model was developed to assess the impact of long-term biocide and QSI exposure on biofilm formation and viability. Changes in bacterial virulence in urothelial cells (HUEPC) after exposure were quantified using a cell invasion model. **Results**: Data shows significant reduction in bacterial cell invasion after long-term biocide and QSI exposure for all isolates. Increased cell invasion was induced by PHMB (1/8 isolates), triclosan (2/8), cinnamaldehyde (1/8) and furanone (1/8). Biocide exposure increased biofilm formation (1 case) and viability (5 cases). QSI exposure increased biofilm formation (2 cases) but decreased viability (3 cases). **Conclusion:** These data indicate the multiple consequences of biocide and QSI adaptation in UPEC that should be considered when selecting an anti-infective catheter-coating agent.

B135

Construction And Test Of An Efficient Biophotonic Imaging (BPI) Reporter System To Study Pneumococcal Biology In Vitro And In Vivo

NADA ALKHORAYEF

leicester university, leicester, United Kingdom

Abstract

Streptococcus pneumoniae is a common nasopharyngeal resident in healthy persons, but remains a major cause of pneumonia, bacteremia and otitis media despite vaccines and effective antibiotics. There is an urgent need for novel therapeutic approaches, but such advances require a detailed knowledge of *S.pneumoniae* biology and its shift from commensal to pathogen. To better understand pneumococcal biology and infections, we need sensitive in vivo imaging technologies. To this end, bioluminescence imaging can be used, for example, to evaluate anti-infectives, intraspecies interaction and pneumococcal virulence non-invasively. A click beetle luciferase (CBR-luc) containing vector pPP3 under the control of putative highly expressed pneumococcal promoters was constructed. The CBR*luc* providing red-shifted light production was integrated into known sites in the *S. pneumoniae* genome. The constructs were compared to a *lux*-based exist system expressing bacterial luciferase using *in vitro* growth experiments.The results revealed that CBR*luc* tagged bacteria, P*phrA::luc*-wt , showed robust activity of bioluminescence in exponential phase that is maintained during stationary phase, whereas, *lux*-expressing pneumococci emitted a light signal with high background that peaked during exponential phase and was significantly reduced in intensity during stationary phase. Initial findings demonstrate that the CBR*luc* reporter system is more efficient than *lux*, providing a potential platform for utilization in understanding of the mechanisms of pneumococcal pathogenesis in vivo system

B136

Antibiotic Susceptibility Pattern of *Salmonella* Isolates from Enteric Fever Suspected Patients

Bijayata Shrestha[1], Gita Sayami[1], Sarita Manandhar[2], Santosh Khanal[2]

[1]HAMS Hospital, Kathmandu, Nepal. [2]National College, Kathmandu, Nepal

Abstract

Background Enteric fever is one of the most common diseases encountered worldwide and is endemic in Nepal. This study was conducted to access antibiotic susceptibility pattern of *Salmonella* isolates from culture positive cases of enteric fever.

Methods Altogether 505 blood samples were collected from patients clinically suspected of enteric fever attending HAMS Hospital. All blood samples were cultured by BACTEC method and sub cultured in blood agar and MacConkey agar plates. All isolates were identified by colony characteristics, biochemical tests and serotyping methods. Antibiotic susceptibility test was performed by modified Kirby Bauer disc diffusion method interpreted with CLSI guideline.

Result Isolation rate of *Salmonella* species was 3.6%. Among 18 *Salmonella* isolates, 10 were *S.* typhi, 8 were *S.* paratyphi A. The prevalence rate of infection was high among the age group 11-20 years (50%) and among the male patients. However, there was no significant association of enteric fever with gender of patients (p=2.47). All 18 isolates were sensitive to Amoxycillin, Azithromycin, Ceftriaxone and Chloramphenicol, Ciprofloxacin and Ofloxacin. Majority of isolates were sensitive to Cefixime (94.4%), Cotrimoxazole (94.4%) and Cephotaxime (90%). There were no any MDR isolates. Higher percentage of isolates was resistant to Nalidixic acid (87.5%).

Conclusion The decreased susceptibility to Fluroquinolones of *S*. typhi and *S*. Paratyphi A can be correlated with resistance to Nalidixic acid. Commonly used third generation Cephalosporins and rolled back first line drugs be the choice in case of NARS isolates.

Key words: Enteric fever, *Salmonella,* Multidrug resistance (MDR), NARS

B137

Antimicrobial Activity of Zamzam Water Against Salmonella typhii in vitro

Amalia Tri Utami

UIN Maulana Malik Ibrahim Malang, Malang, Indonesia

Abstract

Objective: To determine the antimicrobial effect of zam zam water against Salmonella typhi in vitro. The antimicrobial effect was measured from the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of zamzam water against Salmonella typhi. Design: This experimental study used post-test only control group design with four time repetition. Step one was cultivating bacteria in liquid medium with various concentration of extract, that was 1%, 1.25%, 1.5%, 1.75%, 2% with two control, extract control and bacterial control. Results: The MIC (Minimal Inhibition Concentration) is 1.5% concentration of extract. Step two was plating in NAP (Nutrient Agar Plate) medium. The MBC (Minimal Bactericidal Concentration) is 1.75% concentration of zamzam water. The result of experiment was knew there are different average of Salmonella typhi colony from every group. The result experiment was analyzed by One Way Anova Test. The hypothesis test of MBC show significant differentiation, and then was continued with regression test. The conclusion of this study was zamzam water have antimicrobial activity against Salmonella typhii in vitro.

B138

Prevalence, susceptibility patterns and virulence factors of bacterial isolates from neonate-mother pair samples in Benin City, Nigeria.

Samuel Aziegbemhin

Department of Microbiology, Faculty of Life Sciences, University of Benin , Benin , Nigeria

Abstract

ABSTRACT:

Neonatal sepsis remains a major cause of morbidity and mortality in neonates. The prevalence of bacterial isolates in neonates admitted due to sepsis together with mothers and their susceptibility to routinely used antibacterial agents were investigated. Ethical Approval was obtained from the Hospital Management Board while informed consents were obtained from their parents. Forty-five (45) saliva samples from neonates (with signs and symptoms of sepsis admitted at the neonatal unit of Central Hospital, Benin City, Nigeria) were obtained. Vaginal swab samples were also obtained from their mothers to attain a total of ninety samples comprising forty-five saliva-vaginal swab sample pairs. Samples were immediately transported to the Laboratory and processed using standard microbiological protocols. All samples showed significant bacterial growth. At least one similar bacterial isolate were recovered from each neonate-mother pair. Bacteria isolated include *Staphylococcus aureus*, *Klebsiella oxytoca*, *Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp., *Proteus miriabilis*. *Acinetobacter* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Streptococcus agalaticeae*, *Citrobacter* sp. and *Streptococcus pneumoniae*. Both neonatal and maternal isolates were sensitive to unacin, azithromycin, cefotaxime and cefuroxime. Bacterial isolates also showed varying degrees of resistance to bactericidal action of normal serum. Isolates also produced haemolysin. This study gives important insight to the role of saliva in bacteriological analysis of sepsis and has implications for neonatal survival.

Keywords: Bacterial, neonatal, antibiotics.

B139

Investigating the antimicrobial efficacy of MSCs as a potential novel therapy for *Mycobacterium avium* pulmonary infection

Timothy Shaw, Gunnar Schroeder, Anna Krasnodembskaya, Cecilia O'Kane

Queen's University Belfast, Belfast, United Kingdom

Abstract

Non-tuberculous mycobacterial pulmonary disease (NTM-PD) has rapidly increased in global prevalence over the last two decades. NTM-PD occurs mainly in patients with pre-existing structural lung disease and current treatment strategies are often ineffective and poorly tolerated. Outside of the cystic fibrosis population, the most common NTM isolates are from *Mycobacterium avium* complex (MAC). Mesenchymal stromal cells (MSCs) have potent antimicrobial and immunomodulatory properties including direct microbial killing and enhancement of phagocytic function. Their effect on MAC species is unknown.

Human MSCs were infected with *M. avium* at a multiplicity of infection (MOI) of 2. Human monocyte-derived macrophages (MDMs) were also infected with a clinical isolate of *M. avium* at MOI of 2. After 4 hours, MSCs were added at a ratio of 1 MSC:3 MDMs. After 24 and 74 hours, colony counts were performed on supernatants and cell lysates.

MSCs reduced total bacterial counts of *M. avium* by 24% at 24 hours (from $295x10^3$/ml to $225x10^3$/ml, $p<0.05$) and 40% at 72 hours (from $403x10^3$/ml to $243x10^3$/ml, $p<0.05$). MSCs reduced total bacterial counts of *M. avium* in infected MDMs by >40% (from $381x10^3$/ml to $209x10^3$/ml, $p<0.05$) after 24 hours and >70% after 72 hours (from $1050x10^3$/ml to $314x\ 10^3$/ml, $p<0.05$).

MSCs have modest direct antimicrobial effect against MAC, but potently enhance their killing by macrophages. Mechanistic studies are required to understand the mechanisms of the antimicrobial effect, with the aim of exploiting these therapeutically in pulmonary MAC disease.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B140

Phylogenomic analysis of gastroenteritis-associated *Clostridium perfringens* identifies isogenic strains in multiple outbreaks and novel virulence-related features

Raymond Kiu[1], Shabhonam Caim[1], Derek Pickard[2], Gordon Dougan[2], Anais Painset[3], Craig Swift[3], Alison Mather[1], Corinne Amar[3], Lindsay Hall[1]

[1]Quadram Institute, Norwich, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]Public Health England, London, United Kingdom

Abstract

Background: *Clostridium perfringens* is a major enteric pathogen known to cause gastroenteritis in humans. Although major outbreak cases are frequently reported, to date no Whole Genome Sequencing (WGS) based studies have been performed to understand the genomic epidemiology and virulence gene content of *C. perfringens*-associated outbreak strains.

Methods: We have applied both genomic and phylogenetic analyses on a sub-set of 109 newly-sequenced *C. perfringens* strains isolated from gastroenteritis-associated disease cases (including food-poisoning and care-home diarrhoea) from England and Wales between 2011-2017 to probe virulence profiles including toxin and AMR genes, plasmid features and genomic epidemiology of these case isolates.

Results: Our data identified that highly-similar *C. perfringens* strains were associated with 9 care home-associated individual outbreaks over a 5-year interval, indicating potential common sources linked to these distinct outbreaks. Enterotoxin gene *cpe* was encoded in all but 4 isolates (96.4% type-F strains), and it was further determined that virulence plasmids encoding *cpe* were extensively distributed in the isolates (97% care-home isolates carry pCPF5603 plasmid; 60% food-poisoning isolates carry pCPF4969 plasmid). Further virulence factors, such as β2-toxin, were enriched in these isolates (46.7%). Phage proteins were also commonly identified, with additional analysis indicating phages may contribute to spread of virulence determinants.

Conclusion: This study highlights the genotypic and epidemiological relatedness of a large collection of *C. perfringens* strains isolated from gastroenteritis-associated cases from across the UK and Wales. Key points revealed include the potential circulation of disease-associated strains, and impact of *cpe*-encoding-plasmid disseminations, linked to outbreak cases.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B141

Unraveling the role of *C. difficile* S-layer in infection and disease

Filipa Vaz[1], Gill Wilson[1], Joe Kirk[2], Paula Salgado[3], Rob Fagan[2], Gill Douce[1]

[1]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom. [2]Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom. [3]Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle, United Kingdom

Abstract

Clostridium difficile is a gram-positive spore-forming bacterium. Gut colonization is associated with a wide spectrum of gastrointestinal diseases, ranging from mild to severe, acute to persistent. Bacterial survival and multiplication are essential processes dependent in the maintenance of homeostasis, which is, in turn, inherently dependent on the integrity of the cell wall. Bacteria present different mechanisms to maintain cell wall integrity, particularly, to protect it from direct contact with the external environment, such as phosphate polymers, capsule, outer membrane and S-layer. The S-layer is an evolutionary conserved macromolecule present in almost all bacterial types, however its role(s) remain(s) to be clarified. The S-layer is ubiquitous in *C. difficile*strains and is comprised of a conserved and a variable region that confers strain specificity and faces the external environment.

In *C. difficile* FM2.5 strain, the absence of S-layer resulted in impaired pathogenicity. Gut colonization of FM2.5 infected mice was significantly lower than mice infected with the wild-type strain R20291. In addition, FM2.5 showed compromised toxin activity and *in vitro* motility. Accordingly, FM2.5 failed to cause disease in mice and trigger a strong inflammatory response, contrary to mice infected with R20291. Reversion of the S-layer gene restored motility, toxin activity and the abilities to colonize and cause disease. It appears that the S-layer plays a crucial role in various bacterial processes, hence FM2.5 loss of virulence may be due both to the absence of S-layer at the cell surface and its role in other processes that aid to bacterial virulence.

B142

Intracellular survival of *Enterobacter cloacae* complex in human macrophages

Georgiana Parau, Fabiana Bisaro, Helina Marshall, Miguel A. Valvano

Queen's University Belfast, Belfast, United Kingdom

Abstract

The *Enterobacter cloacae* complex (Ecc) is a group of enteric Gram-negative bacteria that also exist as commensals in nature. Ecc bacteria are also responsible for nosocomial outbreaks, targeting primarily immunocompromised patients and causing a wide range of systemic infections. Infection is often fatal as management is complicated by the high-level multidrug resistance expressed by Ecc isolates. Indeed, the Enterobacter species represent the last "E" of the ESKAPE pathogens, which are the global leading causes of nosocomial infections. Despite the clinical relevance of Ecc, little is known about their virulence-associated properties and pathogenicity. Bridging this gap in knowledge is fundamental to provide a blueprint to better tackle Ecc infections. The aim of this study is to explore how *E. cloacae* interact with human macrophages and identify the bacterial and host cell factors involved in this process. Differentiated, human THP-1 monocytic macrophages were infected with fluorescent *E. cloacae* for various lengths of time. Macrophage infection was analysed by confocal microscopy and images processed on ImageJ and LAS X. Data suggests that approximately 90 % of intracellular *E. cloacae* are killed by 5 hours post-infection, but the remaining 10 % of the bacterial population survives and persists up to 48 hours post-infection. We have also observed *E. cloacae* colocalising with early and late endosomes as well as lysosomes. Our current results suggest that *E. cloacae* can subvert normal phagocytic trafficking and possibly adapt to survive inside the acidic lysosomal environment.

B143

Bovine Tuberculosis in Eastern Ethiopia: Prevalence, Risk Factors and Its Public Health Importance

Jelalu Kemal Birmeka[1], Berhanu Sibhat[1], Aklilu Abraham[2], Yitagele Terefe[1], Ketema Tafess Tulu[3,4], Kiros Welay[5], Nejib Getahun[1]

[1]Haramaya University College of Veterinary Medicine, Dire Dawa, Ethiopia. [2]Haramaya University College of Health and Medical Science, Dire Dawa, Ethiopia. [3]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong SAR, China. [4]Arsi University, Department of Medical Laboratory, College of Health Sciences, Asella, Ethiopia. [5]Haramaya University School of Animal & Range Science, Dire Dawa, Ethiopia

Abstract

Bovine tuberculosis is among the primary zoonotic disease caused by *Mycobacterium bovis*. A cross-sectional study was conducted on 315 cattle in selected areas of eastern Ethiopia, aiming to estimate the occurrence of bovine tuberculosis using comparative intradermal tuberculin skin test and assess cattle owners' awareness on its public health implication. Random sampling method was applied in order to select animals from farm/household. Forty three farm/household owners of tuberculin tested animals were interviewed using pre-tested structured questionnaires. The overall prevalence of bovine tuberculosis was 20.3% (n = 64) in dairy cattle at recommended cut off >4mm. From a total of 43 farms/households tested, 22 were positive; each farm exhibited at least one tuberculin positive reactor animal with a total herd level prevalence of 51.2%. The prevalence of bovine tuberculosis in individual animal level was significantly different ($\chi 2$ = 45.2; P-value < 0.001) in different sites. Farming system, herd size and other risk factors were significantly ($p<0.05$) associated with bovine tuberculosis occurrence. Of the total interviewed farm owners, only 33% had the knowledge of or had heard about bovine tuberculosis and 23% respondents were aware of the zoonotic importance of the disease. More than 50% of the interviewees had shown their preference of raw milk consumption. The study showed bovine tuberculosis is highly prevalent. The majority of cattle owners lack awareness about the disease and its public health significance. Awareness rising about the disease, its transmission and zoonotic implication is of great importance for reduction and control measures.

B144

Occurrence and Evaluation of Antimicrobial Susceptibility of *Staphylococcus aureus* Isolated from Chicken Eggs, Eastern Ethiopia

Jelalu Kemal Birmeka[1], Wakene Beji[2], Gebregeorgis Tesfamariam2[2]

[1]Haramaya University College of Veterinary Medicine, Dire Dawa, Ethiopia. [2]Jigjiga University, Jigjiga, Ethiopia

Abstract

Staphylococcus aureus is responsible for a variety of infections in humans and animals that particularly causes staphylococcal food poisoning when it present in foods. This study was aimed to isolate *Staphylococcus aureus* present on the shell surfaces and in the contents of chicken eggs, and determine antimicrobial susceptibility patterns. A total of 335 egg samples were obtained from open market (n = 174) and poultry farm (n = 161). A sterile cotton swab was used to sample the surface of eggs. After sterilizing the shells, the egg contents were sampled. Isolation of *Staphylococcus aureus* was done based on ISO. The isolates were subjected to antimicrobial susceptibility testing using disc diffusion method. Out of the total 335 eggs sample examined, 93(27.8%) samples yielded *S. aureus*. Out of these, 28(17.4%) were from poultry farm while 65(37.4%) were obtained from open market. Similarly, 63(18.8%) were from the shell while 30(8.9%) were from the content. The level of *S. aureus* in egg contents was significantly higher in the open market (CI=0.0962- 0.6085; p = 0.003). All 76 *S. aureus* isolates were resistant to at least one of the antimicrobials tested with overall 3.9-92.0% level of resistance pattern showing higher resistant to penicillin (92%), and ampicillin (89.5%). Multiple drug resistance was detected in 86.8% of the total *S. aureus* isolates. The study showed high level of *S. aureus* with considerable antimicrobial resistant pattern. Further study is needed to better define bacterial resistance to antimicrobial agents with emphasis of multiple drug resistant.

B145

Selection of Specific Peptides Recognised by Polyclonal Antibody from Salmonella Enteritidis Infected Chicken using Next Generation Phage Display Technology

Ibrahim Naqid[1], Ben Maddison[2], Jonathan Owen[2], Kevin Gough[3]

[1]College of Medicine, The University of Zakho, Kurdistan Region, Iraq, Zakho/ Duhok, Iraq. [2]ADAS UK, School of Veterinary Medicine and Science, The University of Nottingham, Nottingham, United Kingdom. [3] School of Veterinary Medicine and Science University of Nottingham, Nottingham, United Kingdom

Abstract

Salmonella Enteritidis is an important cause of human salmonellosis and food-poisoning associated with consumption of contaminated chicken eggs and poultry products. Objective of the study was to develop a serological diagnostic test for the recognition of different *Salmonella* serovars in chickens. Here, phage peptide libraries were screened against 9 IgY samples from chickens infected with *S.* Enteritidis and 9 IgY samples with *S*. Hadar and the individual peptide binders were then identified using NGS. Twenty-nine peptides were identified *in silico* assessment as being enriched specifically against IgY from multiple chickens infected with *S.* Enteritidis compared to those infected with *S.* Hadar. Twenty Nine peptides identified *in silico* assessment were then tested by both training and test cohorts of chicken IgY samples in ELISAs. The training set of samples was made up of IgY from 9 chickens infected with *S*. Enteritidis and 9 infected with *S*. Hadar. Seventeen peptides were selected as the most recognized specific peptides against *S.* Enteritidis infection and were then used against IgY samples from 10 birds infected with *S*. Enteritids and 20 birds with *S.* Typhimurium as a test cohort. Overall, for both training and test cohorts the peptide ELISA assay sensitivity and specificity were 90% for detecting infections. The most discriminatory peptides by ELISA test were AEGEFEPQSARPS and AEGEFFVNRALINQ. The data demonstrated that the NGPD method could identify peptides that represented serovar-specific epitopes/mimotopes, these peptide have potentially important applications for the development of peptide based immuno-diagnostic assays for the recognition of *Salmonella* Enteritidis in chickens.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B146

Molecular epidemiology and antimicrobial resistance of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* strains isolated in the UK between 2004-2017

Zahra Fraz, David Litt, John Duncan, Ginder Mann, Rachel Pike, Meera Chand, Charlotte Gower, Gayatri Amirthalingam, Norman Fry

Public Health England, National Infection Service, London, United Kingdom

Abstract

The genus *Corynebacterium*includes three potentially toxigenic species, *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*, capable of causing diphtheria, a severe disease in humans. The aims of this study were to undertake Multi-Locus Sequence Typing (MLST) on a panel of both toxigenic and non-toxigenic *C. diphtheriae* and *C. ulcerans* strains (20 toxigenic and 29 non-toxigenic *C. diphtheriae*, 17 toxigenic and 14 non-toxigenic *C. ulcerans*) isolated in the UK between 2004 and 2017, and use these results to determine the molecular epidemiology within the UK. Antibiotic sensitivity testing was also undertaken (20 toxigenic and 88 non-toxigenic *C. diphtheriae*, 17 toxigenic and 14 non-toxigenic *C. ulcerans*) and their profiles compared. The MLST results showed that *C. diphtheriae* and *C. ulcerans* isolates formed two distinct genetic populations and that *C. diphtheriae* isolates with intermediate penicillin resistance demonstrated sequence types which were genetically related. The results also showed that ST32 was most prevalent (31%, 9/29 isolates) amongst non-toxigenic *C. diphtheriae*. Non-toxigenic *C. ulcerans* isolates demonstrating intermediate penicillin resistance formed distinct genetic populations and appeared distantly related or unrelated. There were 75% (15 isolates) of toxigenic *C. diphtheriae* isolates, 35% (6 isolates) of toxigenic *C. ulcerans* isolates, 30% (26 isolates) non-toxigenic *C. diphtheriae* and 43% (6 isolates) of non-toxigenic *C. ulcerans* which demonstrated intermediate penicillin resistance. Linezolid and vancomycin were the only antibiotics which demonstrated 100% sensitive profiles for all isolate groups. These data will help inform public health guidance and management of corynebacteria infections caused by these species.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B147

Antimicrobial resistance in non-O157 Shiga-toxin producing *Escherichia coli*

Amy Gentle, Claire Jenkins, Martin Day, David Greig, Anais Painset, Vivienne Do Nascimento

Public Health England, London, United Kingdom

Abstract

Objectives

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic pathogens that cause severe gastrointestinal disease in humans. Monitoring antimicrobial resistance (AMR) in STEC from symptomatic human cases may provide evidence for the extent of transmission of resistant strains and resistance genes from ruminants to humans. The aim of this study was to assess AMR in non-O157 STEC in England and Wales between 2014 and 2016, and to compare phenotypic and Whole Genome Sequencing (WGS) derived AMR profiles.

