

Annual Conference 2018

POSTER ABSTRACT BOOK

**10–13 April,
ICC Birmingham, UK**



@MicrobioSoc
#Microbio18

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P001

Rare and Imported Pathogens Lab (RIPL) turn around time (TAT) for the telephoned communication of positive Zika virus (ZIKV) PCR and serology results.

Zaneeta Dhesi, Emma Aarons

Rare and Imported Pathogens Lab, Public Health England, Salisbury, United Kingdom

Abstract

Background: RIPL introduced developmental assays for ZIKV PCR and serology on 18/01/16 and 10/03/16 respectively. The published ZIKV test TATs were 5 days for PCR and 7 days for serology.

Methods: All ZIKV RNA positive, seroconversion and “probable” cases diagnosed at RIPL up until 31/05/17 were identified. For each case, the date on which the relevant positive sample was received, and the date on which it was telephoned out to the requestor was ascertained. The number of working days between these two dates was calculated.

Results: ZIKV PCR - 151 ZIKV PCR positive results were identified, of which 4 samples were excluded because no TAT could be calculated. The mean TAT for the remaining 147 samples was 1.7 working days. Ninety percent of these results were telephoned within 3 or fewer days of the sample having been received. There was 1 sample where the TAT was above the 90th centile.

ZIKV Serology - 147 seroconversion or “Probable” ZIKV cases diagnosed serologically were identified. The mean TAT for these samples was 2.5 working days. Ninety percent of these results were telephoned within 4 or fewer days of the sample having been received. There were 6 samples where the TAT was more than 4 days.

Conclusion: The TAT for telephoning of positive ZIKV test results has been satisfactory whilst RIPL has had a “Zika registrar”. A smaller scale audit of telephoning of positive ZIKV results should be performed in rotation with other similar audits (ie for leptospirosis, dengue, chikungunya etc).

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P002

The development of a genotypic assay detecting drug-resistance mutations in herpes simplex virus type 2.

Fangyue Chen, Jane Greatorex

University of Cambridge, Cambridge, United Kingdom

Abstract

Background: the emerging herpes simplex virus type 2 antiviral resistance has been of major concern. Genotypic assay involving the identification of resistance-associated mutations in HSV-2 thymidine kinase (*tk*) and/or DNA polymerase (*pol*) is more rapid and less labour-intensive than the current gold standard plaque reduction assay. In this project, a robust genotypic assay using the Next Generation Sequencing technology is in development.

Method: for the sequencing assay PCR amplifying HSV-2 *pol* and *tk* genes, PCR conditions were optimised, focusing on MgCl₂, DMSO concentrations, and annealing temperature. The sensitivity and specificity of the nested PCR for HSV-2 *tk* and *pol* were determined. Finally, a comprehensive search of the existing literature was performed and a HSV-2 *tk* and *pol* drug-resistance mutation database was constructed. Each mutation was linked to a specific drug susceptibility profile.

Results: after optimising the nested PCR sequencing assays for HSV-2 *tk* and *pol* genes, the sensitivity of the assays was determined: for *tk*, the limits of detection was at a viral load of 1000-5000 copies/ml; for *pol*, >=3000 copies/ml. The assays were found to be highly specific. The drug-resistance mutation database was constructed containing 57 mutations for *tk*, 19 mutations for *pol*.

Conclusion: a genotypic assay testing for resistance-associated mutations in HSV-2 is in development. Further work will include the testing of clinical samples, notably those with drug-resistance mutations as tested phenotypically. Based on the database, an automated, searchable tool is currently in development for clinical report generation.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P003

Prevalence of baseline NS5A resistance in patients infected with genotype 1A hepatitis C virus in Scotland.

Amanda Bradley-Stewart, Emily Goldstein, Alasdair MacLean, Rory Gunson

West of Scotland Specialist Virology Centre, Glasgow, United Kingdom

Abstract

Background

Current treatment for hepatitis C virus includes direct-acting antivirals (DAAs). EASL guidelines recommend testing for resistance to NS5A inhibitors prior to treatment in patients infected with genotype 1A (G1A) virus, as resistance-associated amino acid substitutions (RAS) can exist at baseline in treatment naïve individuals, and have been shown to be associated with lower rates of sustained virological response (SVR).

Methods

The aim of the study was to measure the prevalence of baseline NS5A resistance in Scotland. The study population consisted of 525 treatment naïve, G1A infected patients. The patient samples were collected between March and September 2017. The NS5A region was amplified and sequenced using Sanger sequencing and nine amino acid positions associated with resistance were examined.

Results

Eighty-eight patients (16.8%) had NS5A inhibitor resistance at baseline. Seventy-two patients had single mutations, 15 patients had dual mutations and one patient had a triple mutation. These results are comparable to rates reported in a number of previously published studies.

Conclusion

The high rate of baseline RAS in Scotland, together with the high cost of DAAs, supports resistance testing in G1A infected patients prior to treatment. However, given the rate at which new DAAs are currently being licensed, with ever broader genotype efficacy and higher SVR rates, baseline resistance testing may not be required in the near future.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P004

Clinical outcomes of Hepatitis E in a large district general hospital - a 3 year retrospective analysis

Thomas Lamb^{1,2}, Joel Paul¹

¹Pennine Acute Trust, Oldham, United Kingdom. ²University of Edinburgh, Edinburgh, United Kingdom

Abstract

Hepatitis E is a single stranded RNA virus first recognised in India in 1978. Differing from endemic countries where contaminated drinking water can result in large epidemics, UK acquired infection is a most commonly zoonotic transmission resulting in small clusters and sporadic cases. In the UK, Hepatitis E is an emerging disease with case numbers increasing from 124 cases in 2003 to 891 in 2016. Given its increasing disease burden, further clinical information is required particularly related to the dominating UK genotypes, 3 and 4.

We conducted a retrospective analysis of cases tested for Hepatitis E from Pennine Acute Trust, a large district general hospital over a three-year period to November 2017. A total of 900 serum samples were referred to the reference laboratory. We present the clinical outcome of these patients, the recognised risk factors including alcohol consumption and co-morbidities and their correlation with disease severity.

A trend of increased sampling correlates with a rise in case numbers confirmed by Public Health England.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P005

Cytomegalovirus and varicella-zoster virus infections in a cohort of multiple sclerosis patients assessed over a period of one year

Peter Maple, Radu Tanasescu, Cris Constantinescu

University of Nottingham, Nottingham, United Kingdom

Abstract

Background. In the UK multiple sclerosis (MS) is the leading cause of disability in young adults. Most cases of MS initially present with relapsing and remitting episodes (RRMS) which, over time, are superseded by progressive disease. RRMS is characterized by episodes of demyelination as a consequence of autoimmune mechanisms linked to T and B cell dysregulation. Cytomegalovirus (CMV) and varicella-zoster virus (VZV) establish latency following primary infection and reactivation is associated with failing cell-mediated immunity.