Methods

Six hundred and fifty-three non-O157 STEC isolates were analysed. WGS and bioinformatic analysis were performed on 457 isolates in the top 10 Clonal Complexes (CC) (193 were excluded on the basis of CC) and phenotypic susceptibility typing via breakpoint and minimum inhibitory concentration testing was undertaken on 100 isolates exhibiting resistance to at least one antimicrobial.

Results

Of 457 isolates, 332 lacked identifiable resistance genes and were predicted to be fully susceptible to 11 diverse classes of antimicrobials, 125 were found to carry one or more resistance genes and 83 were multi-drug resistant. Four isolates were identified as extended-spectrum b-lactamase-producers. In total, 46 different genes were detected – which conferred resistance to 8 different antibiotic classes. An overall concordance of 97.5% was demonstrated between the two methods.

Conclusions

Phenotypic and genome-derived AMR comparisons showed good correlation for non-O157 STEC. This has added to the evidence base to support the use of genotypic approaches for antimicrobial susceptibility typing, to replace phenotypic typing for surveillance purposes, and guide clinical decision making in the more distant future.

B148

Augmenting Staphylococcal infection: the importance of timing

Oliver Carnell, Joshua Sutton, Simon Foster

University of Sheffield, Sheffield, United Kingdom

Abstract

Staphylococcus aureus (*S. aureus*) acts as a commensal in the microbiome of the skin and nasopharynx. However, on gaining access to the bloodstream it can cause an array of pathogenic outcomes. *S. aureus* can crowdsource the microflora to assist in becoming an opportunistic pathogen as our lab has recently published findings that co-inoculation of *S. aureus* with commensals, acting as pro-infectious agents, leads to a much more robust, virulent infection. This benefits *S. aureus* at doses where it would otherwise be cleared by the immune system. Pro-infectious agents do not need to be live commensals as isolated cell wall peptidoglycan also augments infection. This work aimed to assess the effects of inoculation with pro-infectious agents before and after infection with *S. aureus*. It was found that pro-infectious agents needed to be co-administered in order to fully augment infection. This gives mechanistic insight where *S. aureus* and the pro-infectious agents need to be in the same local environment or phagocyte to augment infection.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B149

Clinical evaluation of the novel Molecular bacterial load assay for real-time monitoring of tuberculosis treatment response

Bariki Mtafya[1,2], Wilber Sabiiti[2], Issa Sabi[1], Yahya Msuya[3], Nyanda Ntinginya[1]

[1]NIMR-Mbeya Medical Research Centre, Mbeya, Tanzania, United Republic of. [2]University of St.Andrews, St.Andrews, United Kingdom. [3]Mbeya Regional TB and Leprosy Control Programme, Mbeya, Tanzania, United Republic of

Abstract

Background

Monitoring the treatment of tuberculosis (TB) relies on less sensitive smear microscopy (SM) and culture methods which are very slow.We evaluated the novel Molecular bacterial load assay (MBLA) for implimentability and real-time monitoring of TB treatment in a clinical setting.

Methods

Therapy naive (Xpert MTB/RIF confirmed) TB positive patients were enrolled in Mbeya, Tanzania. Sputum samples were collected at baseline and thereafter at week 2, month 2, 5 and 6 of treatment. Samples were analysed for *M.tuberculosis (M.tb)* by MBLA and compared to SM, culture and clinical monitoring.

Results

59 TB patients were enrolled for the study. Median age, 37 (18-65) years, 62.7% (37/59) male, 45.6% (27/59) HIV positive and 8.47% (5/59) were re-treatment. Mean BL (±SD) at baseline was 5.48±1.3 declining to 3.42±0.7 at month 2 and 3.51± 0.62 $\log_{10}$CFU/ml at month 6 of treatment. This corresponds MBLA positivity of 92.98%, 65.5% and 7.84% at baseline, month 2 and 6 respectively. In contrast, positivity of SM and culture were 78.95%, 9.62% and 0%, and 85.96%, 25% and 3.39% at baseline, month 2 and 6 respectively. Decline in test positivity reflected resolution of clinical signs. While night sweat, and chest pain resolved earlier on in treatment, resolution of cough was slow and consistent with MBLA. Furthermore, the turn-around-time for MBLA results was 24h compared to median (range) of 14.83 (4.33-42) days for liquid culture.

Conclusion

MBLA exhibited higher sensitivity and shorter turn-around-time than standard tests and clinical signs. This demonstrates the potential of MBLA to offer real-time results for clinical decision making.

B150

Mapping 4D pH Evolution In Streptococcus mutans Biofilms Using Fluorescent Ratiometric pH-Sensitive Nanosensors

Mark Perkins, Birte Blunk, Dean Walsh, Veeren Chauhan, Jonathan Aylott, Kim Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

Streptococcus mutans is a pre-dominant bacterial species found in oral biofilms and participates in the production of dental caries via the generation of organic acids. The production of these acids results from the fermentation of carbohydrates present in a sugar-laden diet. As the acidity of an oral biofilm decreases, the demineralisation of the enamel of a tooth increases; leading to the formation of dental caries. To detect and measure the pH change occurring following a sugar challenge, ratiometric, fluorescent, pH-sensitive nanosensors were incorporated into oral biofilms.

Confocal laser scanning microscopy revealed that the addition of glucose (1%w/v) to an *S. mutans* biofilm resulted in a gradual reduction in the fluorescence intensity ratio during a 30 minute period. This reduction in the fluorescence intensity ratio indicated a reduction in pH of the biofilm over time as the glucose was being fermented, resulting in the production and secretion of acids into the extracellular matrix of the biofilm.

Additionally, a reduction in pH was detected - using widefield microscopy - in starved, planktonic *S. mutans* when treated with glucose. Over the course of 30 minutes, the pH of the medium was reduced from pH 5.3 to pH 3.3 as the glucose was fermented by the bacteria.

These findings will help us map pH changes in oral biofilms as we examine potential methods of preventing the acidification of oral biofilms and the eventual demineralisation of the enamel; leading to the reduction in dental caries and an improvement in the standard of living of those effected.

B151

The O6 antigen of *Escherichia coli* strain CFT073 is a target for *Myoviridae*.

Sinead Moynihan, Stephen Smith

Trinity College Dublin, Dublin, Ireland

Abstract

Extraintestinal pathogenic *Escherichia coli* strain CFT073 is a prototypic urosepsis isolate. CFT073 is of serotype O6:K2:H1 and thus has an O-antigen. The *wzy* gene encodes the O-antigen polymerase Wzy, which catalyses the polymerisation of O subunits into a long chain polysaccharide. A CFT073 Δ*wzy* mutant was constructed using the λ Red recombination system. The lack of O-antigen was confirmed by LPS purification and staining with Pro-Q Emerald 300.

CFT073 Δ*wzy* and wild-type CFT073 were tested for their bacteriophage sensitivity. ΦEB49, a member of the *Myoviridae*, can mediate generalised transduction in strain CFT073. CFT073 Δ*wzy* and wild-type CFT073 mixed with the same concentrations of ΦEB49 phage were compared. Confluent growth was evident on all CFT073 Δ*wzy* plates, whilst lysis was apparent on all CFT073 plates. The inability of ΦEB49 to lyse CFT073 Δ*wzy* suggested that this phage binds to O antigen, hence the decreased O antigen of CFT073 Δ*wzy*, compared to wild-type CFT073, prevented phage lysis from occurring. These data indicate that CFT073 Δ*wzy* is not a susceptible host for ΦEB49.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B152

A micro luminescence-based assay to measure serum susceptibility in *Escherichia coli*.

Aileen Hartnett[1,2], Stephen Smith[1,2], Susan Knowles[3], Sara Lydon[3]

[1]Trinity College Dublin, Dublin, Ireland. [2]The National Children's Research Centre, Dublin, Ireland. [3]National Maternity Hospital Holles Street, Dublin, Ireland

Abstract

Neonatal meningitis *Escherichia coli* (NMEC) is the leading cause of gram-negative meningitis in neonates, and it contributes to neonatal morbidity and mortality globally. The prototypic strain of NMEC, *E.coli* RS218 possesses the K1 capsule and has been widely employed in the study of NMEC pathogenesis. Previously, our laboratory has utilised relatively large volumes of culture to assay serum bactericidal activities, to garner valuable insights into bacterial immune evasion strategies. However, these methods can be labour intensive, time consuming and are not easily adaptable for high throughput applications.

To overcome these limitations, a smaller volume real-time assay was developed. Bacteria were cultured in 100µl volumes and were sub-inoculated for logarithmic growth. A slow kinetic absorbance assay was established on the Optima Fluorostar microplate reader, which enabled the accurate and reliable measurement of bacterial growth. Subsequently, bioluminescence was incorporated into the assay to facilitate the measurement of bacterial viability in real-time. Bacteria were rendered bioluminescent via electroporation of the pIlux plasmid or alternatively by the addition of exogenous beetle luciferin and recombinant firefly luciferase. The utility of these approaches in the determination of bacterial complement evasion is reported below.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B153

Murein from murine: The role of *Staphylococcus aureus* cell wall structure during host-pathogen interaction

Joshua Sutton[1], Oliver Carnell[1], Waldemar Vollmer[2], Simon Foster[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University of Newcastle, Newcastle, United Kingdom

Abstract

The cell wall of bacteria is essential for viability, with peptidoglycan (murein) being the major structural component. Peptidoglycan synthesis is the target of the most important group of antibiotics; the β-lactams. *Staphylococcus aureus*, commonly known by its antibiotic resistant name methicillin resistant *Staphylococcus aureus* (MRSA), is a bacterium that highlights the growing concern of antibiotic resistance, in particular against the β-lactams.

Despite its crucial role, peptidoglycan structure and dynamics during infection are poorly understood. My research uses a combination of *in vivo* infection models and biochemical approaches, to determine the role of this crucial cell wall polymer in infection.

Bacterial growth requires both peptidoglycan synthesis and hydrolysis. *S. aureus* has multiple hydrolases, including four glucosaminidases (*atl, sagA, sagB* and *scaH*) that are required for growth and division. The major enzyme SagB alone is important in infection using both zebrafish and murine models. Conversely the other three enzymes show redundancy, where only a triple *atl sagA scaH* mutant shows attenuation. I am currently dissecting the molecular role of the enzymes in disease.

To correlate my *in vitro* peptidoglycan structural work with what happens during pathogenesis, I have developed a method to isolate material during murine infection. I have found that *in vivo* derived peptidoglycan has reduced cross-linking. Peptidoglycan cross-linking is governed by penicllin binding protein 4 (PBP4), and interestingly a pbp4 mutant, which demonstrates reduced cross-linking, leads to an increased number of bacteria in the livers of infected mice. This suggests a complex role of peptidoglycan structure and dynamics during infection.

B154

Simultaneous control of *Staphylococcus aureus* and *Bacillus cereus* using fusion protein containing two endolysins

Bokyung Son, Sangryeol Ryu

Seoul National University, Seoul, Korea, Republic of

Abstract

As the incidence of antibiotic-resistant bacteria is increasing, phage endolysins have been attracted attention as promising alternatives to antibiotics. The modular structure of the endolysin facilitates the endolysin engineering to develop novel endolysins with enhanced versatility. Here, we constructed fusion proteins consisting of two different endolysins for simultaneous control of *Staphylococcus aureus* and *Bacillus cereus,* which are considered as important pathogens regarding their frequency and seriousness of the infectious disease. The full-length or C-terminally truncated (LysB4EAD) LysB4, an endolysin from *B. cereus*-infecting phage B4, was fused to LysSA11, an endolysin of *S. aureus*-infecting phage SA11, via a helical linker in both orientations. The fusion proteins maintained the lytic activity of their parental endolysins against both *S. aureus* and *B. cereus*, showing successfully extended antimicrobial spectrum. Among them, LysB4EAD-LysSA11 showed significantly increased thermal stability compared with its parental endolysins and was selected for further study. LysB4EAD-LysSA11 exhibited high lytic activity at pH 8.0-9.0 against *S. aureus* and at pH 5.0-10.0 against *B. cereus*, but the lytic activity of the protein was decreased in the presence of 50 mM NaCl. LysB4EAD-LysSA11 could be a potent antimicrobial agent for simultaneous control of multiple pathogenic bacteria and this study will be helpful to design highly specific but multifunctional antimicrobials.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B155

Intracellular replication of pneumococcus in ex vivo-perfused human spleens

Joseph Wanford[1], David Carreno[1], Wen Chung[2], Luisa Martinez-Pomares[3], Ashley Dennison[2], Peter Andrew[1], Richard Moxon[4], Marco Oggioni[1,2]

[1]University of Leicester, Leicester, United Kingdom. [2]University Hospitals Leicester, Leicester, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

Recently, we have demonstrated that the pathogenesis of *Streptococcus pneumoniae* bacteraemia contains a concomitant phase of intracellular replication within splenic macrophages in mice (Ercoli *et al.* NatMicro. 2018). In the present study, we aimed to determine if intracellular replication of pneumococci may play a role in the pathogenesis of sepsis in humans, using two innovative approaches.

We used a model (Chung *et al.* ALTREX. 2018) involving *ex vivo* perfusion of human spleens from elective splenectomy patients (REC reference: 18/EM/0057). Organs were infected with 6.5×10^7 CFU of pneumococci, and serial biopsies and 'blood' samples were taken at predetermined times. Samples were analysed by colony counts, confocal microscopy and flow cytometry. Additionally, infected tissue samples were taken for preparation of organotypic slice culture time courses.

Bacteria injected into the perfusion circuit were rapidly cleared at early time points post-infection, recapitulating what is observed in experimental murine sepsis. Bacterial counts in the spleen increased, providing initial evidence of intracellular bacterial persistence. Microscopy analysis indicated that bacteria could be localised to splenic macrophages, with the size of infectious foci increased over time. Z-stack microscopy localised bacteria within cell membranes, indicating the infection was predominantly intracellular. In *ex vivo* slice cultures increasingly large numbers of pneumococci were cultured over time, further indicating intracellular replication.

In conclusion, we provide evidence for a role of intracellular replication of pneumococci in human splenic macrophages in the pathogenesis of sepsis.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B156

Characterization of a bacteriophage from avian Staphylococcus aureus associated with innate immune evasion

Emily Parr[1], Emily Richardson[2], David Hume[3], J.Ross Fitzgerald[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]MicrobesNG, Birmingham, United Kingdom. [3]University of Queensland, Brisbane, Australia

Abstract

Staphylococcus aureus is an important human and livestock pathogen. An *S. aureus* phage (ϕAvβ) inserted into the chromosome at the beta toxin gene (beta-converting phage) is present in approx. 90% of human strains and contributes to human-specific innate immune evasion. Comparative genomic analysis of *S. aureus* isolates from infected poultry has revealed an avian-specific subfamily of beta-converting phages represented by multiple variants with distinct integrase gene alleles. To investigate the role of the avian beta-converting phages in host-pathogen interactions, a ϕAvβ-deficient strain with non-functional beta-toxin and a ϕAvβ-deficient strain with restored beta-toxin were constructed by allele replacement in an avian pathogenic *S. aureus* strain. Compared to the wild type, both ϕAvβ-deficient strains have reduced net extracellular growth *in vitro* in chicken bone-marrow derived macrophages. Further investigation using GFP-tagged bacteria has revealed that both ϕAvβ-deficient strains show reduced initial phagocytosis and intracellular survival compared to the wild type. Absence of ϕAvβ is also associated with decreased killing of the chicken bone-marrow derived macrophages. We are currently investigating the mechanism underlying this phenotype using deletion mutants of the candidate phage effector genes. Ongoing work also involves using RNAseq to investigate differential host transcriptional response of the macrophages to *S. aureus* in presence/absence of ϕAvβ. Overall, these data will contribute new information relating to the evolution of avian *S. aureus* and mechanisms of bacterial host-adaptation.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B157

The Placental Microbiota in Chorioamnionitis: Increased and varied microbiota in placental membranes with chorioamnionitis

Rochelle Hockney[1], Gareth Waring[2], Caroline Orr[1], Gillian Taylor[1], Stephen Cummings[1], Stephen Robson[2], Andrew Nelson[3]

[1]Teesside University, Middlesbrough, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom. [3]Northumbria University, Newcastle, United Kingdom

Abstract

Chorioamnionitis is inflammation of the foetal compartment of the placental membranes, with links to adverse maternal and neonatal outcomes. The detection of a genuine placental membrane microbiome, plus the microbial composition of foetal membranes with chorioamnionitis remains inconclusive. Thus, the aim of this research is to determine if there is a distinct foetal membrane microbiota associated with chorioamnionitis.

Frozen placental membranes (amnion and chorion) from preterm labour with lowgrade histological chorioamnionitis (n=12) were analysed alongside preterm (n=6) and term labour without chorioamnionitis (n=6). 16S rRNA gene targeted Next Generation Sequencing via Illumina MiSeq was used to determine microbial communities of genomic DNA extracted from placental membranes, with BactQuant 16S qPCR used to assess bacterial load.

Differences in relative abundance were highlighted between chorioamnionitis compared to preterm (p=0.018) and term birth (p=0.035). No significant difference in alpha diversity of specific bacterium was detected between chorioamnionitis and preterm. *Prevotella* and *Ureaplasma* were increased in chorioamnionitis, with a decrease in *Lactobacillus* compared to preterm and term membranes, yet insignificant between groups. 16s qPCR detected a greater bacterial load in chorioamnionitis chorion (7650.977 copies/µl) and amnion (4007.422 copies/µl) compared to preterm and term (<200 copies/µl), with significant differences between chorioamnionitis and preterm amnion (p=0.020), preterm chorion (p=0.042) and term chorion (p=0.025).

Findings indicate the importance of bacterial load in the development of chorioamnionitis, rejecting the theory of a sterile placenta with chorioamnionitis. Further investigation into the potential for bacterium to generate an inflammatory response leading to chorioamnionitis is to be investigated.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B158

Improved molecular typing of toxigenic *Clostridium difficile* strains affecting animal and human health

Madelyn Nowak-Roddy[1], James McKenna[2], Derek Fairley[3,4]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Department of Microbiology/ Regional Virus Laboratory, Belfast Health & Social Care Trust, Belfast , United Kingdom. [3]Department of Microbiology/ Regional Virus Laboratory, Belfast Health & Social Care Trust, Belfast, United Kingdom. [4]3. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast , United Kingdom

Abstract

Clostridium difficile is a Gram-positive, spore forming bacterium, which remains a formidable pathogen as the etiological agent of *C. difficile* infection (CDI). Substantial effort goes into diagnosis of CDI and characterisation of circulating toxigenic *C. difficile* strains for epidemiology and infection prevention and control. Currently, molecular typing of *C. difficile* requires 9 days following diagnosis through PCR ribotyping and multilocus variable number tandem repeat (VNTR) analysis. There is a need for more rapid typing methods to investigate possible linkage between CDI cases in healthcare settings.

This study developed a one-step, closed tube real-time PCR and high resolution melt (HRM) assay targeting the intergenic spacer region (ISR) and several VNTR loci, with results generated in 2.5 hours. The discriminatory power of the PCR-HRM assay was investigated by typing previously characterised toxigenic clinical and animal *C. difficile* isolates (n=90). Through comparison of HRM profiles targeting the ISR of isolates belonging to 17 PCR ribotypes, 13 HRM genotypes were recognised with 11 PCR ribotypes resolved from each other. Using correlation between HRM data and known VNTR repeat numbers at the B7, C6, and G8 loci, VNTR repeat numbers for isolates could be predicted within an average absolute difference of 1.8 at the B7 locus, 2.1 at the C6 locus, and 2.5 at the G8 locus. These results suggest that a PCR-HRM assay with a multilocus panel targeting ISR and selected VNTR loci could form part of an improved molecular typing scheme for toxigenic *C. difficile* strains that is faster than currently available methods.

B159

Detection and quantification of *Staphylococcus aureus* heterogeneity to identify antibiotic-induced persistence

Marwa Hassan[1], Mark Butler[1], Andrea Ranzoni[1,2], Matthew Cooper[1]

[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia. [2]Bioengineering Department, Ellume Pty Ltd, Brisbane, Australia

Abstract

Persister cells are characterised as being viable but non-culturable, a state that preserves their metabolic energy to survive the environmental stress, which allows for recurrent infections. Detection of persisters is, therefore, not possible with standard culture-dependent methods. Furthermore, the effect of antibiotics on the induction of persisters has not been assessed. This study aimed to identify antibiotic-induced persistence and determine the percentage of heterogeneity. Vancomycin, daptomycin and dalbavancin were assessed by standard MIC methods against selected Staphylococcus aureus strains. Replicates of MIC assays were stained with propidium iodide to quantify live/dead and a reactive oxygen species (ROS) dye to detect and quantify persisters using culture-independent single-cell sorting, independently. A comparative analysis was then performed. Dalbavancin showed the lowest MIC values against tested *S. aureus* strains followed by daptomycin and vancomycin. Cell sorting of vancomycin-, daptomycin- and dalbavancin-treated *S. aureus* strains showed a range of 1.9-10.2%, 17.7-62.9% and 7.5-77.6% live cells based on the strain, respectively, in which daptomycin, in particular, was a strong inducer of a persister population. Persisters represented 3.7-16% of the bacterial population. The culture-independent identification of antibiotic-induced persistence through studying at the single-cell level showed different efficacy of antibiotics than standard MIC. Vancomycin was the most effective antibiotic against tested strains followed by dalbavancin then daptomycin as assessed by cell sorting. Therefore, re-evaluation of standard MIC methods may be required to assess the efficacy of antibiotics. Additionally, the detection of daptomycin-associated persisters may provide an elucidation to the reported rapid resistance development *in vivo*.