Methods. Serum samples collected at various time points over one year were available from a cohort of 51 predominantly RRMS patients. The cohort had regular physical examinations and magnetic resonance imaging conducted over the study period. CMV and VZV-IgG levels were measured by enzyme-linked immunosorbent assay.

Results. At the beginning of the study, 17 patients were CMV-IgG positive (geometric mean CMV IgG = 324 PEI units/ml). CMV IgG levels remained stable (at 12 months geometric mean CMV-IgG = 365 PEI units/ml) and there were no new seroconversions. For VZV, all patients were VZV-IgG positive and geometric mean VZV-IgG levels at 0 months and 12 months of 1367 mIU/ml and 1342 mIU/ml, respectively were measured. One patient was diagnosed with zoster sine herpete. A total of 14 patients had clinical relapses during the period of the study; however, none of these were associated with serological evidence of VZV reactivation.

Conclusion. CMV and VZV infection in RRMS patients has been studied and no evidence of an association with clinical relapse was found.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P006

Assessing the potential of a portable DNA sequencer, MinION, as a front line tool to detect and characterise notifiable and emerging animal viral disease.

Charlotte Bickers

Royal Veterinary College, London, United Kingdom. Animal Plant Health Agency, Weybridge, United Kingdom

Abstract

In 2015, Oxford nanopore technologies commercialised a portable DNA sequencer known as minION. In recent years, there has been an increasing interest in sequencing pathogens to diagnose disease out in the field. Despite this, very few studies have successfully developed the minION to sequence unknown viruses due to the device's sensitivity to detect viral nucleic acid. The major objective of this study was to assess the potential of minION to sequence unknown mammal viruses in a clinical sample. Part of the aim of this project is to develop and optimise the sample processing method to enrich and amplify viral nucleic acid in a sample. By employing sample processing methods from commercialised kits on clinical samples, provided by APHA, this study will intend to assess the devices capability to diagnose unknown viruses. The study plans to quantitatively compare nucleic acid of various known virus samples prepared using the sample processing methods such as, RNase H and repli-g. Once established, this preparation will be used to sequence data from the minION device software and compare this data to sequencing obtained from previous data. The minION will then be assessed for the detection of notifiable diseases and samples containing unknown viruses in the APHA archive. This is a Master's project which is ongoing and would have completed 7 months out of 12 by the time of presenting.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P007

Using the Non-Coding Region between the Matrix and Fusion Genes in the Measles Virus Genome to Complement Genotyping and Epidemiology Data.

Ana Penedos, Aurora Fernandez Garcia, David Williams, Kevin Brown

Public Health England, LONDON, United Kingdom

Abstract

Measles is a highly infectious disease caused by measles virus (MeV). Despite the availability of a safe and cost-effective vaccine, measles is one of the world-leading causes of death in young children. Currently, MeV is genotyped on the basis of a 450 nucleotide region of the nucleoprotein gene (N-450) and the hemagglutinin gene (H). However, as the number of circulating genotypes and strains diminishes, this is not sufficiently informative for distinguishing endemic from imported MeV.

In work previously published, we have shown that the non-coding region between the matrix and fusion protein genes of MeV (MF-NCR) yields phylogenetic resolution comparable to that obtained from the whole genome sequence excluding termini (WGS-t) of the virus in an outbreak setting, constituting a potentially useful tool for outbreak characterisation. Since then, we have obtained MF-NCR sequences of over 300 samples. Here, we evaluate the usefulness of this region in conjunction with genotyping and epidemiology data throughout a period of lower circulation of measles and whether epidemiologically-relevant objective measurements can be obtained from the data. We find that the use of the MF-NCR can complement epidemiology and genotyping in determining whether cases are linked, but it cannot be expected to provide definite answers in all contexts.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P008

Laboratory testing for progressive multifocal leukoencephalopathy (PML): Retrospective investigation of UK patients 2003-2017

Li Jin

National Infection Service, Public Health England, London, United Kingdom

Abstract

Background JC virus (JCV) reactivation causes progressive multifocal leukoencephalopathy (PML) in patients with immune-mediated diseases and patients under treatment with immune-suppressants, e.g. multiple sclerosis (MS) patients who are at risk of PML. The objective was to overview the patients with PML and laboratory methodology applied for PML confirmation in last 16 years at the UK national reference laboratory.

Methods JCV quantitative polymerase chain reaction (qPCR) was performed to all cerebrospinal fluid (CSF) and brain biopsy (BB) specimens. Paired serum and CSF samples received were tested with haemagglutination-inhibition (HAI) assay using the recombinant JCV-VP1 antigen to confirm intrathecal JCV antibody production. Albumin levels and total IgG concentration were determined by immunophelometry and the JCV antibody index (JCV-AI) was calculated to exclude the blood barrier dysfunction.

Results Detection of JCV by qPCR in CSF or BB is compulsory for definite diagnosis of PML. However, it can be challenging in some patients with low levels of JCV DNA presented in CSF. Out of 281 patients investigated, 89.7% were JCV detected in CSF or BB using qPCR, 4.6% (13) were JCV-AI >3, and 5.7% (16) were positive by both assays. Patient ages ranged from 18 to 85 years old and 42.4% of the patients were HIV positive. Four of six MS patients developed PML were known under a long term treatment of Natalimuzab.

Conclusion Perform both qPCR and HAI-AI on multiple and follow up specimens contributes to a more comprehensive PML diagnosis and patient management.

Virology Workshop: Clinical Virology

Zone A

Presentations: Wednesday and Thursday evening

P009

Development of ELISAs to detect antibodies to emerging viruses using plant-expressed proteins.

Ge Wu¹, Barnabas King¹, Stephen Dunham¹, George Lomonosoff², Janet Daly¹

¹School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Loughborough, United Kingdom. ²John Innes Centre, Norwich Research Park, Norwich, United Kingdom

Abstract

Transient production of recombinant viral proteins in plants has the advantages of lower cost, lower contamination risk and higher biomass yields in a shorter amount of time compared with bacterial, yeast and animal cell production systems. Extremely high yields of proteins can be produced in plants by agro-infiltration of leaf tissues with specifically designed expression vectors harbouring the sequence of interest. Our group has demonstrated how expression of the nucleoprotein of Schmallenberg virus in plants can be used to develop a sensitive and specific antibody ELISA. The aim of the current study was to develop an ELISA using plant-expressed flavivirus NS1 proteins as a capture antigen to detect specific IgG and IgM specific antibodies. ZIKV NS1 gene was PCR amplified and cloned into the pEAQ-HT plasmid by both restriction enzyme-based and Gibson Assembly cloning methods. In both cases, C-terminal histidine tags were fused to the NS1 sequence for subsequent purification steps using nickel affinity chromatography. The resultant pEAQ-HT-ZIKV-NS1 plasmids will be transformed into plant bacterium *Agrobacterium tumefaciens* which later will be inoculated into *Nicotiana benthamiana* for protein production. Serum samples from dengue and Zika-virus infected humans will be screened using the ELISA. Sera from West Nile virus-infected horses will also be used for assessing flavivirus cross-reactivity.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P010

Use of aciclovir in presumed meningitis: a prospective audit of 195 sequential CSF samples from March to July 2017.