B160

In vitro antimicrobial efficacy of *Cnidoscolus aconitifolius* leaf extract and honey on *Staphylococcus epidermidis* clinical isolate

Hanson Ogbu, Emeka Igboanusi, Nkechi Ezenobi

The University of Port Harcourt, Port Harcourt, Nigeria

Abstract

The search for alternative, potent, cost-effective treatment of ailments caused by resistant microorganisms and the role of plants and their products as essential sources of medicinal agents is receiving increasing attention. Several of these natural products are reported to have capacity to produce natural compounds of high structural diversity that serve as defense agents against invading microorganisms. *Cnidoscolus aconitifolius* also known as tree spinach is an indigenous tropical tree that has gained lot of importance in its nutritive value and traditional use. This study evaluates the possible effect of *Cnidoscolus aconitifolius* leaf extract alone, extract/honey combination on *Staphylococcus epidermidis* clinical isolate. Phytochemical analysis and antimicrobial efficacy of the extract were performed using standard methods. The results of phytochemical analysis reveal the presence of carbohydrate, tannins, alkaloids, steroids, flavonoids, anthraquinone, saponins and carotenoids. Antimicrobial activities showed inhibition zone values of 8.0 ± 0.1 mm for aqueous extract alone, 9.0 ± 0.1 mm for aqueous extract/honey combination. The finding suggests that *C. aconitifolius* might be a good source of compounds that can be used to inhibit the growth of *Staphylococcus epidermidis* pathogen and further supports its popular and wide traditional applications in the treatment of various illnesses. Hence the need for further research to exploit the full potential of *C. aconitifolius* tree in order to influence their extensive consumption, storage, improvement and production.

B161

Focussing on resistance to front-line drugs is the most effective way to combat the antimicrobial resistance crisis

Meghan Perry[1,2], Deirdre McClean[1], Camille Simonet[1], Mark Woolhouse[1], Luke McNally[1]

[1]University of Edinburgh , Edinburgh, United Kingdom. [2]NHS Lothian, Edinburgh, United Kingdom

Abstract

Background: In medical, scientific and political arenas relating to antimicrobial resistance (AMR) there is currently an intense focus on multi-drug resistant pathogens. We question the current emphasis of attention on resistance to last-line antimicrobials, arguing that tackling resistance to front-line antimicrobials has a greater public health benefit.

Methods & Results:

Using AMR monitoring data on 25 drug-pathogen combinations from across Europe, here we show that the presence of front-line pathogen resistance initiates a cascade of resistance selection that ultimately leads to pathogen resistance to last-line antimicrobials.

We then interrogate, by modelling dynamics of resistance evolution with a susceptible-infected model, whether 3 key interventions in the response to AMR are more effectively targeted at front-line or last-line treatment. Interventions that make front-line therapy more effective by use of antimicrobial adjuvants or front-line resistance diagnostics or by introduction of a novel, front-line antimicrobial all lead to larger reductions in mortality and morbidity than the same interventions implemented in last-line therapy.

By combining data for 13 antimicrobial classes on worldwide antimicrobial usage with six different measures of research focus we demonstrate that funding, publications, and attention to those publications do not reflect the importance of front-line antimicrobials and are disproportionately devoted to last-line antimicrobials that account for less than 10% of antimicrobial prescriptions.

Conclusions:

While studying resistance to last-line drugs is undoubtedly important, our work relays a strong message to public health agencies, funding bodies, and researchers that allocating resources to front-line infections can be a more effective way to combat the antimicrobial resistance crisis.

B162

Novel Antimicrobial Agents for Clinical Applications

Nyasha Allen, Daniel P Mulvihill, Jennifer R Hiscock

University of Kent, Canterbury, United Kingdom

Abstract

Since their discovery, antimicrobial compounds have been vital for the treatment and prevention of disease; making many previously fatal diseases treatable or at worst, manageable conditions. The inappropriate use of these compounds has led to the rapid development of resistance mechanisms within bacteria to the majority of compounds currently marketed. A recent UK governmental review predicted that by 2050 global deaths caused by antimicrobial resistant bacteria will outnumber those attributed to cancer [1].

As new resistance mechanisms emerge and resistance within microbial populations increases, so does the need to further understand the molecular basis of resistance, develop new antimicrobial molecules and use better strategies to manage their use [2]. In response to this, we discovered a novel class of antimicrobials and have created 50 structurally related members of this class [3-6]. We sought to understand the structure-activity relationships which will result in the determination of the mode of action of these molecules. Consequently, each variant was screened against *Staphylococcus aureus* and *Escherichia coli* and the minimum inhibitory concentration was calculated for effective compounds.

This will enable us to identify predictive tools that will aid the synthesis of the next generation of these novel therapeutic molecules. We will present our latest findings in the ongoing analysis of the antimicrobial activity for each variant of this new class of antimicrobial compound. In addition, we will discuss the insights provided by the detailed structure-function analysis.

This project is in collaboration with Public Health England and NHS East Kent Trust.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B163

Prevalence of Extended Spectrum Beta-lactamase producing Enterobacteriaceae in urine samples from Thumbay hospitals, U.A.E

NAZEERULLAH RAHAMATHULLAH, Sajith Khan Ahamed Khan, Zahra Arshad Khan, Amina Farrukh, Hamdallah M bashir, Asmau Ahmad

Gulf Medical University, Ajman, UAE

Abstract

Abstract

Beta Lactamases is proven to be one of the leading cause of resistance to β-lactam antibiotics among gram-negative bacteria. Many up to date researches have shown increase in the incidence and prevalence of ESBL worldwide.

This study aimed to determine the prevalence of ESBL strains of *Klebsiella* spp. and *Escherichia coli* species in urinary isolates from the patients admitted in Thumbay hospitals around United Arab Emirates. Furthermore, drug resistant genes (SHV and CTX-M) in the ESBL positive samples were detected. 237 urine samples were collected from November 2017 to January 2018. Based on the lactose utilization, colony morphology, and biochemical utilization of the gram negative bacilli were identified as *E. coli* (53)*, Klebsiella pneumoniae* (10)and *Citrobacter* species (2). Antibiotic sensitivity test, double disc diffusion test and combination disc tests all confirmed that the 65 (27.4%) out of 237 isolates were ESBL producing bacteria. There was high prevalence of bacteria in females than male and the number of *E. coli* strains is higher than *Klebsiella* spp.

DNA isolation was performed on the 65 samples, out of which 50 samples were selected for PCR based on their concentration. The selected DNA samples were used to detect the presence of *bla* $_{CTX-M}$ and *bla* $_{SHV}$ genes. Only 24 DNA samples (48%) contains *bla*$_{CTX-M}$ genes, *bla* $_{SHV}$ or both the genes. 14 samples had *bla* $_{CTX-M}$ gene, 2 *bla* $_{SHV}$ genes, and 8 with both *bla* $_{SHV}$ and *bla* $_{CTX-M}$.

At the rate at which ESBL is spreading, further research, close observation and cautious use of antibiotics is important.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B164

Harnessing Novel Bacterial Peptides for Antimicrobial Activity in the Gut Microbiome.

Amy Sterling, James Dooley, Nigel Ternan, Patrick Naughton

University of Ulster, Coleraine, United Kingdom

Abstract

The Enterococci are a resilient collection of species, found in the human intestine, river sediment and even certain cheeses. Human infection by this genus is dominated by *E. faecalis* and *E. faecium*. Vancomycin resistant enterococci (VRE) are associated with higher mortality rates over non-VRE strains. Enterococci can utilise the highly efficient pheromone responsive plasmid (PRP) system to transfer plasmid DNA between cells. Plasmid containing donor cells respond to small peptide pheromones (7-8 amino acids) and transfer plasmid DNA to pheromone-producing plasmid-free recipient cells. PRP can encode antibiotic resistance (including vancomycin) and virulence enhancing factors.

Investigation into the PRP system between donor and recipient *E. faecalis* environmental isolates has indicated a 40% decrease in PRP transfer in colder environments. Additionally, PRP efficiencies under other conditions, including in presence of synthetic pheromone peptides, have been calculated. Future assays will utilise pheromone imitative fluorescently labelled synthetic peptides to visualise the pheromone binding receptor (PrgZ) on the *E. faecalis* donor cell membrane. Later experiments will focus on varying the synthetic pheromone amino acid composition so to interfere with the PRP system machinery, with the aim of reducing PRP transfer efficiency or preventing PRP transfer completely.

B165

Application of Furanone Compounds for the Modulation of Biofilm Formation in Common Wound Pathogens.

Chris Proctor, Nigel Ternan, Paul McCarron

Ulster University, Coleraine, United Kingdom

Abstract

Chronic wounds are a significant issue in healthcare, presenting a considerable economic burden to the NHS and a serious health risk to patients. The majority of non-healing wounds have been shown to contain a biofilm which prolongs the inflammatory stage of wound healing and significantly delays wound healing. This often causes a normal wound to progress and become chronic, presenting further problems for patients including increased risk of secondary infection, further deterioration of the wound and an increase in treatment intensity.

This project aims to assess the efficacy of several compounds in modulating the formation of biofilms in a number of clinically relevant pathogens when used at sub-inhibitory concentrations. The organisms used in this project include *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We aim to test the efficacy of three plant derived compounds including 4-hydroxy-2,5-dimethyl-3(2H) furanone (HDMF), 2-methyltetrahydrofuran-3-one (MTHF) and L-ascorbic acid. Future work will characterise the efficacy of these compounds when delivered to a biofilm from a hydrogel based delivery system.

At sub-inhibitory concentrations in pure solution, candidate molecules tested to date showed no ability to reduce biofilm formation. Indeed, treatment with HDMF resulted in greater production of biofilm in *P. aeruginosa* and treatment with all compounds showed no difference in biofilm formation by *S. aureus.* To characterise the impact of hydrogel based delivery on compound efficacy all candidate molecules were loaded into a hydrogel and shown to be effectively released from it. Experiments to characterise the modulatory potential of these compounds when released from a hydrogel are currently underway.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B166

The effect of local release antibiotic beads on *in-vitro* bacterial growth from tissue taken from infected diabetic foot ulcers.

Julie Fletcher[1], Robert Porter[2], Sean Aiken[3], Craig Delury[3], Stephen Michell[1]

[1]University of Exeter, Exeter, United Kingdom. [2]Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom. [3]Biocomposites Ltd., Keele, United Kingdom

Abstract

Diabetic foot infection is the main reason for diabetes-related hospitalisation and is a major cause of diabetes-related amputation. Recent figures published by Public Health England show that there are more than 163 diabetes related amputations in England every week. This study investigates the effect of antibiotic loaded calcium sulfate (Stimulan® Rapid Cure) beads on *in-vitro* bacterial growth from tissue taken from diabetic foot infections. Patients were recruited from the Macleod Diabetes and Endocrine Centre at the Royal Devon and Exeter Hospital. Inclusion in the study was based on clinical recognition of an infected foot ulcer requiring wound debridement. Debrided tissue was homogenised and 50 µl spread over the surface of Columbia blood agar and fastidious anaerobe agar. Three replicate calcium sulfate beads containing a combination of vancomycin and gentamicin were then placed on the surface of the agar. Each bead contained approximately 3.4 mg and 1.6 mg of vancomycin and gentamicin respectively. Plates were incubated aerobically or anaerobically as appropriate. Zones of inhibition were recorded at 1 and 4 days. Calcium sulfate beads containing vancomycin and gentamicin were able to inhibit bacterial growth in all tissue homogenates tested with zone diameters ranging from 16 – 40 mm. Local release of antibiotics could have the benefit of achieving high local concentrations within poorly vascularised tissue which may inhibit bacterial growth at the wound site. By improving treatment of diabetic foot infections, it may be possible to prevent amputation, maintain mobility and conserve quality of life.

B167

Epidemiological characteristics of the Manchurian plague pandemic of 1910-1911

Xiangfeng DOU, Meng CHEN, Shuang LI, Yanwei CHEN, Xinghuo PANG, Quanyi WANG

Beijing Center for Disease Prevention and Control, Beijing, China

Abstract

Objective: To investigate the epidemiological characteristics of the Manchurian plague pandemic of 1910-1911 with descriptive epidemiological methods. **Methods** the epidemiological data were distracted from *Report of Manchurian Plague*, which is a summary of local official reports about plague in 1910-1911. The time distribution by day, and the space distribution by country was recorded, and also the source of infection.**Results** The pandemic of plague continued from October 25th, 1910 to April 18th, 1911. There were 46747 dead cases on record in the three provinces of Manchuria. There were 24867 dead cases and 7068 dead cases were reported, and the average mortality rates were 41.21 and 10.25 per ten thousands in Jilin and Liaoning province, respectively. In Heilongjiang province, 14812 dead cases of plague were reported. The huge difference was found in different epidemic regions, the highest mortality rate was 4121 per ten thousands in Binjiang country of Jilin province. Patient zero of pneumonic plague had been infected in Russia and got sick and died in Manzhouli, a northern country in Heilongjiang province. Then the pneumonic plague was mainly spread through railway to other cities. **Conclusion** The epidemiological characteristics of the Manchurian plague pandemic of 1910-1911 were first described with modern epidemiological methods.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B168

Detection and characterisation of bacteria causing lung infection in people with Cystic Fibrosis (CF) by surface-enhanced Raman spectroscopy (SERS)

Danielle Allen[1,2], Jessica Kelly[2], Deirdre Gilpin[1], Steven Bell[2], Michael Tunney[1]

[1]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom. [2]School of Chemistry and Chemical Engineering, Queen's University Belfast, Belfast, United Kingdom

Abstract

Rapid and accurate identification of pathogens in CF could ensure prompt treatment with the most appropriate antibiotic; potentially improving outcomes and shortening hospital stays. As traditional culture methods for detecting bacteria are time-consuming there is a growing interest in SERS as a novel culture-free technique that produces a whole-organism spectroscopic fingerprint at high speed.

Bacterial isolates including *Pseudomonas aeruginosa* (n=32), *Staphylococcus aureus* (n=5), *Streptococcus pneumonia*e (n=5) were incubated for 6 hours at 37°C/180rpm, with a starting optical density (OD) of 0.15. After adjustment of the OD to 0.3, bacterial cells were harvested by centrifugation at 9,000rcf for 3 minutes and washed three times with dH_2O. Bacterial pellets were mixed with citrate reduced silver colloid (CRSC) and dried. Spectra were recorded (4x10 seconds at 785nm) and analysed within GRAMS/Al using Principal Component Analysis (PCA).

Spectra of *P. aeruginosa* isolates (n=32) were separated into two distinct groups; the spectra of one group (n=12) was dominated by the pigment pyocyanin with vibrational brands present at 1350, 1492, 1598 and 1615cm^{-1}. The other group (n=20) had characteristic vibrational bands at 661, 735 and 800cm^{-1} which correspond to guanine, adenine and uracil, respectively. *S. aureus* has a main characteristic band at 735cm^{-1} and *S. pneumoniae* has a characteristic band present at 480cm^{-1}. Bacterial species clustered separately when analysed by PCA.

Reproducible and distinguishable SERS spectra of bacterial isolates were obtained, and it was possible to differentiate between different bacterial species using PCA. These results suggest SERS has the potential to rapidly detect bacteria.

B169

Difficulties in Diagnosis and Treatment of Urinary Tract Infections in an Elderly Population.

Varun Anand Misra, David Oliver Hamilton, Chinari Subudhi

Royal Bolton Hospital, Greater Manchester, United Kingdom

Abstract

Background: Urinary tract infection (UTI) contributes significantly to healthcare burden, accounting for 23% of hospital acquired infections and 2-3% of general practice consultations. Unfortunately, difficulties exist in obtaining an accurate diagnosis, with studies showing misdiagnosis rates above 40% in elderly populations. Furthermore, numerous hospitals across the UK still advocate the use of Trimethoprim for UTI, despite high rates of resistance. These factors combined leads to a sub-optimal experience for patients.

Aim: We aimed to identify the practices surrounding the diagnosis and treatment of UTIs in elderly patients within Royal Bolton Hospital, a large district general hospital in the North-West of England. We also aimed to identify unique patterns of presentation of UTIs in elderly patients which could lead to diagnostic difficulty. Finally, we assessed local antibiotic resistance rates.

Methods: A retrospective case-note analysis of 100 patients, over the age of 65 years, diagnosed with UTI was carried out in 3 cycles between 2016-2018. The final cycle was conducted following removal of Trimethoprim from antibiotic guidance.

Results: Of patients diagnosed with UTI and had MSU (mid-stream urine) sample analysed, only 28.8% displayed microbial growth. 39.1% of patients with confirmed UTI displayed neither signs nor symptoms of UTI. 20% diagnosed with UTI did not have a MSU sample requested. Resistance rates of 39.1% were reported to Trimethoprim, with E.Coli accounting for 56.5% of all UTIs.

Conclusions: Diverse presentation and incomplete diagnostics contributes to misdiagnosis of UTI. Trimethoprim is not an effective treatment option and guidelines should reflect this.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B170

Shining a light on antibiotic selection: optimised live/dead fluorescence spectrometry for rapid antimicrobial susceptibility testing

Robertson Julia[1,2], Fang Ou[1,2], Cushla McGoverin[1,2], Frederique Vanholsbeeck[1,2], Simon Swift[1]

[1]The University of Auckland, Auckland, New Zealand. [2]The Dodd-Walls Centre for Photonic and Quantum Technologies, Dunedin, New Zealand

Abstract

Antibiotic resistance is a serious threat to public health. The empiric use of the wrong antibiotic occurs due to urgency in treatment combined with slow, culture-based diagnostic techniques. Inappropriate antibiotic choice can promote the development of antibiotic resistance.

We propose to use live/dead spectrometry as a rapid alternative to culture-based techniques through application of the LIVE/DEAD® BacLight™ Bacterial Viability Kit. We have developed a spectroscopic device (Optrode) to measure fluorescence from SYTO 9 and propidium iodide stained cells that can be used to enumerate the bacterial load. We propose a procedure using the Optrode that will take bacteria in a clinical sample, challenge with a panel of antibiotics, and measure live/dead ratios to determine the best bactericidal choice.

Using calibration data we optimised the live/dead spectrometry protocol outlined in the kit instructions, improving upon media selection for growth and staining, and analytical parameters. We applied the optimised methodology to detect live and dead *Escherichia coli* in populations challenged with ampicillin. Killing was detected by the Optrode in near real-time when *E. coli* was treated with ampicillin and stained with SYTO 9 and/or PI.

Following on from the promising results generated with ampicillin, live/dead spectrometry of ampicillin challenged cells was characterised in terms of antibiotic concentration, growth phase, and susceptibility to treatment for each treatment time. The generated data demonstrated that reliable detection of *E. coli* knockdown by ampicillin using live/dead spectrometry requires log phase cells challenged with a suitable concentration for a particular treatment time.

B171

The *smvAR* gene locus is involved in biocide susceptibility in *Proteus mirabilis*

Harriet Pelling[1], Lucy J. Bock[2], Emma L. Denham[1], Wendy M. MacFarlane[3], J. Mark Sutton[2], Brian V. Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]Public Health England, National Infection Service, Salisbury, United Kingdom. [3]University of Brighton, Brighton, United Kingdom

Abstract

Proteus mirabilis is a common pathogen of the catheterised urinary tract and often causes serious complications in these patients. This organism is typically considered to be intrinsically resistant to the biocide chlorhexidine, but little is known about the underlying mechanism. Here we identify a role for the SmvAR efflux system, first linked to chlorhexidine tolerance in *Klebsiella pneumoniae,* in the susceptibility of *P. mirabilis* to chlorhexidine and other biocides. Phenotypic and genomic characterisation of clinical isolates showed wide variation in chlorhexidine MICs (8 to ≥512 μg/mL), and indicated *smvR* sequence variation in isolates with elevated chlorhexidine resistance. RT-qPCR revealed a correlation between *smvA* expression and chlorhexidine MIC. The highest *smvA* expression was observed in isolate RS47, which also showed the highest chlorhexidine MIC (≥512 μg/mL) and a *smvR* C-terminal truncation. To confirm the contribution of *smvA* de-repression to chlorhexidine resistance, isolate RS47 was complimented with a functional *smvR* gene from the least resistant *P. mirabilis* isolate characterised (RS50a). Complementation significantly reduced *smvA* expression and reduced chlorhexidine resistance in RS47, however, susceptibility to chlorhexidine was not restored to levels observed in the RS50a *smvR* donor. Resistance to a range of other biocides was also found to be significantly elevated in RS47, and complementation with functional *smvR* was able to reduce MICs to levels comparable with RS50a in most cases. In conclusion, de-repression of the SmvA efflux system plays an important role in modulating *P. mirabilis* susceptibility to a wide range of biocides, but it is likely that other factors also contribute to chlorhexidine resistance.

B172

Role of antioxidant defence system in fluconazole susceptibility and its modulation by berberine in *Candida albican s*

Aijaz Ahmad

University of the WItwatersrand, Johannesburg, South Africa. National Health Laboratory Service, Johannesburg, South Africa

Abstract

Emergence of multidrug resistant species and forms of *Candida* are evolving, which advocates an urgent need for the development of new therapeutic strategies and antifungal drugs. Activation of antioxidant defence system has been known to be forefront mechanism to escape drug toxicity. This study was conducted to evaluate the role of antioxidant defence genes in the susceptibility to fluconazole in *C. albicans* isolates. We also determine the effect of berberine (BER) on growth and antioxidant enzymes in *C. albicans.* Antifungal activity of BER was determined using microdilution method. Gene expression of *SOD1*, *SOD2*, *GPx2*, *GLR1*, *GTT1*, and *CAT1* in untreated and BER treated *C. albicans* cells was measured by RT-qPCR. The activity level of the corresponding enzymes in the presence of BER was determined spectrophotometrically. Antifungal susceptibility showed BER MICs ranging from 125 to 500 µg/ml. Gene expression analysis showed an increase in mRNA expression levels of *SOD1*, *SOD2*, *GPx2*, *GLR1* and *GTT11* genes in fluconazole resistant isolates than in the susceptible group. BER treatment induced significant upregulation in the mRNA expression and enzymatic activities of major antioxidants in a dose-dependent manner. In fluconazole resistant *C. albicans*, excess concentrations caused downregulation of the targeted antioxidants indicating that BER at higher concentrations induced an intense oxidative stress. Overall results revealed an increase in the expression of antioxidant enzymes in response to BER induced oxidative stress, but the antioxidant capacity could not sufficiently combat the toxic effects occurring at higher concentrations. Therefore, BER could serve as a potent ROS-inducing agent in *C. albicans*.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B173

Species-wide investigation of differential 6-methyl-adenine (6mA) DNA methylation by *Sau1* Type I Restriction-Modification in *Staphylococcus aureus*.

Rebecca Mekler[1], Simon Harris[2], Matthew Holden[1]

[1]University of St Andrews, St Andrews, United Kingdom. [2]Wellcome Sanger Institute, Cambridge, United Kingdom

Abstract

Staphylococcus aureus is a prominent global bacterial opportunistic pathogen and a leading cause of nosocomial- and community-associated infections. Prokaryotic DNA methylation is facilitated by methyltransferases, often belonging to Restriction-Modification (RM) systems. The role these systems, and extent to which methylation impacts cell viability, pathogenicity and gene expression remains uncharted.

The main RM system present in *S. aureus* responsible for DNA 6-methyl-adenine (6mA) is the *Sau1 T*ype I system. Bioinformatic analysis was conducted on PacBio sequenced *S. aureus* genomes from the NCTC3000 collection from National Collection of Type Cultures (NCTC) of Public Health England. Modification analysis allowed the characterization of the diversity of *Sau1* systems and corresponding methylation signatures throughout 120 phylogenetically divergent isolates.

To understand the potential effect of differential methylation on gene regulation, comparative genomics was used to identify intra-species variation of the *S. aureus* methylome, namely in different regions of the genome, by different *Sau1hsdMS* elements. Frequency of methylation motifs is preferentially found in the coding sequence, rather than the intergenic regions, as well as the core genome compared to the accessory genome. Additionally, some mobile genetic elements (MGEs) are more densely methylated than others, or in comparison to the core genome.

6mA methylation by *Sau1* systems is an key feature of the *S. aureus* genome, and the distribution of systems defines the predominantly clonal landscape of this organism. Furthermore, we are investigating the importance of *Sau1* in the evolution of successful *S. aureus* lineages and the spread of antimicrobial resistance (AMR), and potential role in epigenetic control.

B174

The Present & The Past: A Review of Newly Accessioned Bacterial Strains into the UK's National Collection of Type Cultures

Jake David Turnbull, Sunita Gurung, Julie E. Russell, Dr. Sarah Alexander

The National Collection of Type Cultures, Culture Collections, National Infection Service, Public Health England, London, United Kingdom

Abstract

The National Collection of Type Cultures (NCTC) is a bacterial culture collection which is taxonomically and biologically diverse. NCTC holds approximately 6000 different strains from over 900 different species; among them strains originally isolated in the 19th century, strains for use as controls as stipulated EUCAST and ISO guidelines, type strains on which the description of bacterial species are based and other strains from a variety of backgrounds. The remit of NCTC is to provide authentic bacterial cultures of medical and veterinary interest to the scientific community, to support and enhance the reproducibility of scientific research and to improve global public health.

To fulfil this remit, remain scientifically relevant and to preserve the legacy of contemporary medical bacteriology for future scientists, NCTC accessions strains of clinical significance: such as recently circulating and outbreak strains, diagnostic escape mutants and strains with novel antimicrobial resistance profiles. In 2018, 166 bacterial strains were accessioned into the NCTC and made available to the scientific community. These include NCTC 14052: a reference strain for emergent hyper-virulent K. pneumoniae, 4 type strains of newly described bacterial species, 82 strains accessioned from the Murray Collection of pre-antibiotic era Enterobacteriaceae and 8 strains with antimicrobial resistance mechanisms previously unrepresented in the collection, including NCTC 14208: a N. gonorrhoeae isolated from an instance of combined ceftriaxone and azithromycin treatment failure. Through literature review we have highlighted their value to the scientific community, both in their own right and in the context of bacterial strains already held by the NCTC.

B175

Efficacy of novel eugenol tosylate congeners as antifungal compounds in combination with fluconazole against *Candida albicans*

JULITHA MOLEPO

University of the Witwatersrand, Johannesburg, South Africa

Abstract

The incidences of *Candida albicans* infections and their changing drug resistance patterns have drastically increased in recent years. Therefore, new drugs and alternative treatment strategies are promptly required. Combination therapy and the use of natural products have been extensively studied as alternative treatment. In this study, we synthesized Eugenol Tosylate Congeners (ETCs 1-6) and evaluated their antifungal activity profile alone and in combination with fluconazole (FLC) against four FLC susceptible and three FLC resistant clinical isolates of *Candida albicans* isolates according to CLSI guidelines. For insight mechanism of antifungal action of ETCs, activity of plasma membrane H^+-ATPase pump of these *C. albicans* isolates was determined by monitoring the pH of the external medium. ETC 1 and ETC 4 were the most active congeners against the resistant isolates with the MIC ranging from 125-250 μg/ml. The MFC of ETCs ranged from 1000 μg/ml to 2000 μg/ml. Results interpreted from fractional inhibitory concentration index (FICI) and isobolograms showed 36% of synergy, 29% of additive, 33% of indifferent and 2% of antagonistic interactions. These compounds also inhibit H^+ efflux activity of H^+-ATPase pump at varying degrees. Our results suggest that these ETCs may be directly binding to this pump and thereby inhibiting H^+-efflux in *Candida* cells. These results advocate the potential of these compounds in developing new antifungal drugs; however, further studies are required to understand the other mechanisms involved and *in vivo* efficacy and toxicity of these compounds.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B176

Pseudomonas aeruginosa antibiogram profiles are poor indicators of genetic relatedness

James McKenna[1], Derek Fairley[1,2], Lesley Neill[1], Lyndsey Crawford[1]

[1]1. Department of Microbiology / Regional Virus Laboratory, Belfast Health & Social Care Trust, Belfast, United Kingdom. [2]2. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, Belfast, United Kingdom

Abstract

Pseudomonas aeruginosa is a significant nosocomial pathogen responsible for severe and life threatening infections particularly in immunocompromised patients. This organism is ubiquitous in healthcare environments paticularly water systems which act as a reservoir of infection. Recognition of a potential outbreak and having the ability to quickly identify and mitigate sources of exposure is critical for effective infection control. Historically analysis of P. aeruginosa antibiogram profiles represents a convenient and frequently used 'first line' indicator of strain relatedness. Reported here is a comparison of *P. aeruginosa* antibiogram profiles with those obtained using rapid Variable Number Tandem Repeat (VNTR) for patient and environmental isolates in in three separate local nosocomial outbreaks. The results demonstrate that antibiogram profiles from *P. aeruginosa* should not be employed as presumptive indicators of relatedness, doing so can falsely re-assure clinicians. Use of rapid molecular typing method VNTR allows genotypically identical strains to be unambiguously identified within 48 hrs.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B177

The Type III CRISPR-Cas System of *Mycobacterium tuberculosis*

Sabine Grüschow[1], Januka Athukoralage[1], Tess Hoogeboom[1,2], Malcolm F. White[1]

[1]University of St Andrews, St Andrews, United Kingdom. [2]Wageningen University, Wageningen, Netherlands

Abstract

Background

Bacteria and archaea have developed a number of strategies to keep the influx of mobile genetic elements (MGEs) such as viruses and plasmids in check. CRISPR-Cas systems provide adaptive immunity; all types of CRISPR-Cas systems have in common the genes for uptake of genetic information from the invading MGE but differ in the composition of the effector complex that directs and enforces the immune response. The genome of *Mycobacterium tuberculosis* encodes a Class 1, type III-A CRISPR-Cas system that has not been studied in detail.

Methodology

We used heterologous production of the mycobacterial CRISPR-Cas genes. Purification of the enzymes enabled *in vitro* biochemical characterisation of the adaptation, maturation and interference proteins. Reconstitution of the system in *E. coli* was used to demonstrate functionality *in vivo.*

Results

Maturation of crRNA through the action of Cas6 proceeded as expected, generating canonical CRISPR RNAs (crRNAs). The type III effector complex, consisting of Csm1-5, was shown to bind crRNA and cleave target RNA with the typical 6 nt spacing, display ssDNase activity and produce the cyclic oligoadenylate signalling molecule. The latter clearly activated the ribonuclease Csm6, an essential element in type III immunity. The *M. tuberculosis* type III CRISPR-Cas system was also reconstituted in *E. coli* where it provided plasmid immunity, demonstrating the functionality *in vivo*.

Conclusions

All elements of the *M. tuberculosis* type III CRISPR-Cas system are functional *in vitro* and *in vivo*. These studies lay the foundation for further investigations into the mechanism of adaptive immunity and possible applications in biotechnology.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B178

Pneumococcal invasive disease preceded by intracellular replication within splenic macrophages

David Carreno[1], Joseph Wanford[1], Zydrune Jasiunaite[1], Ryan Hames[1], Marcos I. Restrepo[2], Carlos J. Orihuela[3], Peter W. Andrew[1], E. Richard Moxon[4], Marco R. Oggioni[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Texas Health Science Center, San Antonio, USA. [3]The University of Alabama at Birmingham, Birmingham, USA. [4]University of Oxford, Oxford, United Kingdom

Abstract

During bacteremic pneumonia, the prevailing dogma is that bacteria seed from the lungs into the blood. Recently, we have shown that experimental murine sepsis is preceded by intracellular replication within splenic macrophages (Ercoli NatMicrobiol 2018), which shed into the bloodstream initiating invasive disease. Here we aimed to investigate a role for the spleen in the pathogenesis of bacteraemia following pneumonia.

We analysed by confocal microscopy the fate of pneumococci during *ex vivo* human spleen perfusions (REC reference: 18/EM/0057), in spleens during pneumonia in non-human primates (Reyes PLOSone 2016) and mice.

During *ex vivo* human spleen perfusion, clusters of pneumococci were observed within macrophages and the size of bacterial clusters increased over time. To associate these infectious foci to invasive pneumococcal disease during pneumonia, we analysed spleens in a baboon pneumonia model, and detected pneumococcal clusters in splenic macrophages. To test the functional relevance of these data, we treated intranasally-challenged mice with a single, non-therapeutic sub-MIC dose of azithromycin, known to concentrate inside macrophages. Data showed that bacterial lung-counts were identical in treated and untreated mice. Untreated mice showed signs of disease, had high blood and spleen-counts, whereas mice treated with the non-therapeutic dose showed no signs of disease, had low spleen-counts and no bacteraemia. Thus, the number of pneumococci in the spleen, not the lung, correlates to blood-counts during bacterial pneumonia.

We hypothesise that after initial control of invasive infection by the spleen, bacteraemia associated with pneumonia arises from a sub-set of splenic macrophages that are permissive for bacterial replication.

B179

Clostridium difficile: Cell Surface Biogenesis

Shauna O'Beirne, Joseph A. Kirk, Robert P. Fagan

University of Sheffield, Sheffield, United Kingdom

Abstract

On the *C. difficile* cell surface is a proteinaceous paracrystalline array, known as the S-layer. The S-layer of *C. difficile* is composed of two proteins: the high molecular weight (HMW) and the low molecular weight (LMW) S-layer proteins, derived from the pre-protein SlpA. PS-II, an anionic polymer found in all *C. difficile* strains examined to date, has been identified as the ligand responsible for the attachment of S-layer and associated cell wall proteins.

Early efforts to knock out *slpA* proved unsuccessful and the genes thought to encode the PS-II synthesis pathway were also thought to be essential. However, by using bacteriocins that specifically target the S-layer, we recently isolated a mutant which had no evident S-layer due to a mutation in the *slpA* gene. As the S-layer was previously thought to be essential, it now brings into question whether PS-II is also essential. In the strain lacking an S-layer, we have now created a deletion mutant in the putative PS-II polymerase and we are attempting to generate additional mutations in the polysaccharide synthesis pathway. Analysis of these mutants will provide insights into the mechanism of PS-II synthesis and shed light on its function in cell morphogenesis.

B180

Nature and consequences of Salmonella infections in cattle

Prerna Vohra, Christina Vrettou, Jayne Hope, John Hopkins, Mark Stevens

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

Abstract

Salmonella enterica is a human and veterinary pathogen of global importance. Cattle are a key reservoir of *S. enterica* serotypes that cause non-typhoidal salmonellosis in humans and infections are often acquired via consumption of contaminated food. Salmonella can survive within the bovine lymphatic system and contaminated peripheral lymph nodes often enter the food chain via ground beef production because they are small and deeply embedded in fat, making it impossible to remove them during food production. *S. enterica* serotypes can also cause acute enteritis and systemic typhoid-like disease in cattle, thereby exerting a significant burden on bovine welfare and productivity. Existing vaccines confer limited serotype-specific protection and a need exists to better understand the host and bacterial factors involved in pathogenesis and protection to inform the design of new vaccines and other intervention strategies. Most of our knowledge about salmonellosis comes from the mouse typhoid model. Here, we sought to understand the effects of infection by *Salmonella* Dublin, which causes typhoid-like disease in cattle, in its natural host. By infecting cattle with *S*. Dublin expressing green fluorescent protein and using flow cytometry we have been able to isolate and characterise the bovine cells infected by *S*. Dublin, study changes in their cell surface marker expression post-infection and compare tropism in the intestine and draining lymph nodes. We have also studied the survival of *S*. Dublin in the main infected host cell type *in vitro* using primary cells to determine the consequences of infection on the pathogen itself.

B181

Structure and interactions of the Ebolavirus Delta-Peptide Amphipathic Helical Domain within the Lipid Bilayer

Diego Cantoni, Jose Ortega-Roldan, Jeremy Rossman

University of Kent, Canterbury, United Kingdom

Abstract

Ebolaviruses are a group of negative sense single-stranded RNA viruses pertaining to the family of filoviridae, of which most members capable of causing severe disease in humans. One of the key proteins the viruses encodes in its genome is glycoprotein, which undergoes proteolytic processing that ultimately gives rise to a series of products including a small peptide named delta-peptide. Recent bioinformatics suggest that this peptide is a viroporin, which suggests it retains beneficial properties with regards to the virus replication and pathogenesis. Delta-peptide contains an amphipathic helical domain (AH), typical of viroporins, for interactions with the cell membrane. Here, we present the first structural data of human pathogenic Zaire and non-human pathogenic Reston ebolavirus delta peptide AH domains and elucidate their interactions and ability to form pores within lipid bilayers using vesicles and nanodiscs.

B182

A novel deep-sea sponge bacterium producing two promising antimicrobial candidates

Abdu Aldarhami[1], Arif Felek[2], Garry Farnham[1], Florie Desriac[1], Kerry Howell[1], Mathew Upton[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]National Institute for Biological Standards and Control, Potters Bar, United Kingdom

Abstract

Background: Natural product screening methods are arguably the most efficient way to identify novel antibiotics. Exploiting obscure, hard to reach environments, implementing the latest high-throughput next generation sequencing techniques, performing *in silico* analysis and synthesis/recombinant expression of promising candidates may increase the discovery of unique agents.

Materials/methods: Deep-sea sponges were collected (~1000m depth) in the North Atlantic Ocean and bacteria were recovered. One strain (EU4) was selected for detailed analysis. Strain EU4 produced an inhibitor into liquid media. This compound was purified using liquid chromatography and matrix-assisted laser desorption/ionization (MALDI) analysis was performed. In parallel, the draft genome was obtained and analysed using BAGEL-3 and antiSMASH-4.0 mining tools. Successful synthetic production was obtained for a candidate identified using antiSMASH.

Results: Analysis of the draft genome (5.8Mbp) indicates that strain EU4 is a novel member of the *Bacillaceae*. To date, the produced compound showed activity towards *Micrococcus luteus* only, while the synthetic compound displays a broad spectrum of activity towards Gram positive and -negative bacteria. In addition, based on MALDI analysis, the synthetic and the naturally produced compounds possess different molecular weights, being approximately 4kDa and 1.7 kDa, respectively.

Conclusions: Bacteria recovered from deep-sea sponges could potentially be a rich source for novel compounds. *In silico* analysis of producer genomes has provided a means of identifying cryptic compounds, not produced in culture. Further study of both compounds, which showed diverse activity spectra, may lead to promising new candidates for development into clinically relevant therapeutics.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B183

Emergence of a non-sporulating secondary phenotype in *Clostridium difficile* ribotype 078 isolated from humans and animals.

Mairead Connor[1], John McGrath[1], Geoff McMullan[1], Nikki Marks[1], Derek Fairley[2]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Belfast Health & Social Care Trust, Belfast, United Kingdom

Abstract

Clostridium (Clostridioides) difficile is a Gram positive, spore forming anaerobic bacterium that is a leading cause of gastroenteritis-associated deaths and currently the most common cause of hospital acquired infection in many developed countries.

C, difficile can be separated in to 8 genetically diverse clades, 5 of which contain clinically relevant, disease causing isolates. *C. difficile* clade 5 is genetically distant from all other *C. difficile* clades, and believed to have diverged from clades 1-4 over a period of approximately 85 million years. Isolates with PCR ribotype 078 (R078) belong to clade 5, and are often associated with *C. difficile* in both humans and animals. Notably, R078 is the most common ribotype found causing human infection in Northern Ireland. Prevalence of R078 in both animals and humans raises questions about colonisation of both hosts by this ribotype as a possible zoonosis, about modes and routes of transmission, and about reservoirs of R078 strains.

Previous work in our laboratory (D. Fairley, personal communication) showed that after anaerobic incubation of ~7 days, some R078 isolates appeared to produce a secondary colony phenotype. These secondary colonies had a smooth, grey appearance, with a much larger, spreading colony morphology. Microscopic examination suggested this secondary phenotype may be non-sporulating, and led to the hypothesis these R078 strains were spontaneously producing rapidly growing non-sporulating variants. To gain further understanding of this secondary phenotype displayed by R078 strains, phenotypic characteristics where investigated further with regards to sporulation, growth rate, antibiotic susceptibility, toxin production, motility and biofilm formation.

B184

The role of Glutamine Synthetase in the Pathogenesis of Neisseria meningitidis

Iyelola Turner, Neil Oldfield, David Turner

University of Nottingham, Nottingham, United Kingdom

Abstract

Neisseria meningitidis is a cause of meningitis and severe sepsis. A moonlighting protein is a protein with the ability to perform additional task(s) alongside its recognised function. These proteins have been identified in both prokaryotic and eukaryotic cells and they represent a highly conserved subset of proteins that typically are either metabolic pathway-associated enzymes or act as molecular chaperones.

To explore the role of GlnA in the pathogenesis of meningococcal disease, the encoding gene designated NMB0359, was amplified from the wild-type meningococcal strain MC58 and cloned into the pQE-30 expression vector. Recombinant glutamine synthetase (rGlnA) was expressed in *E. coli* and purified by immobilised metal affinity chromatograph. Rabbit antisera was raised against purified rGlnA (RαGlnA) and used to investigate the localisation of GlnA at the cell surface. Attempts were also made to generate *glnA* knockout and complemented strains of wild-type *N. meningitidis*.

rGlnA was successfully purified from *E. coli* cell lysates under native conditions. A highly immuno-reactive band of the expected size (52 kDa) was observed when rGlnA immunoblot was probed with RαGlnA. GlnA could be detected on the surface of wild-type encapsulated *N. meningitidis* MC58 using whole-cell enzyme linked immunosorbent assay (ELISA).

Surface localisation of GlnA indicates that it may be a moonlighting protein carrying out function(s) at the cell surface. Future work will investigate possible moonlighting functions which may include adhesion to host cells and proteins, regulation of the host immune response, and contribution to bacterial virulence.

B185

Investigating the nuclear localisation and proteolytic activity of the meningococcal App and MspA autotransporters.

Shoma Dutta, Neil Oldfield, Karl Wooldridge

University of Nottingham, Nottingham , United Kingdom

Abstract

Autotransporter proteins are major secreted virulence factors of Gram-negative bacteria. They are translocated across the inner membrane via the Sec machinery and the outer membrane via the Bam complex and a series of periplasmic chaperones, respectively. The passenger domain may then be proteolytically cleaved and released into the external milieu. The meningococcal autotransporters Adhesion and penetration protein (App) and Meningococcal serine protease A (MspA) are secreted S6-peptidase family autotransporters. Our previous work has shown that FITC-labelled recombinant App or MspA can be taken up by host cells and translocated into the nucleus. App and MspA can also bind to, and cleave recombinant host histones.

We furthered investigate the ability of App and MspA (and their inactive derivatives) to cleave recombinant and host-derived histones. Our data demonstrate proteolytic activity of App and MspA on recombinant H3 and Hep-2 cell-derived H3 (which may undergo post-translational modifications that are not applied to the recombinant protein); no cleavage was observed when the histone proteins were treated with proteolytically inactive mutants of the autotransporter proteins.

We have also further investigated the nuclear localisation of App and MspA by deleting areas of interest within the meningococcal autotransporters and assessing the impact on nuclear localisation in order to identify the autotransporter motifs required to direct App and MspA to the nuclear compartment.

In summary, our results confirm that App and MspA can reach the nuclear compartment of the host cell and clip host-derived histone H3.

B186

Development of a high-throughput ex-vivo burn wound model using porcine skin, and its application to evaluate new approaches to control wound infection

Jonathan Nzakizwanayo[1], Diana Alves[2], Simon Booth[2], Paola Scavone[3], Pascale Schellenberger[4], Jonathan Salvage[2], Cinzia Dedi[2], Naing-Tun Thet[1], Toby Jenkins[1], Ryan Waters[5], Keng Ng[2], Andrew Overall[2], Anthony Metcalfe[6], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Brighton, Brighton, United Kingdom. [3]Department of Microbiology, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. [4]University of Sussex, Brighton, United Kingdom. [5]The Pirbright Institute, Woking, United Kingdom. [6]University of Birmingham, Birmingham, United Kingdom

Abstract

Biofilm formation in wounds is considered a major barrier to successful treatment, and has been associated with the transition of wounds to a chronic non-healing state. Here we present a novel laboratory model of wound biofilm formation using *ex-vivo* porcine skin and a custom burn wound array device[1]. The model supports high-throughput studies of biofilm formation and is compatible with a range of established methods for monitoring bacterial growth, biofilm formation, and gene expression. We demonstrate the use of this model by evaluating the potential for bacteriophage to control biofilm formation by *Staphylococcus aureus*, and for population density dependant expression of *S. aureus* virulence factors (regulated by the Accessory Gene Regulator, *agr*) to signal clinically relevant wound infection. Enumeration of colony forming units and metabolic activity using the XTT assay, confirmed growth of bacteria in wounds and showed a significant reduction in viable cells after phage treatment. Confocal laser scanning microscopy confirmed the growth of biofilms in wounds, and showed phage treatment could significantly reduce the formation of these communities. Evaluation of *agr* activity by qRT-PCR showed an increase in activity during growth in wound models for most strains. Activation of a prototype infection-responsive dressing designed to provide a visual signal of wound infection, was related to increased *agr* activity. In all assays, excellent reproducibility was observed between replicates using this model.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B187

Identification of a nitrite reductase in *Pseudomonas aeruginosa* as a potential antimicrobial target.