Judith Timms¹, Lisa Berry¹, Katie Jones², Taylor Johnathan²

¹Coventry and Warwickshire Pathology Services, Coventry, United Kingdom.

²Katie.Jones2@uhcw.nhs.uk, Coventry, United Kingdom

Abstract

Background

The value of aciclovir in viral meningitis is unproven; we observed aciclovir usage and undertook this prospective audit to determine the extent of this practice locally.

Methodology

Data was recorded from sequential CSF samples. Documentation included iv aciclovir usage, testing decision and subsequent results.

Results

1. 116 patients were not on aciclovir; 79 were on aciclovir.
2. Twenty patients on aciclovir had clinical conditions, including meningitis, for which aciclovir was unjustified.
3. Forty samples were not tested; 35 in the no-aciclovir group and 5 in the aciclovir group.
4. The overall positivity rate was 19% in both groups; there were more enteroviruses and fewer herpes viruses in the no-aciclovir group compared to the aciclovir group.

Discussion

1. A 'ban' on aciclovir in patients with conditions other than possible encephalitis or 'septic' babies would have been safe as only one patient warranted aciclovir and due to the clinical details it is highly unlikely a 'ban' would have been applied to him.
2. No patients in the no-aciclovir group might have benefited from aciclovir. One patient positive for HSV-1 had presented with increased confusion and was known to have leptomenigeal metastases with progressive metastatic disease confirmed on MRI.

Conclusions

19 patients were on aciclovir unnecessarily, one patient each week. This warrants targeted feedback to clinical teams. In adults and children > 2 years we have no evidence of harm if the use of aciclovir was restricted to patients with signs and symptoms of encephalitis or meningitis with rash.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P011

Introduction of a new molecular technique based on the principle of Multiplexed-Tandem PCR and one years' experience of in house testing using the respiratory pathogens assay.

Fiona Shufflebottom

Pennine Acute NHS Trust, Manchester, United Kingdom

Abstract

In January 2017 the microbiology laboratory at Pennine Acute NHS Trust introduced a new molecular assay to test for respiratory pathogens. The AusDiagnostics Respiratory 'C' panel had been selected by the department to give the best range of targets. The assay consists of the following targets:

Influenza A

Influenza typing (H1 and 3)

Influenza B

RSV (A&B)

Rhinovirus/enterovirus

Parainfluenza 1, 2, 3 and 4

Adenovirus

Human metapneumovirus

Bordetella spp

Bordetella pertussis

Mycoplasma pneumoniae

A verification process was completed using samples that had been confirmed positive for the targets by an alternative molecular amplification method. Where clinical samples were not available due to low incidence, either EQA samples or samples from another NHS trust were used. Following the successful completion of this verification the assay was introduced into the department and training was rolled out to specific BMS staff working in the molecular section.

Virology Workshop: Clinical Virology

Zone A

Presentations: Wednesday and Thursday evening

One of the main drivers for this change was to improve turnaround time. Previously samples would be referred to the local PHE reference laboratory which had longer turnaround times and was affecting patient management especially for infection control and bed management. Within a very short time frame the laboratory received very positive feedback from the users including paediatrics, clinical haematology and intensive care to name a few. This greatly helped with rationalising patient's anti-microbial management and patients being discharged from the hospital. There has also been an impact on the timely prophylaxis for patients exposed to influenza.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P012

HIV avidity – Testing from first diagnosis over time, impact on assay performance.

Seb Cotton¹, Gina McAllister¹, Kate Templeton¹, Samantha Shepherd², Rory Gunson², David Goldberg³, Sharon Hutchinson³

¹Edinburgh Specialist Virology Centre, Edinburgh, United Kingdom. ²West of Scotland Specialist Virology Centre, Glasgow, United Kingdom. ³Health Protection Scotland, Glasgow, United Kingdom

Abstract

Background

HIV avidity is now performed on all new diagnosis in UK. The test has known inaccuracy in those with a low level of antibody at the time of a new infection. The aim of this study was to evaluate the effect over time and antiretroviral treatment on HIV avidity and determine if this approach can improve data quality.

Methods

A commercial ELISA adapted for HIV avidity was used to test patient samples and measure HIV avidity. In this study a total of 141 serum or plasma samples from 28 known HIV positive patients were tested. All diagnosis were HIV positive with a low HIV avidity index (<40%). A range of samples were tested for each patient with the mean duration from diagnosis was 542 days (range 73 - 1271 days). HIV treatment data as well as data on HIV subtype, age, sex and sexual orientation was captured.

Results

Of the 28 cases initially reported as low avidity indicating a recent infection 26 (93%) were shown to develop a higher avidity index (>40%). In 7 cases (25%) that had shown antibody maturation, the avidity index decreased while on antiretroviral therapy. In 2 cases (7%) HIV avidity did not mature to a higher avidity index as expected.

Conclusion

This study suggests after long term treatment and an undetectable viral load the HIV avidity index will be affected. Using follow up samples can help clarify HIV incidence data into those initially identified as recent infection.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P013

Immunogenicity evaluation of seasonal influenza vaccine in COPD patients.

Yasser Tatawi¹, George Carnell¹, Simon Scott¹, Nigel Temperton¹, Glen Davison¹, Arwel Jones²

¹University of Kent, Kent, United Kingdom. ²University of Lincoln, Lincoln, United Kingdom

Abstract

Chronic Obstructive Pulmonary Disease (COPD) is a progressive disease characterized by long-term breathing problems and poor airflow. Patients can be divided into frequent exacerbators and infrequent exacerbators, however, the underlying mechanisms behind frequent COPD exacerbations remain undetermined.

The aim of this study is to assess antibody responses to influenza vaccination in frequent and infrequent COPD exacerbators, and healthy participants. Frequent exacerbators, infrequent exacerbators and healthy participants, aged 65-85, were vaccinated with a single dose of the 2016/2017 trivalent inactivated influenza vaccine containing influenza strains A/California/7/2009 H1N1 like virus, A/Hong Kong/4801/2014 H3N2 like virus and B/Brisbane/60/2008 like virus.

Pseudotype neutralization assays were performed with A/California/7/2009 lentiviral pseudotypes on sera collected on day 0 and day 28 post vaccination. Pre and post vaccination antibody titres were high, suggesting extensive exposure to H1 California virus; rendering data analysis difficult to undertake for this strain; in agreement with previous studies indicating presence of pre-existing neutralizing antibodies against 2009 H1N1 in individuals aged above 65 (Ikonen et al., 2010; Stockman, 2011). Among tested samples, 57% showed an increase > 30% in neutralising antibody titres, 28.6% showed an increase < 30%; while 14% showed no change in neutralising antibody titre.