Samuel Fenn, Jean-Frederic Dubern, Michael Stocks, Jonas Elmsley, Miguel Camara

University of Nottingham, Nottingham, United Kingdom

Abstract

The opportunistic pathogen *Pseudomonas aeruginosa* utilizes a wide range of virulence factors to adapt to the host environment. With the antimicrobial pipeline drying up, understanding and targeting virulence factors for therapeutic development is an exciting alternative for the discovery of novel disease inhibitors. An integrated genome-wide transposon mutagenesis screening approach was performed in *P. aeruginosa* using multiple *in vivo* disease models with the aim to identify new virulence factors required for infection. A mutant attenuated in the production of multiple virulence determinants using *in vitro* assays was identified. This mutant also showed severe attenuation using *in vivo* models with up to an 80% increased survival in murine chronic and acute lung infection models. The predicted protein coded by the mutated gene showed homology to nitrite and sulphite reductases. Using a methyl viologen reduction assay, we have shown that this gene encondes a nitrite reductase, operating in a siroheme and 4Fe-4S dependant manner. The preference for nitrite and the requirement of siroheme revealed that product of this gene is an assimilatory nitrite reductase and hence we propose it to be named as NirA. Work is now on-going to understand how NirA contributes to virulence and determine the crystal structure of this protein with a view to screen for novel inhibitors of this enzyme using a drug discovery platform available in our laboratories.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B188

Investigation of a hospital *Enterobacter cloacae* NDM-1 outbreak using whole genome sequencing.

Derek Fairley[1,2], Yuri Protaschik[1], Ruth Finn[1], Jane Turton[3], Michel Doumith[3], Wesam Elbaz[1], Sara Hedderwick[1], Declan Bradley[2]

[1]Belfast Health & Social Care Trust, Belfast, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom. [3]Public Health England, London, United Kingdom

Abstract

Caprbapenemase-producing Gram-negative micro-organisms are emerging as a major clinical problem. The infections caused by these highly resistant and hospital-adapted pathogens may become untreatable using existing antibiotics.

Over a three year period, six patients at a large UK tertiary-referral hospital were colonised or infected with carbapenem-resistant *Enterobacter cloacae* carrying the $bla_{\text{NDM-1}}$ metallo-*β*-lactamase gene. Environmental isolates were also obtained from a clinical wash-hand basin and taps. The isolates had very similar pulsed-field gel electrophoresis profiles, suggesting they were related, although only four of the cases had epidemiological links. Whole genome sequencing showed the isolates had the same genomic background (sequence type ST114). Genes encoding seven different extended-spectrum and inhibitor-resistant β-lactamase and carbapenemase enzymes ($bla_{\text{NDM-1}}$; $bla_{\text{CTX-M-15}}$; $bla_{\text{ACT-16}}$; $bla_{\text{VEB-1}}$; $bla_{\text{TEM-1}}$; $bla_{\text{OXA-1}}$ and $bla_{\text{OXA-10}}$) were present, in addition to multiple genes and mutations conferring resistance to aminoglycosides, quinolones, trimethoprim, tetracycline , sulphonamide, chloramphenicol, rifampicin and fosfomycin. Phenotypic testing indicated sensitivity only to colistin and tigecycline. Genome-wide single nucleotide polymorphism analysis showed the four linked isolates were closely related, and differed from the unlinked isolates by 16-24 SNPs. Moreover, resistance encoding plasmids had been lost in the two unlinked isolates. This suggested these isolates, although sharing a recent common ancestor, had evolved in different environments. Whole genome sequencing allowed resolution of very closely related *E. cloacae* strains, and confirmed the outbreak did not extend beyond the linked patients. Sequencing also confirmed the same highly resistant *E. cloacae* strain had persisted within a clinical unit for over two years, despite rigorous efforts to eradicate it.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B189

Development of Anti-virulence polymers targeting mycobacteria

Bala Madduri[1], Oliver Creese[2], Dr Francisco Fernandez-Trillo[2], Dr Luke Alderwick[1]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham , Birmingham, United Kingdom. [2]Institute of Microbiology and Infection, School of Chemistry, University of Birmingham, Birmingham, United Kingdom

Abstract

Modern medicine is under the excruciating pressure of drug resistant bacterial strains which are ever advancing with the introduction of every new class of antibiotics. Traditional bactericidal and bacteriostatic drugs, while effective in eliminating the susceptible bacterial strains, also impose a selective pressure on bacteria which often leads to the emergence of antimicrobial resistance. An alternative approach is the development of anti-virulence therapies, which aims reduce bacterial pathogenesis while avoiding the selective pressure of classical antimicrobial inhibitors, thus rendering bacteria harmless and potentiating natural elimination from the host by innate immunity defence mechanisms. We have synthesised a selection of functional polymers of poly(acryloyl hydrazide) using a panel of aldehyde functionalisation groups and evaluated their anti-virulence properties on both *Mycobacterium bovis* BCG and *Mycobacterium smegmatis* mc^2 155, two surrogate organisms to study *Mycobacterium tuberculosis,* the etiological agent responsible for tuberculosis. Using a combination of microscopy and *in vitro* studies, we have shown the effectiveness of anti-virulence polymers in reducing mycobacterial phagocytosis in J774 macrophages with minimal antimicrobial activity.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B190

The validation of VIPcheck™ plates to screen Aspergillus fumigatus isolates for phenotypic resistance to triazole antifungal agents in St. James's Hospital, Dublin.

Auveen Griffin, Katie Dunne, Alida Talento, Tom Rogers

St. James's Hospital, Dublin 8, Ireland

Abstract

Triazole resistance is an emerging problem in *Aspergillus fumigatus* (AF) resulting in failure of azole therapy. Triazole resistant AF is acquired through one of two routes - previous exposure to triazole therapy or an environmental source.

In vitro antifungal susceptibility testing (AFST) on all AF strains isolated in a microbiology laboratory would be both labour intensive and impractical. A method to screen for triazole resistance would be more favourable.

VIPcheck™ plates provide a simple agar based screening method. Each 4-well plate contains a growth control (GC) well and 3 wells containing itraconazole (4mg/L), voriconazole (2mg/L) and posaconazole (0.5 mg/L). Briefly, 25ul of a 0.5-2McF suspension AF is inoculated into each well and plates are read after 48hrs incubation at 37◦C. Any growth in a triazole containing well is suggestive of resistance.

Currently in SJH, AFST is carried out using gradient strips (Liofilchem™) and results are interpreted using EUCAST breakpoints. We validated the VIPcheck™ plates with the intention to include this screening method as part of our AFST for AF isolated from clinical samples.

A total of 18 isolates (clinical and environmental) of AF were tested using the VIPcheck™ plates (n=2 wild type, n=18 resistant to ≥ 1 triazole drug as previously determined by AFST and/or molecular methods). The wild type isolates showed growth only in the GC well while the resistant strains all showed growth in one or more of the triazole containing wells.

Our results suggest that the VIPcheck™ plate is a reliable screening method for triazole resistance.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B191

A SLIC answer to a continuing problem

Robert Hammond

University of St Andrews, St andrews, United Kingdom

Abstract

Background: The burden of Anti-Microbial Resistance (AMR) is a growing problem globally. Here we present a device that determines susceptibility rapidly **from primary human or animal samples** and could turn the tide of AMR. SLIC (Scattered Light Integrating Collector) is a sensitive device for the detection of microbes based on the scattering of laser light.

Methods: Proof of concept studies were carried out initially to establish the lower limit of detection. This was found to be 10-50 cfu/mL. This exquisite sensitivity allowed us to commence work establishing rapid MICs. Starting with an inoculum of 10^5/mL bacteria and using a relevant range of antibiotic concentrations the MIC can be established in less than one microbial doubling period.

Results: The rapid and sensitive detection SLIC affords allows for fast growing organisms such as *E. coli* and *S. aureus* to have their MICs established in less than 10 minutes, for any antibiotic. For slow growing organisms such as *M. bovis* we are able to establish an MIC in <2 hours. The technology can also be used for fastidious and difficult to grow organisms such as *H. influenzae* and *Mycoplasma spp.*

Conclusion: As bacterial quantification is continuously monitored we are able to see the action of antibiotics in real time. Using this facility, we can readily distinguish between lytic antibiotics & bactericidal but non-lytic antibiotics. This provides the opportunity to gain new insights into the mechanisms of action and the effect antibiotics have on microbes in a new way in a **novel Point-of-Care device**.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B192

Functional evolutionary genomics of *Legionella pneumophila* reveals the molecular basis of serum resistance among clinical isolates

Joana Alves[1], Bryan Wee[1], Diane Lindsay[2], Andrew Smith[2], J. Ross Fitzgerald[1]

[1]The Roslin Institute, Edinburgh, United Kingdom. [2]Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, Glasgow, United Kingdom

Abstract

Since the first reported Legionnaires' disease outbreak in the UK in 1984, the Scottish microbiology reference laboratory has maintained a comprehensive collection of isolates of *Legionella spp*.
Whole genome sequencing of the extensive Scottish *Legionella pneumophila* collection, combined with the publicly available genomes, represents a powerful tool for investigating Scottish outbreaks and also the relationships between clinical and environmental isolates.
Our data indicate that *Legionella* infections in Scotland over the last 30 years originated from diverse lineages that are representative of the global diversity of this species. Genome-wide association analysis (GWAS) on this large and diverse genome dataset highlighted genes that are over-represented in clinical isolates; namely, subsets of genes involved in lipopolysaccharide (LPS) biosynthesis. More than twenty Scottish strains were selected to represent the breadth of diversity of the *L. pneumophila* species as well as the presence/absence of top gene identified by GWAS. The interaction of these strains with components of the innate immune response was evaluated and we found an association between the expression of an LPS biosynthesis enzyme and *L. pneumophila* resistance to complement killing. The complementation of natural mutants of this enzyme confirmed its role in *L. pneumophila* evasion of complement. Furthermore, by selectively inhibiting the different complement pathways, we show the essentiality of activation of the classical pathway in the killing of *L. pneumophila* in non-immune serum.

Overall, we show that human pathogenic potential is distributed across the *L. pneumophila* phylogeny and that LPS modification contributes to the ability to escape the innate immune response.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B193

Propionibacterium acnes infection of the prostate gland as a risk-factor and biomarker of prostate cancer.

Andrew McDowell[1,2], Emma Barnard[2], Derek Fairley[3], Lorraine Martin[4], Declan O'Rourke[5], Sheila Patrick[2]

[1]Centre for Stratified Medicine, School of Biomedical Sciences, Ulster University, Londonderry, United Kingdom. [2]Centre for Experimental Medicine, School of Medicine, Dentistry & Biomedical Sciences, Queen's University, Belfast, United Kingdom. [3]Department of Microbiology, Belfast Health & Social Care Trust, Belfast, United Kingdom. [4]School of Pharmacy, Queen's University, Belfast, United Kingdom. [5]Histopathology & Cytopathology Laboratory, Belfast City Hospital, Belfast Health and Social Care Trust, Belfast, United Kingdom

Abstract

Prostate cancer (PCa) is the most common male cancer in the UK, killing approximately 11,000 men every year. There is now increasing interest in the role played by the anaerobic bacterium *Propionibacterium acnes* in the aetiology of this condition via chronic, intracellular and asymptomatic infection of the prostate gland leading to oncogenesis. To investigate this, we developed a novel quantitative real-time PCR assay for retrospective detection of *P. acnes* in formalin-fixed paraffin-embedded tissue sections prepared from archived prostate biopsy samples. A total of 81 biopsy samples were examined from 53 patients with prostate carcinoma, versus 111 samples from 60 patients whose biopsies were histologically normal. Our assay revealed that 35% of men with PCa were positive for the presence of *P. acnes* in either one or both prostate lobes compared to only 8% of the normal tissue (Fisher's exact test, 2-sided; $p<0.001$). This rate of detection is in keeping with previous culture-based studies, and equates to a 2-fold relative risk (95% CI: 1.45-2.75; $p<0.0001$) after adjustment for age; this level of risk approximates to having one first-degree relative diagnosed with the condition. No statistical association between the presence or absence of infection/ colonization and age, blood prostate specific antigen (PSA) levels or Gleason score was observed. Our studies suggest *P. acnes* infection of the prostate gland may be a potential risk factor for PCa development. Furthermore, the presence of *P. acnes* in cancerous tissue is also a highly specific biomarker for the condition versus PSA measurement (92% versus 65%).

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B194

Understanding the Pathogenic Process of Uropathogenic *Escherichia coli* ST127 using Proteomics on Uroepithelial Co-culture Samples

Nizar Saeedi[1,2], Vikram Sharma[1], Simon Jackson[1], Mathew Upton[1]

[1]University of Plymouth, Peninsula Schools of Medicine and Dentistry, Plymouth, United Kingdom. [2]University of Tabuk, Faculty of Applied Medical Sciences, Tabuk, Saudi Arabia

Abstract

Background: Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infection (UTI). Strains of sequence type (ST) 127 exhibit the highest virulence potential of most UPEC strains, but little is known about pathogenicity during infection. We sought to investigate this using a quantitative proteomics approach.

Material/Methods: Three strains of UPEC ST127 (EC18, EC41 and SA189), in addition to non-pathogenic strain *E. coli* K12, were analysed in co-culture with the uroepithelial cell-line HT1197 for 5 hours. We analyzed the bacterial and uroepithelial proteome along with the secreted proteins in the medium (secretome). The digested proteins and peptides from all fractions were separated on a Dionex Ultimate 3000 RSLC nano flow system and analyzed in an Orbitrap Velos Pro FTMS. Data were processed using Persues software.

Results: Label free quantitative proteomics revealed different proteomic profiles of the co-cultured strains. Gene Ontology enrichmentanalysis showed upregulation in the pentose phosphate pathway and glycolysis/glycogenesis in EC18 (an O-antigen deficient mutant). These two pathways could be important routes of carbon flux through the central metabolic pathways during growth in urine. Co-culture of SA189 with HT1197 cells leads to apparent cytotoxic effects in HT1197 cells not seen with other UPEC strains. Analysis of the SA189 secretome revealed highly abundant bacterial proteins, some of which (e.g. aromatic-amino-acid aminotransferase) were uniquely found during co-culture conditions.

Conclusion: Proteomics is crucial towards increasing our understanding of the pathogenic potential of UPEC ST127 strains and may facilitate identification of novel diagnostic or therapeutic targets to reduce UTI.

B195

Purification and characterisation of antimicrobial agents isolated from a member of the *Paenibacillus* genus

Vicky Bennett[1,2], Matthew Koch[2], Abdu Aldarhami[2], Mathew Upton[2]

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

Abstract

Antimicrobial resistance (AMR) poses an ever-increasing threat to public health; the prevalence of resistant bacterial strains has reduced the clinical efficacy of many existing therapeutics and is therefore contributing to rising mortality rates due to difficult to treat bacterial infections. Two key approaches used to mitigate the threat of AMR are the discovery of novel therapeutics with activity against these resistant strains, and educating the wider public about the impact of AMR, and steps that can be taken to reduce the development of resistance. We are combining both approaches to enhance the impact of our public engagement activities. During a recent event at the University of Plymouth, a member of the public isolated the bacterial strain "36A" from the button of a lift control panel. Simultaneous antagonistic screening identified antimicrobial activity against a range of both Gram-positive and Gram-negative bacteria. 36A was then subjected to draft genome sequence determination via the MinION platform (Oxford Nanopore). Growth media were optimised to enhance antimicrobial activity, with fermentation in LB broth and subsequent purification of the culture supernatant via multi-stage column chromatography resulting in the isolation of four putative antimicrobial compounds. Initial characterisation has shown that each compound has a peptidic component, all showing stability and potency at a relatively low concentration against MRSA, *E.coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Structural characterisation has been carried out using mass spectrometry, with further characterisation and cell toxicity studies ongoing. The producing strain has been identified as a member of the *Paenibacillus* genus.

B196

Induction and characterisation of a 25-hydroxycholesterol associated immune response to Gram positive and negative bacteria in a whole blood model of sepsis

Heather Chick, William J. Griffiths, Yuqin Wang, Tom. J. Humphrey, Thomas S. Wilkinson

Swansea University, Swansea, United Kingdom

Abstract

Early diagnosis and treatment of sepsis is one of the biggest challenges to ICU clinicians. Globally, 19million cases occur annually and it is the third biggest cause of death in the UK. Sepsis is characterised by an uncontrollable, non-specific immune response to an infection, and as a result is difficult to diagnose. Recent research has found that 25-hydroxycholesterol (25-HC) plays a crucial role in the immune response to viral infection. Less is known about the role of sepsis-associated bacteria in this response. To identify novel biomarkers in bacterial sepsis a whole blood model was used and the cellular and molecular responses measured to well-characterised bacteria (*Escherichia coli* K12 and *Staphylococcus epidermidis* RP62A) using flow cytometry, ELISA and high performance liquid chromatography-mass spectrometry (LC-MS).

Following bacterial infection, mononuclear cells and granulocytes decrease rapidly in response to both K12 and RP62A. This corresponds to a concomitant increase in total CD45 and CD19 expression and the concentration of the proinflammatory cytokines IL-6, CCL3 and CCL20. Proinflammatory responses were significantly more pronounced in K12 infection. There were significant increases in 25-HC in response to K12 infection, and this effect was partially blocked through inhibition of TLR2 or TLR4. Our results suggest the importance of using both cellular and humoral screening to identify unique pathways induced by sepsis causing bacteria. In addition, the current study provides some of the first evidence that 25-HC may be involved in a bacterial driven immune response. This study has importance when designing novel biomarkers to predict sepsis.

B197

Towards a clinically relevant model for investigation of host-microbe interactions in ventilator-associated pneumonia

Alexander Tompsett, Lan Ngo Ngoc, Ian Goodhead, Sarah Withers, Joe Latimer

University of Salford, Manchester, United Kingdom

Abstract

Ventilator-associated pneumonia (VAP) is amongst the most common healthcare-associated infections worldwide. Current understanding of the underlying mechanisms has focussed on either the microbiological or physiological elements but host-microbe interactions, which are instrumental in pathogenesis, have received less research focus. This work aims to explore clinically relevant and reproducible models to investigate these interactions in the context of VAP.

A clinical isolate of *Pseudomonas aeruginosa*, a pathogen common in VAP, was investigated in systems of increasing complexity. Sensitivity to key antibiotics (LVX, MEM, and TZP) used for treatment of VAP was unaffected by the presence of cytokines (IL-1β, IL-6, and TNFα) *in vitro*. Larvae of *Galleria mellonella*, an *in vivo* insect model with a rudimentary immune system, was used to test virulence of *P. aeruginosa, Staphylococcus aureus*, and *Klebsiella pneumoniae*. *P. aeruginosa* killed 100% of larvae within 24 hours. *S. aureus* and *K. pneumoniae* killed 43.5% and 50% of larvae respectively, within 8 days. An *ex vivo* mammalian model was developed, which demonstrated abundant *P. aeruginosa* proliferation on lung tissue. After validating inactivation of host lung tissue, we identified changes in the expression of *P. aeruginosa* quorum sensing genes *LasI* and *RhlI*, specifically induced by host interaction.

Our results suggest that host factors may influence bacterial growth and gene expression. We will use these early data to validate and expand our models prior to investigation of clinical samples from VAP patients. We will report our most recent findings in the development of clinically relevant models to investigate VAP.

B198

A Case Report of *Fusobacterium necrophorum* masquerading as *Neisseria* in septic arthritis

Derek Fairley[1,2], Hannah McCormick[1], Conor O'Neill[2], Mark Leith[2], Louise McCorry[1], James Mckenna[1], Adrian Pendleton[1], Yuri Protaschik[1], Anne Loughrey[1], Peter Coyle[2]

[1]Belfast Health & Social Care Trust, Belfast, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

A 17 year old female presented to the rheumatology department with a history of left wrist pain, stiffness and swelling, with associated flu-like symptoms. An initial microscopy report of "Gram negative diplococci" in aspirated joint fluid suggested infection with either gonococcus or meningococcus. Empirical antibiotic therapy (Ceftriaxone) was used to cover for potential meningococcal disease. A bacterial 16S rDNA real-time PCR assay detected a significant bacterial load in the aspirate, although specific real-time PCR testing was negative for both gonococcus and meningococcus. The patient did not clinically improve, and all specimens remained culture negative, so 16S PCR and sequencing was applied directly to the aspirate. The sequences recovered were identified as *Fusobacterium necrophorum*. Necrobacillosis was confirmed by isolation of this organism from the joint aspirate after prolonged incubation.

The outcome was favourable following antibiotic treatment. However, reliance on microscopy findings alone could easily have led to an incorrect diagnosis of gonococcal septic arthritis (a condition with very low complication rates and excellent prognosis) in this case. In contrast, nongonococcal septic arthritis is a medical emergency with significant morbidity and mortality. Infective arthritis with *Fusobacterium* spp. is a rare and difficult to diagnose infection that can be associated with life-threatening thrombophlebitis of the internal jugular vein and/or bacteraemia (Lemierre's Disease).

This highlights the risk of initial microscopy results being misleading, and the utility of bacterial 16S sequencing from normally sterile sites, allowing accurate diagnosis and appropriate treatment.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B199

Propionibacterium acnes induces dysregulation of protease-activated receptor (PAR) gene activity in chronically infected prostate epithelial cells.