Immunogenicity data for A/California/7/2009, B/Brisbane/60/2008 and A/Hong Kong/4801/2014 in parallel with cognate HI assay data will enable a comprehensive immunogenicity profile to be built up. This has the potential to inform new strategies for the annual vaccination of this important risk group.

Virology Workshop: Clinical Virology
Zone A
Presentations: Wednesday and Thursday evening

P014

Control Response to a Measles Outbreak in an Acute Care Trust.

Bozena Poller, Naomi Meardon, Cariad Evans

Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

Abstract

Background

During autumn 2016, Sheffield Teaching Hospitals (STH) NHS Foundation Trust experienced a measles outbreak around its Emergency Department (ED) totalling 17 cases, including 7 staff. Numerous exposures occurred, with technology and responsive teams crucial in responding to the outbreak.

Methods

An outbreak response team met daily with key Trust Management and departmental representatives, allowing comprehensive and consistent dissemination of information. Telephone support systems were established for concerned staff.

Contact tracing was prioritised to identify and protect any vulnerable patients or employees, and assist workforce planning around mandatory staff exclusions. STH's electronic patient and medical record systems were exploited, allowing rapid listing and interrogation of ED attendances; flagging of contacts' records in case of re-attendance; generation of alert letters to those discharged; and remote immunity assessment of inpatients, reducing disruption to clinical areas.

A reactive virology service was vital, with prompt immunity results assisting decisions around patient placement and staff exclusion. Development of in-house measles PCR testing reduced turn-around from 2 days at a reference laboratory, to 4 hours. Home sampling packs were created, enabling testing of excluded symptomatic staff.

Results

Over 4000 patient contacts were identified, with almost 900 alert letters distributed. Nearly 1050 frontline staff underwent immunity check, 175 requiring further assessment, and around 100 vaccinated. Despite these numbers, the outbreak was contained within 9 weeks and no inpatient infections were observed.

Conclusion

STH delivered an effective and swift response to a highly contagious infection and challenging situation. Many lessons were learnt, applicable to other scenarios and Trusts.

P015

ANTIDIABETICS AND ANTIBACTERIAL POTENTIAL OF METHANOL LEAF EXTRACT OF *Eucalyptus camaldulensis*

Idris Abubakar^{1,2}, Enoch N. Gana¹, Talatu S. Bosso¹, Amos T.N³

¹Department of Biological Science, Niger state Polytechnic, Zungeru, Zungeru, Nigeria. ²Department of Microbiology, Kaduna State University,, Kaduna, Nigeria. ³1Department of Biological Science, Niger state Polytechnic, Zungeru, Zungeru, Nigeria

Abstract

This study investigated the antidiabetics, antimicrobial, phytochemical property as well as evaluated the MIC and MBC of methanol extract of *Eucalyptus camaldulensis*, against 3 bacterial isolates: *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*. Antidiabetic study of the plant extract was conducted using alloxan induced diabetic rat by administrating the extract invivo to the rats for 21 day. The phytochemically analyzed reveled the presence of phenol, tannin, alkaloid, saponin, flavonoi, terperes, steroids, cardiac glycosides and anthraquinon. It shows effectiveness in both gram positive and gram negative bacteria with the highest concentration having MIC and MBC 1.28×10^{-3} and 2.56×10^{-4} respectively on *Salmonella typhi*. MIC and MBC for *Escherichia coli* is 6.4×10^{-3} and 2.56×10^{-4} at the highest concentration (1.0mg/ml) at the same concentration the extract is not effective on *Staphylococcus aureus*. however, the antidiabetic study showed percentage decrease in fasting blood sugar at 37.56%, 57.97% and 59.24% for group A which received 100mg/ml, B which received 200mg/ml and C which received 400mg/ml respectively after treatment for 21 days, while for the positive control which receive 10mg/ml of Glibenclamide, negative control and normal the percentage decrease in fasting blood sugar are 26.00%, -49.54 and 10.98 respectively. It can then be concluded that the methanolic leaf extract of *Eucalyptus camaldulensis* is active against the two test organisms out of the three tested and also effective more than the standard drug (glimbenclimide) used against diabetes therefore can be recommended for therapeutic use.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P016

Functional expression of raw starch degrading enzyme from *Laceyella sacchari* LP175 in *Escherichia coli* and its application for hydrolysis of dried cassava chips without the heating process

Thanasak Lomthong^{1,2}, Sophie Duquesne³, Alain Marty³, Vichien Kitpreechavanich¹

¹Kasetsart University, Bangkok, Thailand. ²Rajamangala University of Technology Thanyaburi, Pathum-Thani, Thailand. ³LISBP, Université de Toulouse, Toulouse, France

Abstract

The application of raw starch degrading enzyme (RSDE) is currently of interest in various starch processing industries since RSDE could hydrolyse raw starch granules at below gelatinization temperature without the heating process. This could reduce the energy consumption and also reduce the cost of operations. In this work, RSDE produced by *Laceyella sacchari* LP175, was purified with a 14.7 purification fold and 40.5 % yield. The first 15 N-terminal amino acids were sequenced and showed a 100% homology with α -amylase from *Laceyella* sp. DS3 and *Thermoactinomyces vulgaris*. The RSDE gene was functionally annotated with the *L. sacchari* strain GS1-1 available genome which showed the presence of a putative gene of 1362 bp encoding 453 amino acids. The RSDE gene was amplified from *L. sacchari* LP175 genomic DNA and cloned for expression in *Escherichia coli* which showed the highest activity on raw cassava starch at pH 6.5 and a temperature at 50 °C. The recombinant LsA175 could hydrolyze raw cassava chips at below gelatinization temperature and showed higher efficiency for hydrolysis of raw cassava chip than commercial α -amylase (Termamyl) at 50 °C. These results suggested that the raw starch degrading gene from *L. sacchari* LP175 could be expressed in *E. coli* BL21 (DE3), thus providing an alternative choice for enzyme production at an industrial level in terms of reduce the energy consumption, operation cost and also reduce the global environmental problems.