Andrew McDowell[1], Declan McCafferty[2], Lorraine Martin[2]

[1]Centre for Stratified Medicine, School of Biomedical Sciences, Ulster University, Londonderry, United Kingdom.
[2]School of Pharmacy, Queen's University, Belfast, United Kingdom

Abstract

There is now increasing interest in the role played by the anaerobic bacterium *Propionibacterium acnes* in the aetiology of prostate cancer (PCa) via chronic, intracellular and asymptomatic infection of the prostate gland leading to oncogenesis. To date, our understanding of how this bacterium could cause PCa remains unclear, although *in vitro* studies do demonstrate its ability to widely stimulate gene dysregulation and induce cellular transformation of prostate epithelial cells. We have taken a pathway-focused approach to understanding how this bacterium may effect RWPE-1 prostate epithelial cells via dysregulation of protease-activator gene activity (PAR), and associated downstream effectors and target genes. PARs are G-coupled cell surface proteins and are overexpressed in many different cancer types, including PCa where they are associated with tumour growth, invasion, metastasis and biochemical recurrence. Using a PAR signalling gene expression array, our results show that after a chronic 2-week infection *in vitro* with *P. acnes*, both PAR-1 and PAR-2 are significantly overexpressed, alongside significant dysregulation of genes encoding potential cancer mediators associated with inflammation, proliferation, angiogenesis and migration. Functional PAR-1 and PAR-2 upregulation was confirmed using a calcium mobilisation assay, which measures an ephemeral rise in intracellular calcium when cells are treated with agonist peptides against the receptors. These results provide additional evidence that *P. acnes* in the prostate gland may be a clinically relevant observation and a potential risk factor for PCa development.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B200

Development of biofilm-penetrant antimicrobial delivery system to counter *Burkholderia infections*

Adam Pattinson[1], Louis Laurence[1], Michael McArthur[1,2]

[1]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [2]Procarta Biosystems, Norwich, United Kingdom

Abstract

Melioidosis is caused by the Gram-negative, intracellular bacterium *Burkholderia pseudomallei.* The epidemiology of the disease is unclear- it is limited to tropical regions, where monitoring can be sparse and further complicated by the multifaceted nature of the condition. The most common indication of acute melioidosis is pneumonia (51%) that can progress to acute fulminant sepsis with multifocal infiltrates with high rates of mortality. Meliodosis has been estimated to cause 89,000 deaths annually with an estimated mortality rate of approximately 50%. Due to ease of the dissemination of the pathogen, its ability to form biofilms, the high degree of antimicrobial resistance and paucity of effective treatments, controlling the infection is a major challenge. Our approach is to develop nanoparticles capable of effectively delivering antimicrobials to biofilms formed by *Burkholderia* species. Formulations of a proprietary lipidic delivery agent, CM2, have been tested against two species: *B. cepacia* UCB717 that has no capsule and *B. thailandensis* (that is used as a surrogate for *B. pseudomallei*), including strains with and without capsules both of which readily form biofilms. Confocal laser scanning microscopy was used to monitor the uptake of the nanoparticles and the MIC and MBEC of CM2 alone (and analogues), in combination with a panel of antimicrobials and a novel oligonucleotide antimicrobial, termed a Transcription Factor Decoy (TFD), were measured. The most efficacious combinations will be formulated for delivery by inhalation prior to testing in animal models.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B201

The vaginal microbiota differs between women who deliver preterm relative to those who deliver full-term

Conor Feehily[1,2], David Crosby[3], Raul Cabrera-Rubio[1,2], Shane Higgins[3], Fionnuala M McAuliffe[3], Paul D Cotter[1,2]

[1]Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland. [2]APC Microbiome Ireland, University College Cork, Cork, Ireland. [3]UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin, National Maternity Hospital, Dublin 2, Ireland

Abstract

Changes in the vaginal microbiota, and more specifically bacterial vaginosis (BV), have been linked to preterm birth (PTB). The risk of PTB is reported to increase up to 2-fold in pregnant women with BV. BV is characterised by a shift in healthy populations of lactobacilliin the vagina towards a more mixed-species microbiota that mainly includes *Gardnerella vaginalis*, *Atopobium vaginae* and *Prevotella* sp., among others. Despite the reported link between BV and PTB, controversy remains. Our study aimed to use next-generation sequencing to examine the relationship between the vaginal microbiota and the onset of pre-term birth. 37 pregnant women after 10 weeks gestation who were at risk of pre-term delivery and a further 11 pregnant women deemed not at risk were recruited and the associated vaginal microbial composition (16S rRNA) determined. Distinctly different patterns of *Lactobacillus* populations were evident within samples collected from the 8 women who subsequently delivered preterm compared to those who had full-term pregnancies. *L. crispatus* was significantly reduced in the preterm birth group. It was also evident that the preterm birth group clustered into two distinct community state types, CST-V & CST IIIa, dominated by *L. jensenii* and *L. iners,* respectively. There was no significant difference in microbial diversity across groups nor was a distinct BV associated community identified in the preterm group.

B202

Acquisition of fluoroquinolone resistance in *Campylobacter jejuni* leads to an increase in biofilm formation and virulence.

Matthew Whelan, Jeremy Simpson, Tadhg Ó Cróinín

University College Dublin, Dublin, Ireland

Abstract

Campylobacter jejuni is the leading cause of bacterial gastroenteritis with over 550 million cases reported yearly. The World Health Organization has listed *C. jejuni* as one of 12 microorganisms on a global priority list for antibiotic resistance due to a rapid increase in the number of strains resistant to fluoroquinolone antibiotics. This fluoroquinolone resistance is conferred through a single point mutation in the QRDR region within the *gyrA* gene which is also involved in DNA supercoiling homeostasis. We recently revealed that changes in DNA topology play a major role in the regulation of virulence in *C. jejuni* with relaxation of DNA supercoiling associated with increased attachment to and invasion of human epithelial cells. The aim of this study was to investigate whether fluoroquinolone resistant strains of *C. jejuni* displayed altered supercoiling associated phenotypes. A panel of mutants were derived against nalidixic acid and ciprofloxacin and shown to have a greater ability to form viable biofilms under aerobic conditions and that this phenotype was associated with changes in DNA supercoiling levels. These mutants were also shown to have an increased ability to attach to and invade epithelial cells *in vitro* and conferred an increase in the killing efficiency of *Galleria mellonella*. We report for the first time that fluoroquinolone resistance in *C. jejuni* is associated with an increase in virulence and the ability to form viable biofilms in oxygen rich environments. These altered phenotypes may play a critical role in the continued increase in fluoroquinolone resistance observed for this important pathogen.

B203

Investigating the uptake mechanism of S type pyocins

Faisal Albaqami, Christopher Penfold, Kim Hardie, Philip Bardelang

University of Nottingham, Nottingham, United Kingdom

Abstract

Pseudomonas aeruginosa (P.aeruginosa) is an opportunistic pathogen with a high mortality and morbidity rate. It has many mechanisms of resistance to antibiotics, which makes it hard to treat. Pyocin is a protein that is produced by *P.aeruginosa* that kills related strain bacteria. pyocin type S bind to ferrisiderophore receptors that uses the TonB system to translocate into the bacterial cell. The aim of this study was to express and purify receptor and translocation (R+T) domains of pyocin S1, S2 and S3 plus the TonB1 receptor and ToLAIII receptor of *P.aeruginosa* to determine if pyocins S uses these to transverse the inner membrane of target cells. IPTG was used to induce the protein in the expression and analyzed by SDS-PAGE gel, giving fragment size of 11 kDa (ToLAIII), 23kDa (TonB1), 45 kDa (S2 R+T) and 79 kDa (S3 R+T). Protein purification was cried on the four proteins using affinity chromatography technique by His-tag in C-terminal of S2 and S3 R+T domain, N-terminal of TonB1 and ToLAIII proteins. In addition, Gel Filtration chromatography was used to further purify the proteins so that the interaction between them could be tested.This study confirmed that the proteins used is expressed and purified, so in the future study the gel filtration would be carried out for R+T domains of the other S pyocins and TonB1 protein so that the interaction would be tested between the proteins in the study.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B204

Gut microbiota derived mitochondrial inhibitors cross the blood brain barrier and localise white matter

Heather Hulme[1], Maya Kamat [1], Lynsey Meikle[1], John Swales[2], Ryan Bragg[2], Victor V. Torres[3], Michael Ormsby[1], Saverio Tardito[3], Gillian Douce[1], Justin Van Der Hooft[1], Julia Edgar[1], RuAngelie Edrada-Ebel[4], Richard J. A. Goodwin[2], Richard Burchmore[1], Daniel M. Wall[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]AstraZeneca, Cambridge, United Kingdom. [3]Cancer Research UK Beatson Institute , Glasgow, United Kingdom. [4]University of Strathclyde, Glasgow, United Kingdom

Abstract

The microbiome-gut-brain (MGB) axis is a bi-directional route of communication that exists between the brain and the microbes that reside in the gut. The MGB axis is becoming of increasing importance as significant alterations in the gut microbiota are now linked to numerous neurological conditions, however, little is currently known about the microbiome derived mediators of communication. Here we used mass spectrometry imaging (MSI), a label free imaging technique, to identify bacterial products that cross the blood brain barrier in specific pathogen free (SPF) mice. We identified two bacterial molecules abundant in white matter regions of the murine that were absent in the brain and gut in germ free (GF) mice. We have identified the primary gut microbial producers of these metabolites to be members of the *Lachnospiraceae* family. Both molecules were found to be structurally similar to carnitine and localise with carnitine in the SPF mouse brain. Using a primary murine cell culture model of the central nervous system white matter we show that these molecules are capable of significantly impairing mitochondrial basal respiration. Given their systemic presence in the mouse and their presence in human biological samples, these metabolites may have significant implications for diseases associated with mitochondrial dysfunction and an altered gut microbiota. These results are the first to describe a direct molecular inter-kingdom communication between prokaryotes and the mammalian brain that can facilitate functional inhibition in mammalian brain cells.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B205

The UK Public Health Rapid Support Team: A Novel Programme Integrating Outbreak Response, Operational Research, and Capacity Building

Jonathan Ashcroft[1,2], Benedict Gannon[3,2], Hilary Bower[4,5], Emilio Hornsey [6,2], Matt Knight[4,2], Jimmy Whitworth[6,5], Daniel G. Bausch[4,5]

[1]Public Health England, Porton Down, United Kingdom. [2]London School of Hygiene and Tropical Medicine , London, United Kingdom. [3]Public Health England , Porton Down, United Kingdom. [4]Public Health England, London, United Kingdom. [5]London School of Hygiene and Tropical Medicine, London, United Kingdom. [6]Public Health England , London, United Kingdom

Abstract

The 2013-16 epidemic of Ebola virus disease underscored the shortcomings of the international community to both respond to outbreaks and conduct critical research in complex humanitarian crises. To address these concerns, the UK Government has formed the UK Public Health Rapid Support Team (UK- PHRST). The UK-PHRST is a collaboration between Public Health England and the London School of Hygiene & Tropical Medicine with the University of Oxford and Kings College London as academic partners. The UK-PHRST has a novel triple mandate to work in low- and middle-income countries (LMICs) to:

- Respond to outbreaks
- Conduct innovative operational research during and between outbreaks to generate evidence on best practices
- Build LMIC and regional capacity for outbreak response

B206

Mobilome and resistome analysis of multidrug-resistant *Escherichia coli* isolates from urinary tract infections

Maria Getino, Arnoud van Vliet, Amanda Fivian-Hughes, Roberto La Ragione

University of Surrey, Guildford, United Kingdom

Abstract

Urinary tract infections (UTIs) are one of the most common clinical presentations in health care facilities worldwide. The most common aetiology of UTIs is *Escherichia coli*, a widespread bacterium often carrying multiple genes to survive antibiotic treatment, usually encoded by mobile elements that can be distributed to a wide range of bacteria. In the studies presented here, a collection of 245 uropathogenic *E. coli* strains were isolated from three different hospitals in the South England area to study the most prevalent genetic elements involved in the transmission of antimicrobial resistance. The collection is mainly composed by multi-drug resistant isolates conferring resistance to extended-spectrum beta-lactam antibiotics, based on the resistance profile carried out by the corresponding hospital microbiology laboratory. The entire set of isolates were genotypically characterised by multiplex PCR for the presence of genes conferring resistance to beta-lactams (*blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M*, and *blaAmpC* genes) and colistin (*mcr-1* and *mcr-2* genes). Most of the isolates (78%) were positive for one or more beta-lactam resistance genes, while all of them were found negative for the colistin resistance genes *mcr-1* and *mcr-2*. A set of 96 isolates was selected for whole genome sequencing in order to further analyse the presence of antimicrobial resistance determinants, especially those carried by mobile genetic elements. The bioinformatics analysis will provide additional information including phylotype, serotype, virulence traits, metal resistance genes, mobility genes, and plasmid content.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B207

Bacterial Responses to Candidate Antimicrobials from Historical Pharmacopeias

Jessica Furner-Pardoe[1], Ricky Cain[2], Catharine Ortori[3], David A. Barrett[3], Freya Harrison[2]

[1]the University of Warwick , Coventry, United Kingdom. [2]The University of Warwick , Coventry, United Kingdom. [3]The University of Nottingham , Nottingham , United Kingdom

Abstract

Combatting the rise in antimicrobial resistance is one of the major challenges in modern science. Ethnopharmacology (the study of traditional pharmacopeias) could help reveal new antibiotics. However, the standard approach of purifying individual compounds from natural materia medica rarely produces clinically-useful products. Historical medical manuscripts, in contrast, prescribe complex preparations of several ingredients to treat infections and it is suspected their efficacy may rely on creating a cocktail of natural products. A reconstructed 1000-year-old remedy, containing onion, garlic, wine and bile salts, can kill methicillin-resistant *Staphylococcus aureus* in a mouse chronic wound model, and *Pseudomonas aeruginosa* in an ex vivo chronic lung infection model. Despite garlic's well characterised antimicrobial activity, our initial results have demonstrated that the efficacy depends on the combination of ingredients and here we demonstrate the role of each ingredient within the remedy. In the interests of understanding the remedy's action at a chemical level, techniques including HPLC, liquid chromatography and mass spectroscopy have been used to identify known antimicrobial molecules and narrow down potential new antimicrobial molecules.

B208

Functionalised liposomal formulations for delivery of antibiotic agents

Rosa Catania, Oran Maguire, Chris Moore, Franco H. Falcone, Weng Chan, Giuseppe Mantovani, Snow Stolnik, Alan Huett

University of Nottingham, Nottingham, United Kingdom

Abstract

Antimicrobial resistance is a major global healthcare challenge. Beyond the discovery of novel antimicrobial agents, the development of novel formulations for enhancing current antibiotics is a promising strategy to reduce the rate of treatment failure. Drug delivery nano-carriers can achieve high local concentration of antimicrobial agents, reduce toxicity, and improve biodistribution and pharmacokinetics.

We have taken two approaches to enhance antibiotic delivery and effectiveness. Firstly, we used a bespoke targeted liposomal system for intracellular antibiotic delivery to phagocytic cells. This enables treatment of an intracellular Gram-negative infection with a cell-impermeable antibiotic. Targeted liposomes were found to significantly enhance uptake compared to uncoated liposome control formulations both in *in vitro* and *in vivo* (zebrafish model).

Secondly, liposomal nanoformulations were utilised to deliver peptide antibiotics, where liposomes protect peptides from degradation, and allow potential co-delivery of combination therapeutics. We investigated different liposomal formulations and drug combinations against *E. coli* and *S. aureus*. We show that peptide-loaded liposomes are more efficient compared to free drug in inhibiting the growth of both Gram-negative and Gram-positive bacteria.

These initial results suggest that liposome-mediated delivery can be utilised for the repositioning and repurposing of existing antibiotics, potentially allowing for the treatment of diverse infections in a more effective manner.

B209

Continuous culture of *Escherichia coli*, under selective pressure by a novel antimicrobial complex, does not result in the development of resistance

Lilit Tonoyan[1], Ruairi Friel[1], Gerard Fleming[2], Vincent O'Flaherty[2]

[1]Westway Health Ltd., Galway, Ireland. [2]National University of Ireland, Galway, Galway, Ireland

Abstract

Antibiotic resistance is a major global health problem. Preservation of antibiotics, underpinned by the availability of novel antimicrobials that avoid the emergence of antimicrobial resistance and antibiotic cross-resistance is an important societal goal. We have developed a novel biocidal complex (iodo-thiocyanate complex or ITC), drawing the inspiration from naturally occurring peroxidase-catalysed systems. This study was aimed to reveal the potential of ITC for induction of resistance and cross-resistance, and, thus, different aspects of resistance were explored. We show that the repeated exposure of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and methicillin-resistant *S. aureus* to sub-inhibitory levels of ITC during serial passage of batch cultures did not generate ITC resistance. By comparison, *E. coli* and *S. aureus* developed low-level and high-level resistance to levofloxacin (LVX), respectively. Further, we attempted to generate *de novo* resistance in antimicrobial-sensitive *E. coli* during 20-days of continuous culturing when exposed to gradually increasing concentrations of ITC and LVX. The exposure of *E. coli* to ITC did not induce resistance to ITC, or cross-resistance to LVX. No distinct mutational pattern was evidenced from whole-genome sequence (WGS)-based analysis of ITC-challenged bacterial populations. By contrast, the resistance to LVX was rapidly induced, selected for high-level and enriched with a distinctly characteristic genome mutational pattern. WGS of LVX-challenged population revealed that the majority of mutations appeared in the genes of LVX target proteins and drug influx. This study suggests that the usage of ITC may not trigger the emergence of facile resistance or cross-resistance, in contrast to common antibiotics.

B210

Do Streptococcus pneumoniae and Respiratory Syncytial Virus synergise to promote invasive disease?

Khandaker Shadia, Marie Yang, Aras Kadioglu

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

Abstract

Streptococcus pneumoniae (S.p.) and Respiratory Syncytial Virus (RSV) are two major pathogens commonly found to coexist in respiratory secretions in patients with acute respiratory infection. Though there is increasing evidence of a synergistic interplay between these two pathobionts, the exact mechanisms remain obscure.

The aim of our study was to decipher how coinfection with RSV alter pneumococcal growth dynamics and host immune response and how this impact on the colonisation and invasive properties of S.p.

Using *in vivo* mouse model, we made the key observation that upon coinfection with RSV, the density of pneumococcal colonisation in the nasopharynx and dissemination to the lower respiratory tract were significantly higher in mice previously colonised with S.p. These mice also presented more severe weight loss and delayed recovery compared to mono-infected animals as well as significantly heightened pro-inflammatory cytokine profiles. Measurement of *in vitro* transepithelial electrical resistance (TEER) showed that, upon RSV coinfection, S.p. transmigrate through the epithelial barrier without altering epithelial integrity suggesting a transcellular mechanism rather than paracellular migration. Moreover, RSV-pneumococcal coinfection of human primary nasal epithelial cell demonstrated major changes in host protein expression involved in the catalytic activity, ubiquitination, cytoskeletal organisation, and endocytosis. Simultaneously, significant upregulation observed in bacterial proteins involved in the ribosomal activity, streptococcus-induced tissue inflammation, DNA supercoiling, and bacterial viability during oxidative stress, affecting both the survival and the virulence of S.p.

Our results explain the complex interactions between pneumococci, RSV and host and help towards further understanding the significance of viral-bacterial co-infection in clinical settings.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B211

Effect of metronidazole on microbiomes associated with asymptomatic bacterial vaginosis

Makella Coudray, Daniel Ruiz-Perez, Brett Colbert, Karl Krupp, Hansi Kumari, Giri Narasimhan, Kalai Mathee, Purnima Madhivanan

Florida International University, Miami, USA

Abstract

Asymptomatic Bacterial Vaginosis (BV) damages vaginal epithelium increasing risk of sexually-transmitted infections. *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella amnii, P. bivia,* and *Candida albicans* are associated with BV. Presence of *Lactobacillus* spp is indicative of a healthy microbiota. Symptomatic BV is treated with metronidazole. The role of microbiota and metronidazole treatment in the recurrence and persistence of asymptomatic BV remains to be elucidated. This study uses whole genome sequencing (WGS) to determine the microbiota changes with metronidazole treatment.

DNA from 20 vaginal swabs was obtained at four time points over 12 months from five African American women and was subjected to WGS. The first time point is the untreated baseline. All subjects were tested every 4 months and received a course of metronidazole for each episode of BV during the 12 months period. Nugent scores were used to classify BV status. The microbial profiles were analyzed along with the sociodemographic metadata.

Despite treatment, the participants did not recover from BV — two participants experienced persistent BV, and the rest had recurrent BV. WGS analyses show that *G. vaginalis* was the most abundant organism as compared to *Lactobacillus sp*ecies. The metronidazole treatment resulted in the loss of *Lactobacillus* and *Prevotella* species. One participant scored healthy based on Nugent score at one time point, during when Lactobacillus species dominated the microbiome.

Based on this pilot longitudinal study, metronidazole may not be an effective treatment for asymptomatic BV. Studies with larger cohorts can lead to statistically significant conclusions to develop alternative interventions for asymptomatic BV.

B212

Development of 'smart' wound dressings for biofilm sensing and control

Erin Magee, Nathan Wells, Andrew Mills, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

Chronic wounds affect approximately 2% of the worldwide population and incur healthcare costs in the billions. Key to their persistence is the formation of microbial biofilms, which are accounted for in nearly 80% of all non-healing wounds. The smart dressing presented herein aims to detect a range of volatile infection protagonists, with a striking colour change that can be visualised with the naked eye, providing 24/7, non-invasive monitoring of infection development and antimicrobial treatment efficacy.

A range of coloured indicator films housing dyes responsive to volatile analytes in the wound headspace were developed and tested against porcine skin inoculated with *Pseudomonas aeruginosa*. Digital images of the indicator film were captured at regular time intervals and the resulting images were aligned and split into red, green and blue (RGB) colour channels to yield semi-quantitative data. vAPCI-MS was exploited to identify additional volatiles for incorporation into the smart dressing design.

A CO_2-sensing film comprising xylenol blue dye underwent a marked colour change from blue to yellow within 12 hours of inoculation with PAO1, whilst indicators monitoring uninoculated control skin remained blue (no colour change). In addition, vAPCI-MS identified putrescine as an additional volatile of interest, and responsive indicator films were developed for its detection.

The marked colour change exhibited by each indicator film is easily visualised by eye and can be digitally analysed to provide semi-quantitative data. This early warning, point-of-care technology is a promising candidate in combatting biofilm development in wounds.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B213

Advanced titanium dioxide-polytetrafluorethylene (TiO2-PTFE) nanocomposite coatings on stainless steel surfaces exhibit significant antibacterial and anti-corrosion properties

Shuai Zhang[1], Xinjin Liang[1,2], Geoffrey Gadd[1], Laszlo Csetenyi[1], Qi Zhao[1]

[1]University of Dundee, Dundee, United Kingdom. [2]University of South Wales, Treforest, United Kingdom

Abstract

Bacterial infection and corrosion are the two of the most significant causes of metallic implant failure. In our study, we innovated a facile two-step approach to synthesising a TiO2-PTFE nanocomposite coating on stainless steel, which endows the implant surface with both antibacterial and anticorrosion properties. By harnessing the adhesion and reactivity of bioinspired polydopamine, the TiO2-PTFE coating was uniformly deposited onto substrates by using a sol-gel dip coating technique. The TiO2-PTFE coating exhibited minimal bacterial adhesion against both Gram-negative Escherichia coli WT F1693 and Gram-positive Staphylococcus auerus F1557. Moreover, it was observed that an increasing TiO2 concentration in the bath enhanced antibacterial activity. Benefiting from the synergistic effect between TiO2 and PTFE, the TiO2-PTFE coating showed improved corrosion resistance in artificial body fluids comparing with the sole TiO2 and PTFE coatings. The TiO2-PTFE coating also demonstrated extraordinary biocompatibility with fibroblast cells in culture, making it a prospective useful strategy to overcome current challenges in the use of metallic implants.