P017

Influence of Lactic Acid Bacteria (LAB)-Consortium Fermentation on the Antinutritional Composition of Bambara Groundnut Flour

Alloysius Chibuike Ogodo¹, Ositadinma Chinyere Ugbogu², Reginald Azu Onyeagba²

¹Federal University Wukari, Wukari, Nigeria. ²Abia State University Uturu, Uturu, Nigeria

Abstract

Antinutritional factors of bambara groundnut flour fermented with lactic acid (LAB)-consortium was evaluated. Bambara groundnut was processed into flour, fermented spontaneously and with LAB-consortium previously isolated from maize (*Lactobacillus plantarum* WCFS1 + *Lactobacillus rhamnosus* GG, ATCC 53/03 + *Lactobacillus nantensis* LP33 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus reuteri* DSM 20016) and sorghum (*Pediococcus acidilactici* DSM 20284 + *Lactobacillus fermentum* CIP 102980 + *Lactobacillus brevis* ATCC 14869 + *Lactobacillus nantensis* LP33 + *Lactobacillus plantarum* WCFS1) to evaluate their effects on the antinutritional factors of the flour at 12 h intervals using standard techniques. The result shows significant ($p < 0.05$) decrease in tannin content with increasing fermentation period ranging from $4.63 \pm 0.06\%$ (unfermented) to $0.08 \pm 0.00\%$ (48h LAB-consortium from sorghum fermented). Phytate decreased significantly ($p < 0.05$) from 46.01 ± 0.04 mg/100g (unfermented) to 13.04 ± 0.06 mg/100g (48 h LAB-consortium from maize fermented). The polyphenol content decreased from the original value of 684.86 ± 0.30 mg/100g to 167.46 ± 0.06 mg/100g. The variations differ significantly ($p < 0.05$). The LAB-consortium fermentation decreased trypsin inhibitor activity significantly ($p < 0.05$) from 7.40 ± 0.06 mg/100g (unfermented sample) to 0.99 ± 0.03 mg/100g (48h LAB-consortium from maize fermented). Comparison of the fermentation parameters of LAB-consortium from maize and LAB-consortium from sorghum fermentation set-ups showed no significant difference in most of the parameters. This suggests the potential of LAB-consortia fermentation in decreasing the antinutritional factors associated with bambara groundnut more than spontaneous fermentation.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P018

Effect of temperature on the metabolism of *Escherichia coli* in view of gene expressions

Chowdhury Mohammad Monirul Hasan

University of Chittagong, Chitagong, Bangladesh

Abstract

Biological systems are known to be robust and adaptable to the culture environment. It is strongly desired to understand the metabolic changes and its complicated mechanism upon heat shock in practice for the efficient metabolite production by temperature up-shift in *Escherichia coli*. In the present research, therefore, we investigated the effect of temperature up-shift from 37°C to 42°C on the metabolism in view of gene expressions. The results of aerobic batch and continuous cultivations of *E. coli* indicate that more acetate was accumulated with lower biomass yield and less glucose consumption rate at 42°C as compared to the case at 37°C. The down-regulation of the glucose uptake rate corresponds to the down-regulation of *ptsG* gene expression caused by the up-regulation of *m/c* gene expression. In accordance with up-regulation of *arcA*, which may be caused by the lower oxygen solubility at 42°C, the expressions of the TCA cycle-related genes and the respiratory chain gene *cyoA* were down-regulated. The present result clarified the mechanism of metabolic changes upon heat shock from 37°C to 42°C based on gene expressions of heat shock genes, global regulators, and the metabolic pathway genes. This information is useful for a variety of applications such as temperature-induced heterologous protein productions or simultaneous saccharification and fermentation (SSF) etc.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P019

Resistance of isolated *Acidithiobacillus sp.* 13Zn and *Leptospirillum ferriphilum* CC to copper and zinc

Anna Khachatryan, Narine Vardanyan, Arevik Vardanyan

Institute of Microbiology of SPC "Armbiotechnology" NAS of Armenia, Yerevan, Armenia

Abstract

Influence of copper and zinc on the oxidation of Fe(II) by isolated *Acidithiobacillus sp.* 13Zn and *Leptospirillum ferriphilum* CC was studied in the concentration range from 10 to 250mM. It was revealed that oxidation of Fe(II) by *L.ferriphilum* CC was inhibited by 50% even in the presence of 10mM copper and zinc, whereas inhibition of Fe(II) oxidation by *Acidithiobacillus sp.* 13Zn was only 45% in the case of 100mM copper in the medium. In the presence of Zn(II) up to 150mM in the medium, the oxidation of Fe(II) by *Acidithiobacillus sp.* 13Zn was stimulated by 10-30%. The inhibition of Fe(II) oxidation by *Acidithiobacillus sp.* 13Zn was observed in the concentration of zinc 200mM and more. Zinc ions inhibit Fe(II) oxidation by *Acidithiobacillus sp.* 13Zn in the concentration of 200mM and higher. Cultivation of *Acidithiobacillus sp.* 13Zn in the medium with gradually increasing concentrations of copper from 20 to 150mM allowed to enhance the oxidation rate of Fe(II) of the primary culture from 32.4 to 63.6 mg/l hour. The use of the above mentioned method allowed to obtain culture of *L.ferriphilum* CC adapted to 75mM copper and about 2 times increase the oxidation rate of Fe(II) in comparison with the primary culture. Thus, cultivation of *Acidithiobacillus sp.* 13Zn and *L.ferriphilum* under condition of gradually increasing concentrations of copper resulted in bacterial cultures adapted to 150mM and 75mM copper, respectively.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P020

In vitro antimicrobial activity of phytic acid in elimination of endodontic pathogen

Rania Nassar¹, Mohannad Nassar², Abiola Senok¹

¹Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, UAE. ²RAK Medical and Health Sciences University, Dubai, UAE

Abstract

Background: *Enterococcus faecalis* is the most common bacteria associated with failure of root canal treatment. During this procedure, an endodontic irrigant with antibacterial effect against *E. faecalis* during a short contact time and with minimal adverse effects on dentin is recommended. Phytic acid (IP6) is a natural agent which has been suggested as a potential irrigant. This study was designed to determine the contact time required for IP6 to eliminate *E. faecalis* and compare it with the currently used ethylenediaminetetraacetic acid (EDTA).

Methods: The membrane filtration method was used to assess the contact time needed for the irrigants to eliminate *E. faecalis* (ATCC 29212). This was done according to BS-EN-1040:2005. The irrigants were assayed at concentrations of 0.5, 1% and 2% IP and 18% EDTA and contact time of 30 s, 1 min, 2 min and 5 min was maintained. The contact time for each irrigant to produce negative bacterial culture (100% inhibition of growth) was determined.

Results: IP6 showed bactericidal effect at all concentrations used. However, the contact time needed for the bactericidal effect of 0.5% and 1% IP6 was 5 min which was significantly higher than 30 s contact time for the 2% and 5% IP6. EDTA was not able to eliminate *E. faecalis* at any contact time.

Conclusions: The study shows that IP6 can exert bactericidal effect on *E. faecalis* at low concentration and minimal contact time. These findings provide new insight into the potential for IP6 to replace EDTA as irrigant.

P021

Comparative studies of the antibacterial properties of *Salvadora persica* and mouth washes against oral isolates

Pauline Nnagbo, Stanley Dike

Imo State University, Owerri, Nigeria

Abstract

Background: Oral health is an integral part of overall health. In many traditional cultures, plastic-bristle brushes are not used; herbal chewing sticks are used instead.

Materials/methods: The comparative antibacterial efficacy of methanol extract of *Salvadora persica* and different mouth washes was studied against different carcinogenic and pathogenic bacteria including *Streptococcus* species, *Staphylococcus* species, *Enterococcus* species and *Escherichia coli* by agar well diffusion method.

Results: Methanol extract of *Salvadora persica* inhibited all the test bacteria at the highest concentration tested (50 mg/ml); with the highest zone of inhibition observed against *Staphylococcus* species (15.8 mm), followed by *Enterococcus* species (12.3 mm), *Streptococcus* species (10.7 mm) and *Escherichia coli* (10.4 mm). Oral B and listerine exhibited highest antibacterial activity at the highest concentration used (50 mg/ml) against *Staphylococcus* species (16.2 mm, 18.6 mm) followed by *Streptococcus* species (14.6 mm, 16.3 mm), *Escherichia coli* (14.7 mm, 15.0 mm) and *Enterococcus* species (14.1mm, 13.5mm) respectively. Listerine mouth wash showed highest bactericidal activity compared to oral B and *Salvadora persica*. The methanol extract of *Salvadora persica* had an MIC of 50 mg/ml which was similar to those of commercial mouth washes examined in this study. It has antibacterial effects and could be used as therapeutic agent and a potent oral hygiene tool.

Conclusions: We recommend further pharmacological studies to discover non-synthetic tooth paste and mouth washes for maintaining good oral health.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P022

Activity of natural products against *Neisseria gonorrhoeae*.

Colin Churchward, Polliane Arruda, Andrew Sears, Venetia Galanaki, Archana Arutsothy, Puspaltha Raja Naidu, Damithi Perera, Ali Al-kinani, Lori Snyder

Kingston University, Kingston upon Thames, United Kingdom

Abstract

Neisseria gonorrhoeae, the cause of the sexual transmitted disease gonorrhoea, is resistant to many antimicrobial agents. Strains have been isolated that are resistant to recommended antibiotic treatments. As we enter a post-antibiotic era for gonococcal infections, it becomes increasingly urgent to explore other options to either treat or prevent *N. gonorrhoeae* infection. Selected oils (flax seed, hemp, and coconut oil) and essential oils (bay laurel, black pepper, cinnamon, citronella, clove, eucalyptus, holy basil, galangal, ginger, lemongrass, lime, marjoram, neem, nutmeg, oregano, peppermint, patchouli, rosemary, spearmint, star anise, tea tree, and turmeric) were tested for their antimicrobial properties against *N. gonorrhoeae*. Untreated flax seed oil, hemp oil, and coconut oil were not bactericidal nor did they prevent growth of the bacteria. Treatment of these three oils with a lipase produced large zones of inhibition and one was bactericidal by log reduction assay, able to kill 10^7 gonococcal cells within two minutes. Diluted essential oils produced zones of inhibition and were bactericidal, even when heavily diluted. These natural oils show promising anti-gonococcal activity that could potentially be exploited for treatment or prevention of *N. gonorrhoeae* infections in the future.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P023

Effects of different enrichments on microbial freshwater sediment used as inoculum in microbial fuel cells

Caterina Armato^{1,2}, Daniyal Ahmed², Deborah Traversi¹, Valentina Margaria², Marzia Quaglio², Giorgio Gilli¹, Guido Saracco², Tiziana Schilirò¹

¹Department of Public Health and Pediatrics, University of Torino, Torino, Italy. ²Centre for Sustainable Future Technologies (CSFT@PoliTo), Torino, Italy

Abstract

The characterization of anodic microbial communities is of great importance in Microbial Fuel Cells (MFCs) field.

A high abundance of Anode Respiring Bacteria (ARB) in the anode chamber of MFCs is required condition for optimal performances.

The effect of the enrichments of freshwater sediment with ferric citrate (FeC) and without it (Gen) in the medium was evaluated studying the microbial community structures during preculture steps and during operational condition.

We characterized the microbial population using a combination of 16S rDNA analysis including DGGE-based analysis, sequencing and qPCR to determine differences between the enrichment procedures.

The enrichment effect was visible on the microbial community composition both during precultures and in the MFCs. Proteobacteria, Bacteroidetes and Firmicutes resulted the main Phyla.

Gen-MFCs showed better performance than the FeC-MFCs (shorter start-up time, lower anode potential, higher current and power density). Both the approaches affected the community diversity: Shannon diversity decreased, especially during FeC enrichment ($p < 0.01$), as well as β -Proteobacteria and γ -Proteobacteria percentages.

Bacteroidetes, γ -Proteobacteria and δ -Proteobacteria resulted statistically higher in the attached component of Gen-MFCs than in FeC-MFCs; Firmicutes in FeC-MFCs than in Gen-MFCs ($p < 0.05$).

Geobacteraceae and *Pseudomonas spp.* decreased more during the FeC enrichments and their DNA concentration was higher in the Gen-MFCs and FeC-MFCs, respectively.

Our data suggests that FeC enrichment reduced diversity of the anode community and the relative abundance of ARB, decreasing the system performance. Therefore, a heterogeneous community dominated by ARB could improve the performance of the MFCs and allow their application *in situ*.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P024

The use of Solid-Phase synthesised nano-sized Molecularly Imprinted Polymers for the specific targeting and treatment of bacterial species.

Liam Crawford

University of Leicester, Leicester, United Kingdom

Abstract

Background: Solid-Phase Nano-sized Molecularly Imprinted Polymers (nanoMIPs) are synthesised through the polymerisation of monomers that polymerise around a template in solid-phase, such as a peptide, forming a nanoMIP that is specific to the template, or templates, through electrostatic interactions and space complementarity. The nanoMIPs are double imprinted to the template and to antibiotics to target the conserved surface protein Lpp20 of *Helicobacter pylori* or the surface layer protein SlpA of *Clostridium difficile*. We are targeting *Helicobacter pylori* and *Clostridium difficile* as specific delivery of antibiotics would be expected to improve the: specificity of drug delivery, patient benefit, and ultimately treatment outcome.

Methods: Synthesis of the first nanoMIPs was performed by immobilising peptides to glass beads. The polymerisation was catalysed by production of free radicals. Low affinity nanoMIPs were removed through washing with H₂O at 0°C, whilst the high affinity nanoMIPs were eluted at 60°C. Imaging of nanoMIP binding to the bacteria was performed by confocal microscopy.

Results: Synthesis allowed to produce with a high yield Lpp20 specific or SlpA specific fluorescent tagged nanoMIPs at a nanoscale. The binding of nanoMIPs to specific bacteria was confirmed by differing microscopy techniques showing binding of single nanoMIPs to bacteria.