B214

Old drugs learn new tricks - Repurposing phenothiazines to uncover effective antimicrobial

Daniela Alves Ferreira, Marta Martins

Trinity College Dublin , Dublin , Ireland

Abstract

Thioridazine (TZ) is an antipsychotic drug that acts against antibiotic resistant bacteria. The main aim of this study was to uncover the mechanism of action of TZ using *Salmonella enterica* serovar Thypimurium as a model bacterium.

The antibacterial activity of TZ was initially determined based on its minimum inhibitory concentration (MIC). Membrane permeability assays were performed and fluorescence measured using the Ethidium Bromide accumulation assay. *Salmonella* was exposed to TZ and its effects on membrane potential and cell wall assessed by flow cytometry and Transmission Electron Microscopy, respectively. Effects on the bacterial proteome were assessed through 2D gel electrophoresis. Infection assasys were performed in THP-1 ad RAW 264.7 cells treated and non-treated with TZ.

The MIC of TZ against Salmonella was 200 mg/L. Our *in vitro* data demonstrates that TZ mechanism(s) of action involves primarily *Salmonella*'s membrane by affecting its permeability and potential after 15 minutes of exposure to TZ. At half of the MIC, and only after 15 minutes, TZ disrupts the bacterial membrane leading to leakage of the cellular contents and lysis of *Salmonella*. Proteomic profiling revealed 75 upregulated and 62 downreuglated proteins. Infected macrophages treated with sub-MIC of TZ, showed a reduction on intracellular CFU/mL. This may be indicative of TZ's ability to enhance the killing activity of infected macrophages.

The results obtained suggest that TZ may act *in vitro* by targeting the bacterial cell-envelope. Due to its effect on infected macrophages, TZ may be considered a useful adjuvant to current therapeutics.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B215

The impact of a horizontally acquired virulence plasmid on Bacillus cereus G9241, the causative agent of an anthrax-like illness.

Grace Taylor-Joyce, Tom Brooker, Nick Waterfield

University of Warwick, Coventry, United Kingdom

Abstract

Bacillus cereus, *Bacillus anthracis* and *Bacillus thuringiensis*, are Gram-positive, spore- forming bacteria and principle members of the *Bacillus cereus* sensu lato complex. The species are highly similar at a chromosomal level, but are phenotypically diverse due to the presence of different plasmids. The anthrax pathogen, *B. anthracis* contains two virulence plasmids, pXO1 and pXO2. The pXO1 plasmid carries the anthrax toxin genes which are involved in intracellular survival and suppression of immune cell function while pXO2 carries capsule genes which are required for the pathogen to evade phagocytosis. Both plasmids are required to allow *B. anthracis* to act as a highly virulent mammalian pathogen. As well as encoding toxins, the pXO1 plasmid encodes *atxA*, a transcriptional regulator that is able to control gene expression from both the plasmid and chromosome. It is proposed that AtxA is incompatible with the chromosomally encoded PlcR, a global transcriptional regulator which controls expression of secreted haemolytic and cytolytic toxins. This has led to the genetic inactivation of *plcR* in all *B. anthracis* isolates and driven the evolution of high mammalian virulence. Interestingly, there are several *B. cereus* isolates that possess a pXO1-like plasmid, called pBCXO1, which are capable of inducing an anthrax like illness. Importantly, genome sequencing of one such strain, *B. cereus* G9241, revealed intact copies of both *atxA*and *plcR* genes. This project aims to understand how G9241 has evolved to accommodate both regulators. We have used a pBCXO1-cured strain of G9241 to study the influence of AtxA and pBCXO1 on the biology of G9241 and how it interacts with human macrophage.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B216

Staphylococcus aureus Targets Corneodesmosin to Initiate Skin Colonisation and Infection in Atopic Dermatitis

Aisling Towell[1], Pauline Vitry[2], Cecile Feuillie[2], Alan Irvine[1,3], Yves Dufrêne[2,4], Joan Geoghegan[1]

[1]Trinity College Dublin, Dublin, Ireland. [2]Université catholique de Louvain, Louvain-la-Neuve, Belgium. [3]National Children's Research Centre, Dublin, Ireland. [4]Walloon Excellence in Life sciences and Biotechnology (WELBIO), Wavre, Belgium

Abstract

An association between the chronic inflammatory human skin disease atopic dermatitis (AD) and the bacterium *Staphylococcus aureus is well established*. AD patients with severe disease frequently carry a high burden of *S. aureus* on their skin and have reduced levels of natural moisturizing factor (NMF) in the stratum corneum. Despite this association, there is a lack of understanding of the molecular determinants of skin colonization and infection in AD. The aim of this study was to investigate the molecular basis of bacterial adherence to skin corneocytes in AD. We have recently shown that *S. aureus* adheres more strongly to corneocytes on skin with low NMF levels. Low NMF corneocytes from AD, unlike normal corneocytes, are covered with villus-like projections (VPs). The tips of VPs are decorated with corneodesmosin, a cohesion protein normally confined to the junctions between corneocytes in the uppermost layers of the epidermis. Here we show that corneodesmosin is the major ligand for *S. aureus* in AD skin. The cell wall-anchored proteins FnBPB and ClfB mediate bacterial adherence to recombinant corneodesmosin *in vitro*. *S. aureus* bound strongly to low NMF corneocytes while a mutant deficient in both FnBPB and ClfB did not bind. High-resolution imaging revealed that the binding of *S. aureus* was mostly concentrated on the VPs, where corneodesmosin is located. Consistent with this, blocking with antibodies against corneodesmosin reduced adherence of *S. aureus*. In summary this study identifies a novel interaction between *S. aureus* and corneodesmosin providing new insights into skin colonisation in AD.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B217

A liposomal drug delivery system for improved eradication of *Helicobacter pylori*

Nicola Osborne, Rosa Catania, Snow Stolnik-Trenkic, Franco H. Falcone, Karen Robinson

University of Nottingham, Nottingham, United Kingdom

Abstract

Helicobacter pylori is the leading cause of peptic ulcers and gastric cancer. Eradicating *H. pylori* infections is becoming more difficult due to increasing antibiotic resistance and poor patient compliance. We aim to develop a novel liposomal drug delivery system that encapsulates antibiotics and the antimicrobial fatty acid linolenic acid (LLA). We hypothesise that *H. pylori* will have much lower resistance rates to this dual formulation.

Liposomes consisting of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), sphingomyelin, and cholesterol were produced using the thin film hydration method. The size of our liposomes was assessed using dynamic light scattering (DLS), and their antimicrobial activity was assessed using a viable count assay.

The liposomes had a particle size ranging from 95-150 nm. We have successfully loaded LLA up to 20% of total lipid composition, and shown stability of the particles in storage at 4°C for up to 3 months. The LLA liposomes achieved complete eradication of *H. pylori in vitro* at 150 μg/mL, whereas control liposomes containing no LLA had no antimicrobial effect. We have recently formulated liposomes encapsulating LLA and amoxicillin at 2.2 mg/mL and 0.99 mg/mL respectively, and are currently assessing their antimicrobial activity against *H. pylori.*

In conclusion, it is possible to prepare LLA- and amoxicillin-containing liposomes and LLA has antimicrobial activity against *H. pylori.* In future work we will utilise strategies to enhance gastric retention which will provide local bactericidal activity, improving on inefficient systemic uptake mechanisms with current therapies.

B218

Isolation and Characterization of *Clostridioides difficile* spores from contaminated single-used surgical gowns.

Shannon Waterfield[1], Lee Hutt[1], Robert Burky[2], Lovleen Tina Joshi[1]

[1]University of Plymouth, Plymouth, United Kingdom. [2]Adventist Health, Yuba City, USA

Abstract

Clostridium difficile is the primary cause of antibiotic associated diarrhoea globally. In the UK there has been a decline in the prevalence of *C. difficile* due to implementation of surveillance and infection control procedures. At Rideout Hospital, USA, however, there is a high incidence of *C. difficile* infection, which has been partly attributed to poor infection control measures. Other factors include the ability of spores to adhere to fomites such as surgical gowns. It has been demonstrated that the single-use polypropylene surgical gowns used at Rideout can 'trap' hydrophobic epidemic spores of *C. difficile* within the fibres, which can then be transferred to stainless steel surfaces and hospital floor vinyl; even with use of appropriate sporicides such as sodium dichloroisocyanurate. This study sought to establish the strains of *C. difficile* present on the gowns and thus inside the nosocomial environment. Contaminated gowns from Rideout were cultured for 5 days anaerobically in Brain –Heart Infusion broth supplemented with 0.1% Sodium taurocholate. Broth culture was screened for the presence of *C. difficile* using CCFA media, C. DIFF QUIK CHEK COMPLETE®, 16s-23s RNA analysis and toxin PCR. Once isolated, strains were sequenced and tested for biocide susceptibility to in-use concentrations of Sodium dichloroisocyanurate. In total 23 suspected *C. difficile* samples were isolated from the gowns; of which 8 were confirmed. Sporicide susceptibility testing is ongoing. Once infective strains have been identified measures can be taken to enforce appropriate infection control procedures in order to limit the prevalence of spores and reduce infection rates.

B219

Understanding the ecology and evolution of polymicrobial wound infections

Ana da Silva[1], Tim Sloan[1], Paul Williams[1], Steve Diggle[2]

[1]University of Nottingham, Nottingham, United Kingdom. [2]Georgia Tech University, Georgia, USA

Abstract

Chronic wounds (CW) are a common complication of diabetic ulcers (DUs), which are a major burden to health care systems worldwide and can result in lower limb amputation due to the intractability of the infection. In DUs there is a high probability of the infecting bacteria evolving considerable phenotypic and genetic diversity, as has previously been shown for chronic lung infections. However, it is not known whether this is also the case for chronic DUs, and whether diversity impacts on virulence and antibiotic resistance. To study this, bacterial populations were isolated from different samples from patients with DUs. Phenotypic diversity was investigated in *P. aeruginosa* populations through the analysis of phenotypes traditionally associated with pathogenicity, and through a whole genome study.

Phenotypic variation in *P. aeruginosa* isolates taken from different patients was observed, but little variation within the same CW (with exception of one patient with a leg ulcer). Antibiotic resistance was found to increase during the course of infection, and it became apparent that *P. aeruginosa* colonisation in DUs is via a single strain per ulcer, and potentially per patient, even though some sample-specific phenotypic profiles were found to arise from a homogenous population. For this case, a detailed genomic analysis between bone and blood isolates was done, including a comparison of their transcriptomes using RNAseq. The results suggest that the loss of flagellum facilitated evasion of the innate immune system, which allowed bacteria to go undetected and spread systemically causing the rapid decline in the patient's health.

B220

Incidence of Pseudomonas aeruginosa Resistance in a Tertiary Hospital

Oluwatosin Ajibade[1], Oladipo Kolawole[2], Idowu Adeosun[3], kolade Aina[1], Titilayo Olotu[1], Olubukola Oyawoye[1], Olutoyin Bamigboye[1]

[1]Adeleke Univeristy, Ede, Nigeria. [2]Ladoke Akintola University of Technology, Ogbomosho, Nigeria. [3]Adeleke, University, Nigeria

Abstract

Antibiotic is a formidable remedy to infections caused by diverse microbial agents. This assertion is however questioned in the wake of antimicrobial resistance. Fifty clinical isolates of *Pseudomonas aeruginosa* were obtained from both in and out-patients using standard procedure. The isolates were identified using standard biochemical tests. The antibiotic susceptibility pattern of each isolate was examined in accordance to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the Kirby-*Bauer's* disc diffusion method. The antibiotics used in the study includes: Ciprotab, Colistin-sulphate, Meropenem, Ceftraxone and Cefepine. Out of the clinical isolates obtained, a total of 48 per cent male and 52 per cent females were the population under study. The percentage ratio of in -patient and out-patient examined were 32% to 68%. The percentage distribution of the administration class for medical and surgical was 34% and 66% respectively. The highest incidence of *Pseudomonas aeruginosa* was from patients that have undergone cesarean section (28%). Highest susceptibility was observed in Ciprotab (82%) Meropenem (64%) and Ceftraxone (46%). Highest number of resistance was observed against Cefepine and Colistin Sulphate while less than 5% were resistant to Ciprotab and Meropenem. Meropenem and ciprotab were the two classes of drugs that showed highest activity against *Pseudomonas aeruginosa*. Commonly used antibiotics must be continuously examined for its efficacy. Anti-microbial susceptibility monitoring is necessary inorder to guide physicians in prescribing the right combinations of anti-microbials to limit and prevent the emergence of multi-drug resistant strains of *P. aeruginosa*.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B221

No Clinical Benefit of Empirical Antimicrobial Therapyfor Pediatric Diarrhea in a High-Usage, High-ResistanceSetting

Tuyen Ha Thanh, Stephen Baker

Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam

Abstract

Pediatric diarrheal disease presents a major public health burden in low- to middle-income countries. The clinical benefits of empirical antimicrobial treatment for diarrhea are unclear in settings that lack reliable diagnostics and have high antimicrobial resistance (AMR). In this study, conducted a prospective multicenter cross-sectional study of pediatric patients hospitalized with diarrhea containing
blood and/or mucus in Ho Chi Minh City, Vietnam. Clinical parameters, including disease outcome and treatment, were measured. Shigella, nontyphoidal Salmonella (NTS), and Campylobacter were isolated from fecal samples, and antimicrobial susceptibility profiles were determined. Statistical analyses, comprising log-rank tests and accelerated failure time models, were performed
to assess the effect of antimicrobials on disease outcome. Among 3166 recruited participants (median age 10 months; interquartile range, 6.5–16.7 months), one-third (1096 of 3166) had bloody diarrhea, and 25% (793 of 3166) were culture positive for Shigella, NTS, or Campylobacter. More than 85% of patients (2697 of 3166) were treated with antimicrobials; fluoroquinolones were the most commonly administered antimicrobials. AMR was highly prevalent among the isolated bacteria, including resistance against fluoroquinolones and third-generation cephalosporins. Antimicrobial treatment and multidrug resistance status of the infecting pathogens were found to have no significant effect on outcome. Antimicrobial treatment was significantly associated with an increase in the duration of hospitalization with particular groups of diarrheal diseases. Our results imply a lack of clinical benefit for treating diarrhea with antimicrobials in a setting using high antimicrobials; adequately powered randomized controlled trials are required to assess the role of antimicrobials for diarrhea.

Infection forum

Presentations: Wednesday evening and Thursday lunchtime

B222

Immunoregulatory Properties of Helicobacter pylori Molecules

Ekene Ogbodo[1], Joanne Rhead[1], Darren Letley[1], Kazuyo Kaneko[1], Jody Winter[2], Karen Robinson[1]

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

Abstract

Helicobacter pylori infection is one of the most prevalent worldwide, and humans have co-evolved with these bacteria. Infection rates have been declining in recent decades, and this is linked to increased occurrence of immune and inflammatory diseases such as asthma and multiple sclerosis. We previously reported that *H. pylori*-infected patients have higher frequencies of IL-10-secreting immunosuppressive regulatory T cells (Tregs) in their gastric mucosa and peripheral blood.

Three components of *H. pylori* which induced IL-10 secretion from Tregs were identified: catalase (KatA), Peptidyl-prolyl cis-trans isomerase (PPT) and g-Glutamyl transpeptidase (GGT). These were cloned, expressed in ClearColi^{O} BL21(DE3) *E. coli* to minimise effects from LPS*,* purified and characterised. LPS content in the recombinant proteins was assayed, using an E-TOXATE assay, and shown to be <0.1 EU/ml. Jurkat T-cells and THP-1 monocytic cells were incubated for 1 hour with 10, 25 and 50 mg/ml of the recombinant proteins, prior to activation with PMA/Ionomycin or LPS. After 24 hours IL-2 (Jurkat cells) and IL-6 (THP-1) concentrations were quantified by ELISA.

All three proteins induced a dose-dependent reduction in cytokine production, compared to controls treated only with PMA/Ionomycin or LPS. KatA most strongly suppressed IL-2 secretion by Jurkat cells (79.5% reduction with 50 mg/ml, $p<0.05$), whereas GGT was most effective in suppressing IL-6 from THP-1 cells (68.07% reduction with 50 mg/ml, $p<0.05$). There was no accompanying decrease in cell viability.

H. pylori KatA, GGT and PPT are immunomodulatory and may contribute to the suppression of inflammation during infection.

B223

COMPARISON OF METHODS FOR DETECTION OF *Candida* IN BRONCHOALVEOLAR LAVAGE (BAL) OF CANCER PATIENTS

Samra Shoukat, Dr Basit Zeeshan

University Of Central Punjab, Lahore, Pakistan

Abstract

An accurate and rapid identification of *Candida* species in cancer patients with pulmonary symptoms can provide important information for effective treatment.*Candida* infections represent an increasing cause of morbidity and mortality in patients receiving immunosuppressive chemotherapy for cancer, organ transplantation or in immunocompromised. We used conventional methods such as culture, fermentation reactions,morphology and molecular methods based on the ribosomal DNA repetitive regions or the Internal Transcribed Spacer (ITS) for the identification of *Candida* species. Seventy bronchial specimens of Bronchoalveolar Lavage (BAL) from cancer patients at Shaukat Khanum Memorial Cancer Hospital & Research Center. Lahore, Pakistan were included in this study. Seventy cancer patients were diagnosed on the basis of histological profile. *Candida* detected by conventional methods using Sabouraud Dextrose Agar (SDA), potassium hydroxide (KOH) preparation,germ tube test,fermentation reactions and Gomori methanamine-silver stain (GMS).Thirty (42%) positive isolates of Candida species were obtained by culture, twenty (28%) isolates were germ tube test positive while thirty isolates (42%) were positive by PCR method. In conclusion, the results of our study showed that the PCR based detection methods are significantly better and can detect *Candida* with more accuracy and specificity as compared to conventional methods. Our study would pave the path for optimization of protocols for detection of *Candida* in cancer patients.

Irish Fungal Society session

Presentations: Wednesday evening and Thursday lunchtime

B224

Film-forming Agents as Potential Barriers to Fungal Skin Infections

Kaho Ho, Begona Delgado-Charro, Albert Bolhuis

University of Bath, Bath, United Kingdom

Abstract

Background: Superficial fungal infections are one of the most common causes of human disease caused by dermatophytes or yeasts. Dermatophyte infections are caused by fungi that can digest keratin, infecting the keratinised tissues e.g. skin, hair and nails. It has a higher prevalence than the other superficial mycoses, and its incidence has increased continuously over the last few decades, probably because of the change in lifestyle and frequent usage of antibiotics. Therefore, the aim of the project is to develop a physical barrier that can prevent the early stages of infection to the skin, to avoid development of antifungal resistance and cross-contamination.

Materials/methods: We developed an *ex vivo* model using porcine skin to study the potential of film-forming agents in prevention and treatment of dermatophytosis caused by *Trichophyton rubrum*. We used cell viability assays, confocal and electron microscopy to study the effects of film-forming agents on *T. rubrum*, followed by using QTOF-LCMS and NMR to analyse the carbohydrates binding and chelation to study its mechanism of action.

Results: A cationic polymer used in pharmaceutical and cosmetic products inhibited growth of *T. rubrum* on porcine skin. Viability assays indicated that the polymer has a fungistatic activity and microscopy imaging indicated it formed a coating on top of *T. rubrum*. The QTOF-LCMS and NMR indicated the polymer inhibits fungal growth by removing the carbohydrate content and chelation.

Conclusions: The present study suggests this cationic polymer has considerable antifungal activity against *Trichophyton rubrum* by preventing the supply of nutrients to the fungi.

Irish Fungal Society session

Presentations: Wednesday evening and Thursday lunchtime

B225

Probing the role of histone modifications in the evolution of pathogenicity in *Candida glabrata*

Callum O'Kane[1], Mary O'Connell[2], Edel Hyland[1]

[1]Queen's Univeristy Belfast, Belfast, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

Abstract

Candidiasis is one of the most prevalent mycoses worldwide, and there are few successful treatments for this disease. In the UK, *Candida glabrata* accounts for 25% of *Candida* infections, and due to the increasing incidence of multidrug resistance, *C. glabrata* poses an eminent threat to public health, exacerbated by the limited range of antifungal therapies. Our aim is to elucidate the molecular mechanisms underlying the emergence of pathogenicity in the *C. glabrata* lineage, to identify novel therapeutical targets.

Using comparative phylogenomics, we putatively identified 19 genes under positive selection in the *C. glabrata* lineage. Each of these genes influences chromatin structure by regulating histone post-translational modifications (PTMs). To assess the contribution of these genes to virulence, we monitored the phenotypic consequences of individually removing each gene in clinically-relevant assays. To date, we have focused on 3 genes, cg-SPP1 (regulates histone H3 methylation), cg-HAT1 (histone H4 acetyltransferase) and cg-AHC1 (subunit of Ada histone acetyltransferase complex). Preliminary data show that the individual deletion of these genes increases biofilm formation and fluconazole resistance in *C. glabrata*. Furthermore, acetyltransferase knockout strains show hypervirulence in an *in vivo Galleria melonella* infection model, and phenotypic differences in abiotic stress assays. RNA-sequencing was performed on the type-strain, *cg-hat1Δ*, and *cg-ahc1Δ*, in the presence and absence of fluconazole to determine the molecular bases for these phenotypes, revealing commonality between differentially expressed genes in both mutants. Together, our data suggest that histone PTMs play a significant, and overlapping, role in dictating virulence in *C. glabrata,* and detail the global transcriptomic response to fluconazole.

Irish Fungal Society session

Presentations: Wednesday evening and Thursday lunchtime

B226

*Candida albicans TLO*s and fitness: phenotypic analysis of a *TLO* null strain of *C. albicans* generated via CRISPR-Cas9 mutagenesis

Jessica Fletcher, Peter Flanagan, Derek Sullivan, Gary Moran

Trinity College Dublin, Dublin, Ireland

Abstract

Background: *Candida albicans* is a fungus that is both a commensal and opportunistic pathogenic in humans. Genomic analysis has highlighted the expansion of the telomere associated ORF (TLO) gene family as unique in *C. albicans*. In *C. albicans* there are 15 different members of the TLO family present, compared to only two in its closest relative, *C. dubliniensis.* Here we show that deleting all *TLO*s from *C. albicans* reduces fitness and effects multiple phenotypes.

Method: A guide RNA (gRNA) with sequence with homology to the *TLO* genes was introduced to *C. albicans* AHY940 (a*LEU2/*Δ*leu2*), along with the other components of the CRISPR-Cas9 (Nguyen *et al.* 2017).