Conclusion: The use of nanoMIPs for delivery of antibiotics is a novel, cheap and effective way of treating bacterial infections. We envisage that our work will demonstrate that this specific delivery will optimise antimicrobial delivery thus contributing to the maintenance of a normal microbiota.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P025

Community composition changes during bioleaching of chalcopyrite

Sarah Jones¹, Karen Hudson-Edwards², Joanne Santini³

¹Earth and Planetary Sciences, Birkbeck, London, United Kingdom. ²Camborne School of Mines, University of Exeter, Exeter, United Kingdom. ³Structural & Molecular Biology, UCL, London, United Kingdom

Abstract

Chalcopyrite (CuFeS₂) is a sulfide mineral that has been mined as a source of copper for a large portion of human history, and is still the primary mineral used for the extraction of copper to this day. The high lattice energy of chalcopyrite makes chemical copper extraction expensive. Traditional methods of copper extraction from chalcopyrite can also result in the concentration of toxic metals in waste products. Conversely, the process of bioleaching is a cost-effective, low input method of metal extraction. Bioleaching exploits the sulfur and iron metabolisms of acidophilic prokaryotes to extract metals from ore. To potentially optimise chalcopyrite bioleaching, it is necessary to determine which organisms, and therefore metabolisms, are contributing to the dissolution process. SC3 is a bioleaching consortium enriched from a mining environment in a working copper mine in Skouriotissa, Cyprus. Bacterial species present within the consortium include: *Leptospirillum ferrodiazotrophum*, several *Acidithiobacillus* species, and one member of the order *Rhodospirillales*. There are also archaeal species present: *Ferroplasma acidarmanus*, *Ferroplasma type II* and the *Thermoplasmatales* member dubbed "G-plasma". The consortium was grown on chalcopyrite in a minimal acid medium, at pH 1.5. Changes in the community composition of the consortium were determined by 16s rRNA gene amplicon sequencing and compared to the dissolution of copper, iron and sulphur over time. The outcomes of this work will help inform community dynamics during the bioleaching of chalcopyrite, knowledge which could help improve the practical and commercial viability of bioleaching.

P026

Characterisation of a novel *Herpetosiphon* species (Llansteffan) with a particular focus on its predation

Paul Livingstone, David Whitworth

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Background: *Herpetosiphon spp* are ubiquitous, chemoheterotrophic, filamentous gliding, predatory bacteria comprising of 4 species *H.geysericola*, *H.giganteus*, *H.gulosus* and *H.aurantiacus*. We isolated a novel *Herpetosiphon spp* strain(CA052B) and studied its predatory activity against clinically important organisms.

Methods: CA052B strain was isolated from soil at the edge of a stream at Llansteffan, West Wales, United Kingdom. The isolate was identified by 16SrRNA sequencing and the genome was sequenced using Illumina Hiseq2500. Biochemical characterisation was carried out using Gram negative CRYSTAL and API20NE kits. Morphology was studied with transmission and scanning electron microscopy. Lawn culture method was used to analyse predatory activity against 10 prey organisms of clinical relevance. Outer membrane vesicles were extracted and their killing activity against the prey organisms was assayed by flow cytometry.

Results: 16SrRNA sequencing of CA052B showed similarity of around 98-99% with other *Herpetosiphon* species. However comparing the genome of CA052B with the publically available genomes of *H.aurantiacus* and *H.geysericola*, the average nucleotide identity was 84.29% and 92.03% while genome-to-genome distance calculation(GGDC) showed 28.2% and 46.6% identity, respectively. Besides, biochemically CA052B was distinct from *H.gulosus* and *H.giganteus*, therefore seems to fit the criteria for being a novel species. CA052B predate efficiently on *E.coli*, *K.pneumoniae*, *P.mirabilis*, *S.aureus*, *S.epidermidis*, *S.saprophyticus* and *E.faecalis*, its purified outer membrane vesicles also exhibit killing activity against the same prey organisms.

Conclusion: The predatory *Herpetosiphon* isolate CA052B is an efficient predator against clinically important pathogens. Secondary metabolite biosynthetic clusters are apparent in the CA052B genome, encouraging further exploration of its antibiotic potential.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P027

Source tracking of fecal pollution in Lake Pontchartrain, Louisiana, USA

Samendra Sherchan

Tulane University, New Orleans, USA

Abstract

Water quality in Lake Pontchartrain was deteriorating and recreational activities along the beach were restricted by the end of the 20th Century. A microbial source tracking (MST) study was conducted to determine the fecal contamination sources at public beach of the lake, so that effective pollution control strategies can be developed. Water samples were collected over an eight-month period at ten locations along the lake in 2016 and 2017. *E. coli* and *Enterococcus* were detected in 90.6% (culture) and 97.5% (qPCR), 95.8% (culture) and 91.8% (qPCR) of water samples from all sampling sites, respectively. Significant positive relationship between *E. coli* and *Enterococcus* results was observed for both qPCR and culture methods. HF183 marker was detected in 94.3% water samples (149 of 158), with concentrations ranging from 29.0 to 6073.5 GC/100 ml and from 129.8 to 38465.6 GC/100 ml in summer and winter, respectively. The results also indicate that significant rainfall events have the potential to supply considerable loads of fecal bacteria to lake waters. Further research is needed to determine the contribution of other animals to fecal contamination in the region.

P028

Bio-catalytic upgrading of heavy and pyrolysis oils: optioneering of fossil, biorefined, and renewable resources

Sophie A. Archer¹, Joe Wood¹, Brajendra K. Sharma², Lynne E. Macaskie¹

¹University of Birmingham, Birmingham, United Kingdom. ²University of Illinois, Champaign, USA

Abstract

As fossil fuels deplete, attention is turning to intensively carbon-emitting and environmentally-damaging extraction methods to use heavy oils and bitumens. In situ catalytic upgrading can use platinum group metals (PGMs) in a once-through process, which decreases oil viscosity in situ and is cleaner but is prohibitively expensive, while the once-through process wastes limited PGM resources. Current developments involve the recovery of otherwise-lost PGMs from waste sources using bacteria, to make low grade, effective, biologically based nanoscale sacrificial catalysts. Biorefined catalyst was comparably effective to other commercial catalysts in heavy oil upgrading and suffered less fouling via accumulated 'coke'.

For ecological alternatives, pyrolysis oil from renewable biomass can produce similar liquid fuels to fossil sources after upgrading and refinery but the upgrading challenges are greater, including the need for catalytic deoxygenation. Pyrolysis oils from wood and algal sources were successfully upgraded using bio-PGM neo-catalysts. This makes a carbon neutral fuel, due to carbon sequestration during photosynthetic biomass growth, while the bacterial components supporting the catalyst become assimilated into the fuel. This improves the environmental impact of the pyrolysis oil fuels, which is currently being assessed and quantified within a life cycle analysis to compare the fossil and biomass derived routes to fuel oils in order to underpin respective business cases taking into account environmental as well as techno-economic factors.