Results: The Δ*tlo* strain was found to be generally pseudohyphal in morphology, compared to the wild type. It also showed defective growth in nutrient rich YEPD and YEP-Galactose. Growth on YEPD agar produced colonies similar in appearance to WT, however hyphal induction via growth on Spider agar was greatly reduced in the mutant strain compared to WT. Resistance to oxidative stress was examined and the Δ*tlo* strain was more susceptible to stress induced by both H_2O_2 and tBOOH. In tests to determine the resistance of the Δ*tlo* strain to cell wall perturbating compounds it was seen that this strain is much less resistant to Congo Red and Calcofluor white than WT. Biofilm formation on plastic surfaces also was reduced in the Δ*tlo* strain compared to that of the WT.

Conclusions: Deletion of the *TLO* genes in *C. albicans* greatly impacts phenotypes associated with virulence, and generally results in a less fit strain of *C. albicans*.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B227

Impact of biofilms on complexant driven transport of radionuclides.

Kim Patel, Paul Humphreys

University of Huddersfield, Huddersfield, United Kingdom

Abstract

The current solution for radioactive waste with the UK involves the safe storage of the hazardous material in containers at a geological disposal facilities. One option for the disposal of intermediate level radioactive waste (ILW) is disposal to a cementitious facility where the ambient pH will be highly alkaline. Cellulosic materials present within ILW is expected to degrade via alkaline hydrolysis leading to the production of cellulose degradation products (CDPs), the main component being isosaccharinic acid (ISA). There are concerns surrounding the impact of CDPs on radioactive waste disposal due to their ability to form complexes with certain radionuclides (e.g. Pu) enhancing their mobility. Here we investigate the use of microbial biofilms to metabolise ISA at alkaline pH leading to the release of radioelements from the ISA complexes and their subsequent immobilising via sorption. These results demonstrate how the sorption of naturally occurring nickel, an orthologue of radioactive ^{63}Ni, changes in the absence and presence of CDP and how when biofilm producing microorganisms are introduced into the system isosaccharinic acids are degraded which release the complexed radionuclide.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B228

The communities that colonise the cold

Melanie Hay[1,2], Arwyn Edwards[2], Andrew Mitchell[1]

[1]Department of Geography and Earth Sciences, Aberystwyth University, Aberystwyth, United Kingdom. [2]Institute of Biology, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom

Abstract

The Polar Regions have excellent potential for bioprospecting because the microorganisms that live there have adapted to extreme environmental conditions; and these adaptations can be harnessed in a variety of biotechnological applications. In this study, we describe the community structure of a range of different cryospheric habitats to identify environments appropriate for specific bioprospecting activities.

Samples were collected from Midtre Lovénbreen glacier and surrounds in Spitzbergen, Svalbard in late June to early July 2017. Many cryospheric environment types were sampled including air, snow, slush, meltwater, cryoconite, proglacial water, soil and seawater. DNA was extracted and prepared for 16S amplicon sequencing. Amplicon sequence variants (ASVs) were assigned using DADA2 and contaminants were removed using the Decontam package in R. We searched the scientific literature for biotechnological applications of abundant community members in each environment.

Each environment type displayed a unique community structure, with some physically linked environment types, like glacial snow, slush and meltwater showing continuity through space, and synchronised changes over time. The snow, slush and meltwater habitats were low in biomass and dominated by Gammaproteobacteria, with Cyanobacteria increasing in abundance as melt progressed. Cryoconite was dominated by Cyanobacteria, while sea water was dominated by Bacteriodetes, Gammaproteobacteria and Cyanobacteria. Soil was by far the most biodiverse habitat, with many phyla represented, and a large number of Actinobacteria suitable for antimicrobial discovery. Many of the current EPS, cold-active enzymes, fatty acids, antioxidants and antifreeze proteins are sourced from Proteobacteria and Cyanobacteria, the most abundant phyla in this study.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B229

Engineered extremes: Microbial interactions with steel and bentonite in a Geological Disposal Facility

Megan Barnett, Kay Green, Andy Kilpatrick, Lorraine Field, Simon Kemp, Simon Gregory

British Geological Survey, Nottingham, United Kingdom

Abstract

The conditions of the deep subsurface, combined by perturbations caused by geological disposal of radioactive waste create multiple extreme conditions (limited space availability, high pH, high temperature etc.) for microbial communities. Microbial activity has the potential to cause corrosion of steel and alteration of bentonite clays used in geological disposal facilities. To understand the limits on microbial growth, and the potential for microbial activity to affect the swelling behaviour of the bentonite and metal corrosion, a suite of laboratory experiments is being conducted. In situ repository conditions have been replicated in the MIND (Horizon2020) project. Preliminary results show evidence of corrosion in all experiments, an increase in the basal spacings of smectites in the zone immediately surrounding the steel and inoculated samples had evidence of calcite crystal formation, accompanied by differences in the iron phases. These experiments simulate the in situ conditions well, but the complex nature of this experimental design (high pressure and flow) reduces the practicality of varying the environmental conditions. To complement these investigations, a low-tech solution has been implemented with unpressurised, hydrated bentonite batch experiments. The simpler nature of this set-up allows for investigation of more parameters. Microcosms with artificial groundwater used in the MIND set-up are being compared to the MIND groundwater composition, modelled to represent permafrost conditions. The effect of incubation temperature is also being investigated. Combined, these experiments will help to understand the influence of microbes under the extremes of geological disposal facilities and how their behaviour may change by external parameters.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B230

Motilimonas cestriensis sp. nov., Isolated From a Cheshire Brine Spring

Matthew Kelbrick[1], Raeid Abed[2], André Antunes[1]

[1]Edge Hill University, Ormskirk, United Kingdom. [2]Sultan Qaboos University, Muscat, Oman

Abstract

The Cheshire Salt District (UK) is home to a wide range of largely unexplored inland brine springs, whose increased salinity originates from subterranean Triassic salt rock deposits. Our study focused on the Anderton Brine Spring System, a set of pools of varying salinity which are subjected to regular salinity fluctuations depending on drainage and evaporation (recorded salinities have ranged from 1.2% to 13% NaCl).

Preliminary investigation of samples obtained from one of these pools (with 4.2% NaCl) revealed a wealth of novel isolates which were analysed by 16S rRNA gene sequencing. Within these isolated strains, we have detected that strain MKS20 had a 97% similarity to its closest relative, *Motilimonas eburnea*, which is currently the only characterised species in a newly discovered genus of the order *Alteromonadales*. All reported strains within this genus have been isolated from marine environments, namely marine-sediments and the gut of a sea cucumber. MKS20 is the first strain in the genus to be reported from an inland brine spring and thus significantly extends the ecological range of the genus *Motilimonas*.

The distinctiveness of our strain is further supported by preliminary results from polyphasic taxonomic characterisation (e.g. metabolic and enzymatic profiling). Based on these, strain MKS20 represents a putative new species within the genus for which we propose the name *Motilimonas cestriensis*, referring to Cheshire, the place of isolation.

B231

Vertically transmitted symbionts in the hadal snailfish bridge Mycoplasma and Ureaplasma

Li-sheng He, Chun-ang Lian, Yong Wang

Institute of Deep-sea Science and Engineering, Chinese Academy of Science, Sanya, Hainan, China

Abstract

The hadal snailfish was an endemic vertebrate in the trench, whose survive strategy was always the hot topic pursued by scientists. In this study, we found that ureaplasma named as *Ureaplasma liparidae* were dominant in the midgut and hindgut of the Liparidae sp.1, which were collected from the Marina and Yap Trench around 8000m depth. Phylogenetic analysis showed that *U.liparidae* was clustered with other known ureaplasma pathogens. However, through comparing the gene content with ureaplasma pathogens, common virulence genes were absent in the *U.liparidae* genome. Moreover, the genome contained all the genes related to glucose degradation and subsequent synthesis of vitamin B_2, which was never reported in ureaplasmas. In *U.liparidae*, there were only 9 genes responsible for amino acids. Instead, the genome harbored genes coded for clustered regularly interspaced short palindromic repeats (CRISPRs) system, composed of three *cas* genes and 118 CRISPR spacers. Besides, the draft genome possesses genes involved in binding chitin, which was required for chitin digestion. All of these mentioned above supported a symbiotic lifestyle of *U.liparidae* in the gut of Liparidae and provide nutrition and protection for its host. Interestingly, by detecting 16S rRNA and proteome in the egg of Liparidae, results indicated that ureaplasma could be passed on to next generation through vertical transmission and restriction endonuclease derived by *U.liparidae* may help the host to defense foreign DNA. This study was the first report for ureaplasmas as mutualistic symbionts, and highlight the important role of symbiont for hadal organism survive.

Extremophiles: living life on the edge
Presentations: Wednesday evening and Thursday lunchtime

B232
Viable metabolisms in a simulated martian chemical environment

Macey C. Macey, Nisha K. Ramkissoon, Victoria K. Pearson, Susanne P Schwenzer, Karen Olsson-Francis

The Open University, Milton Keynes, United Kingdom

Abstract

There is evidence that water may exist on Mars as brines in the subsurface. The chemistries of these brines will be greatly influenced by the local lithologies, which would impact on the organisms that could potentially live within them. There are multiple metabolisms that are theoretically viable under martian chemical conditions. In order to better establish which of these are capable of supporting persistent growth under martian conditions, we performed a series of enrichments using four geological simulants for Mars: a global composition, an early and unaltered basaltic composition, a sulfur-rich composition, and a haematite-rich composition.

Enrichments were inoculated with sediment from Pyefleet mudflats in the Colne estuary (Essex, UK). Mudflat sediment was added to the simulant materials and a brine based on the chemistry of Rocknest. Enrichments were supplied with a H2/CO2 headspace at 1 bar pressure and incubated for twenty days. The enrichments were repeatedly subcultured into fresh simulant material and brines in order to effectively select for a community actively growing in this chemical environment. The enriched community was characterised through the isolation and identification of microbes, microscopy and the amplicon sequencing of 16S rRNA genes amplified from DNA extracted from each stage of the enrichment. Biotic and abiotic experiments were conducted to identify geochemical changes that occur due to the presence of microbial activity

We will present details on the communities enriched on the different martian simulants, metabolisms identified as present within the actively growing community and geochemical changes that were identified.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B233

Biodiversity and antibiotic resistance profiling of a Triassic halite deposit in Northern Ireland

Julianne Megaw, Stephen Kelly, Thomas Thompson, Timofey Skvortsov, Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

Kilroot salt mine, a Triassic halite deposit located in County Antrim, Northern Ireland, is the only hypersaline environment in the island of Ireland. We profiled the microbiome of this unstudied environment using conventional isolation approaches (with the addition of some augmented techniques) and metagenomics. Based on 16S rRNA gene sequencing, 89 extremely halophilic archaea from six known genera, and 55 halophilic and halotolerant bacteria from 19 genera were isolated. The archaea were highly similar to what has been previously isolated from other ancient halite deposits, and as expected, numerous genera were identified from metagenomic analysis which were not among the isolates, indicating the limitations of culture-based approaches. We also observed very high levels of antimicrobial resistance (AMR) among a selection of isolates from this ancient environment based on minimum inhibitory concentration (MIC) assays. A growing body of evidence suggests AMR is not a modern phenomenon, but that its origins are ancient, greatly predating modern antibiotic use, and implies that the halophiles obtained may provide reservoirs of AMR genes. Despite this, annotation of whole genome sequences and the metagenome identified few genes which would explain the highly resistant phenotypes, which may suggest that novel, as yet unidentified resistance mechanisms may be utilised by these microorganisms. Studying antibiotic resistance in environments that have had little or no exposure to anthropogenic antibiotic use provides a critical measure of the natural diversity of AMR, which has significant implications in our understanding of its prevalence and evolution.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B234

Mysterious microbes with mysterious molecules: polyphosphate cycling in the Archaea

Jason Chin[1], Jasmine Connolly[2], Fabiana Paula[2], Vincent O'Flaherty[2], John McGrath[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]National University of Ireland Galway, Galway, Ireland

Abstract

Every organism which has ever been examined for the presence of polyphosphate - from bacteria to humans - has been found to produce this mysterious molecule. Potentially formed from thousands of polymerised phosphate residues, these granules have been studied extensively in the Bacteria and are known to play key roles in a range of areas such as life cycle control, antibiotic resistance and global nutrient cycling. Knocking out known genes for polyphosphate production (polyphosphate kinases, PPK) or degradation (exopolyphosphatase, PPX) results in severe deficiencies in behaviours such as stress tolerance, pathogenicity and growth.

Despite the wealth of studies of Bacterial polyphosphate almost nothing is known about Archaeal polyphosphate cycling. There are several studies which have made serendipitous observations of polyphosphate granules in Archaea, and a limited number of observations of the presence/absence of PPK or PPX in Archaeal genomes have been made, but no focussed study of the synthesis or degradation of polyphosphate by Archaea has been performed. Here we examine the distribution of the known polyphosphate metabolism genes across the Archaea, and show that distinct patterns of gene distribution exist across ecosystems. We also show that despite unconventional patterns of gene distribution in individual isolates these organisms are still clearly capable of the synthesis and degradation of polyphosphate, suggesting that novel enzymes and regulation systems are present in the Archaea which may explain previous anomalous data in the Bacteria. This suggests that our understanding of the role of polyphosphate in global nutrient cycling, antibiotic resistance and growth is significantly incomplete.

Extremophiles: living life on the edge
Presentations: Wednesday evening and Thursday lunchtime

B235

Biomineralization of microbes in natural and restored saltmarshes: A missing link in restoration efforts?

James D'Arcy, André Antunes

Edge Hill University , Ormskirk, United Kingdom

Abstract

Saltmarshes are threatened coastal ecosystems, which are subject to cyclic variation in conditions which create a gradient-rich environment (e.g. salinity, pH). They support very diverse communities of plants and animals, protect the coast from erosion, and play an important role in element cycling and bio-remediation. The importance of saltmarshes has only been recently recognised, which has led to significant efforts in restoring sites that had been previously converted to Agriculture. Such restoration attempts have had limited success in returning these sites to their original biodiversity and biological structure. While not yet fully understood, it is thought that several factors are at play, including persisting differences in soil structure and quality.

Coastal environments have been previously shown to harbour significant microbial populations capable of producing $CaCO_3$biominerals. On the other side, $CaCO_3$ biomineral production is known to affect the texture and overall properties of soil, a property currently used in the improvement of soils for Agriculture.

We suggest that these factors could be linked, and that the limitations of saltmarsh restoration efforts might result from differences in biomineral production. Our results provide an overview on differences between samples that we've collected in natural and restored sites, based on cultivation and screening efforts. Our insights might provide an important step forward in saltmarsh restoration and contribute to more successful approaches to protect these vital biotopes and increase biodiversity.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B236

Metabolic Profiling and Environmental Characterisation of Salterns in the Islands of Cabo Verde

Bryn McCulloch[1], James Rowson[1], Aires da Mour[2], Hélio Rocha[2], Marta Filipa Simões[1], André Antunes[1]

[1]Edge Hill University, Ormskirk, United Kingdom. [2]Universidade Jean Piaget de Cabo Verde, Praia, Cape Verde

Abstract

The Cabo Verde Islands constitute a biodiversity hotspot, encompassing a wide range of different biotopes. Thus far, most surveys and programmes have focused exclusively on the flora and/or fauna of the islands and no effort has been made to systematically assess and preserve locally existing microbial biodiversity. The absence of such studies is especially troubling regarding their traditional salterns, as many of them have been abandoned, and increase of construction in coastal areas has already started to indelibly change some of these unique ecosystems and will likely obliterate some of them.

Here we provide an overview of the preliminary data recently collected from salterns in the islands of Sal, Maio, and Boavista and include details on their previously unreported physical-chemical characteristics (salinity, pH, temperature and ionic composition), as well as first results on microbial metabolic profiling. Our data has shown a wide diversity of environmental niches (particularly noticeable in Maio), and showcased differences in substrate use both between and within different salterns. These are the first step of our ongoing efforts in assisting in the survey of several threatened extreme environments in Cabo Verde, characterising their geochemistry and microbiology, and identifying their potential biotechnological applications.

Extremophiles: living life on the edge

Presentations: Wednesday evening and Thursday lunchtime

B237

Production of cross-domain signalling molecules by Halophilic Archaea

Thomas Thompson[1], Stephen Kelly[1], Víctor Fuentes[2], Julianne Megaw[1], Brendan Gilmore[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Centro de Investigaciones Biológica, Madrid , Spain

Abstract

Cell-cell communication through the production of autoinducer molecules has been widely studied in bacteria and found to play a pivotal role in biofilm formation and gene regulation. Quorum Sensing (QS) within the domain Archaea is understudied compared with their bacterial counterparts. The aim of this study was to determine whether archaea are capable of cross-kingdom signalling through the production of QS inducing compounds and QS inhibitory activities.

A combination of culture-dependent (crude extracts from archaeal isolates) and culture-independent (genomic mining) techniques were employed to investigate the production of compounds capable of modulating bacterial QS. Crude archaeal extracts were screened for activity using the bioreporter strains *Agrobacterium tumefaciens* ATCC BAA-2240, *Escherichia coli* JM109 pSB536, pSB401 and pSB1142, *Chromobacterium violaceum* CV026, and *Pseudomonas aeruginosa* MW-1. Active strains were further characterised using initial bio-assay guided fractionation.

Preliminary results revealed different strains were capable of eliciting a QS response in the bacterial bio-reporters. Initial characterisation using LC-MS, TLC-overlays, and other biochemical tests, suggests the possible production of Butyryl Homoserine Lactones or homologs of this molecule by at least one of the strains. Conversely, other halophilic archaea were capable of inhibiting the production of QS-controlled pathways, as demonstrated by the reduction in virulence factor production by *P. aeruginosa*.

Further characterisation of these compounds will prove to be an invaluable insight into the poorly understood mechanisms behind archaeal QS and equally will reveal the role of these compounds in cross-kingdom signalling.

Marine protists as emergine models for functional genomics and cell biology
Presentations: Wednesday evening and Thursday lunchtime

B238

Development of a Genetic Modification System for the Dinoflagellate *Amphidinium carterae*

Isabel Nimmo, Adrian Barbrook, Ellen Nisbet, Chris Howe

University of Cambridge, Cambridge, United Kingdom

Abstract

Dinoflagellates are important marine algae, being an essential symbiont in corals. Loss of the dinoflagellates causes coral bleaching, and ultimately death of coral reefs. Current efforts to study dinoflagellates are greatly hindered by the lack of a reliable method of genetic transformation.

The chloroplast genome of dinoflagellates is fragmented into multiple plasmid-like minicircles, each carrying at most a few genes. We have used 'artificial' minicircles, based on fusions between endogenous minicircles and *E. coli* plasmids to establish a transformation system and optimize parameters for it.

We have determined the sensitivity of wild type dinoflagellate strains to possible selective agents. We have compared the performance of a number of transformation methods, including electroporation and particle bombardment, and find that electroporation is not effective for transformation, whereas particle bombardment ('biolistics') is. We have assessed different parameters for biolistics and subsequent selection.

We have shown the successful maintenance of sequence from an artificial minicircle within a dividing cell population over a period of six months, as well as evidence (using RT-PCR) of transcription and the expected phenotype for the inserted gene.

This represents a significant step forward in developing the genetic modification of *Amphidinium carterae.* We are currently testing the protocol with other dinoflagellates.

Marine protists as emergine models for functional genomics and cell biology
Presentations: Wednesday evening and Thursday lunchtime

B239

The unorthodox chromosomal organisation of the dinoflagellates

I Ian Hu, Ross Waller

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

Abstract

Dinoflagellates nuclei are unlike any other. They have: 1) highly inflated genome sizes, 2) practically lack histones and nucleosomal organization; 3) have permanently condensed liquid crystalline chromosomes throughout the life cycle, and; 4) contain a novel a major new nuclear DNA-binding protein. This protein, called Dinoflagellate/Viral NucleoProtein (DVNP) is small in size (10-20 kDa), highly positively charged (30-40% R+K), and its gene is one of the most highly transcribed in dinoflagellate cells. It has no homology to histone proteins, has no homologues in either eukaryotes or prokaryotes, but is found in a number of marine large DNA viruses. To understand the role of DVNP in the dinoflagellate nuclei we have expressed and purified DVNP and are studying the properties of this novel protein and its interaction with DNA. We show that DVNP is a monomer in solution, but upon exposure to DNA it rapidly binds to and compacts DNA into complexes micrometers in size. Using single-molecule imaging and optical tweezers, DVNP is seen to compact DNA a rates of over 50 μm/sec and change the mechanical properties of DNA. Most interestingly, the DVNP/DNA aggregates show a propensity to travel along the DNA strand en masse. Together, these observations suggest that DVNP plays a central role in the novel model for chromatin management found in dinoflagellates.

B240

Development of genetic tools for *Perkinsus* species: parasites at the evolutionary interface between apicomplexan pathogens and dinoflagellate algae

Elin Einarsson, Jana Zielinski, Imen Lassadi, Ross Waller

Cambridge University, Cambridge, United Kingdom

Abstract

Perkinsus species lie at the evolutionary intersection between dinoflagellates and apicomplexan parasites, and, themselves, are important marine parasites of mollusks with significant commercial and environmental impact. *Perkinsus* enables the study of character evolution relevant to both apicomplexans and dinoflagellates of traits such as invasion mechanisms of parasites; reductive evolution of organelles e.g. plastids; and radical changes in chromatin biology of dinoflagellates. We are developing the tools needed to establish *Perkinsus* as an additional model system for marine organisms to dissect essential questions regarding its biology.

Transfection of *Perkinsus* spp. has previously been reported, but only one promoter has been characterized and used to drive gene expression. In order to gain knowledge about additional promoter regions used in this genus, we have selected multiple promoters and evaluated their timing and expression level by fusion to luciferase. This has allowed development of a toolbox of promoters with different expression properties that provide greater power for molecular-genetic experimentation. Drug-selection of transfectants has been achieved, but current selection takes months. Therefore, we are optimizing the best selection regime using puromycin on *P. marinus* transfectants. The newly characterized promoters will allow further tuning to optimize recovery of transfected cells during selection. Finally, we have introduced CRISPR-Cas9 in this organism to promote genome engineering and genetic studies for the first time in *Perkinsus*. The tools developed in this project are promoting *Perkinsus* to a powerful model organism that will shed light on both the biology of this genus as well as that of its near relati

Charles Darwin House,
12 Roger Street, London,
WC1N 2JU, UK
www.microbiologysociety.org

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