P029

Impact of Sulphidogenic and Methanogenic Biofilms on Steel Surfaces Under Alkaline Conditions

Richard Wormald, Christopher Charles, Paul Humphreys

University of Huddersfield, Huddersfield, United Kingdom

Abstract

The disposal of intermediate-level radioactive waste within a cementitious geological disposal facility is likely to result in the formation of an extremely alkaline environment (pH 10.0 – 13.0). The potential for sulphide and/or methane generation by colonising microbes could impact on the long-term gas volumes through gas generation and the promotion of corrosion.

CDC biofilm reactors were employed to investigate the impact of sulphidogenic and methanogenic biofilms on stainless steel surfaces. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) revealed steel surfaces were colonised by microbial cells embedded in a complex organic matrix composed of polysaccharides, eDNA, lipids and protein. The biofilms formed on the steel surfaces were capable of the simultaneous generation of methane and sulphide from a range of substrates. However, classical sulphate reduction was not evident and traditional sulphate-reducing bacteria were absent from the associated microbial communities. At these pH values sulphide generation appeared to be associated with microbial degradation of the proteins present in the biofilm matrix. A route confirmed through the generation of sulphide in the absence of sulphate when a sulphur containing protein was provided as a carbon source. The extent to which the route of sulphide generation enhances corrosion is currently being investigated.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P030

Efficacy and formulation of products aiding the prevention and/or removal of microbial biofilms

James Delaney Delaney

Swansea University, Swansea, United Kingdom

Abstract

Incorrect maintenance of hot tubs and swim spas can lead to the formation of microbial biofilms on spa surfaces and piping. This in turn leads to poor water quality, excessive chemical consumption and potential public health concerns should pathogens can become established in the biofilm. It is also possible that the inappropriate use of biofilm control agents could lead to risks of antimicrobial resistance (AMR).

The objective of this study is to analyse the efficacy of three currently manufactured products aiding the removal of gross bacteriological contamination and/or biofilms in the wet leisure industry, particularly in the maintenance of hot tubs, spas and swim spas. Data derived from testing will provide a greater understanding of; recommended dose rates and product concentrations, optimal flushing frequencies, possible efficacy enhancement and the spread of AMR.

Biofilms of faecal/ non-faecal derived microbial strains affecting the wet leisure industry (*ex. Legionella*) are currently being established under static and dynamic shear conditions, following which a variety of product formulations and concentrations will be assessed for efficient biofilm removal, including time of exposure and frequency. Materials of coupons to be tested are those that are used to manufacture hot-tubs/ swim spas and are known to be suffering from biofouling. Selected isolates will then be exposed to different compounds from products to determine Maximum Tolerance Concentrations (MTC) and Minimum Inhibitory Concentrations (MIC).

The outputs of the biofilm analysis will then be studied for antagonistic and synergistic biofilm removal efficacy using benchmark dose response analysis

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P031

Radiofrequency radiation-injury of bacteria enables them to make altered palladium nanoparticles with enhanced catalytic activity

Iryna Mikheenko¹, Jaime Gomez-Bolivar², Mohamed Merroun², James Bennett³, Joe Wood¹, [Lynne Macaskie](#)¹

¹University of Birmingham, Birmingham, United Kingdom. ²University of Granada, Granada, Spain.

³University of Hull, Hull, United Kingdom

Abstract

Bacteria can make nanoparticles of palladium metal (Pd(0)) by uptake of Pd(II) ions and enzymatic reduction to Pd(0) to make a supported and bio-scaffolded metal catalyst. Nanoscale bio-Pd has high catalytic activity in chemical synthesis reactions that typically use palladium catalysts. These reactions include hydrogenations where *cis*-alkene products are highly favoured over the *trans*-alkenes. Such bio-Pd was found previously to be comparably active to commercial hydrogenation catalysts in terms of reaction rate and product selectivity in the hydrogenation of 2-pentyne and soybean oil.

Application of low-dose radiofrequency radiation (RF) (and microwaves) is known to cause transient damage to cell membranes and promote ingress of solutes into bacterial cells by bypassing the cellular permeability barrier. Cells of *Desulfovibrio desulfuricans* that had been injured in this way produced more, and smaller, Pd(0)-nanoparticles than uninjured counterparts. The two populations of Pd(0)-loaded cells were compared with respect to their ability to hydrogenate 2-pentyne. The catalyst produced by the injured bacteria showed a 2-3 fold increased rate of hydrogenation with no compromise in selectivity to *cis*-pentene. Application of the RF before exposure to Pd(II), during uptake of Pd(II) and during its subsequent enzymatic reduction to Pd(0) gave similar results, suggesting that the injury predisposed the cells to make a better catalyst. Possible reasons for this are discussed in the light of other work that revealed upregulation of some bacterial nickel uptake and processing functions arising from RF radiation from a commonly used source.

P032

Understanding the Genome-wide Response of *Streptomyces coelicolor* to the Glycopeptide Antibiotic Teicoplanin

Sam Connelly

Oxford Brookes University, Oxford, United Kingdom

Abstract

Antibiotics are indispensable in modern healthcare; they serve both prophylactic and therapeutic roles in clinical medicine and have drastically reduced patient mortality. However, our reliance on antibiotics for minor infections and agriculture has led to the release of vast amounts of antibiotics into the environment. There is a large body of evidence that links this environmental pollution to the dissemination of genes increasing antimicrobial resistance (AMR) in bacteria. A decline in antibiotic innovation coupled with the decreasing efficacy of our available drugs makes AMR a serious public health concern.

Our group is interested in prolonging the clinical use of the glycopeptides by improving our comprehension of how they function. These drugs play a vital role in the treatment of difficult infections, such as those caused by *methicillin-resistant Staphylococcus aureus (MRSA)*, for which glycopeptides are usually the last resort. But, their increased usage has led to a higher prevalence of resistance across the country.

This work focuses on improving our comprehension of the poorly understood glycopeptide teicoplanin. We have employed the non-pathogenic model organism *Streptomyces coelicolor* to characterise how teicoplanin influences its transcriptomic profile, in an effort to indirectly pinpoint which genes are required in the teicoplanin stress response. Using this approach, it has been possible to identify possible genes involved AMR to teicoplanin. This information will improve our understanding of the specific mechanism of teicoplanin and offer insight into improved antimicrobial therapies.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P033

Environmental Characterisation and Metabolic Profiling of The Anderton Park Brine Springs: An Unexpectedly Diverse Hypersaline Environment

George Gray, Molly Barsby, Matthew Kelbrick, Catherine Cooney, André Antunes

Biology Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

The Cheshire salt district is associated with a Triassic halite layer, which is at the genesis of a wide range of different hypersaline environments (e.g. salt mines, brine springs, brine fields). Despite being historically well documented, and explored for the production of salt since pre-Roman times, very few studies have looked into the microbiology of such locations (e.g. [1]).

Among the least know sites within the Cheshire salt district, we have a group of brine springs, located in the Anderton Nature Park. Here we provide an overview of this spring system and include full data on their physico-chemical characteristics (salinity, pH, temperature and concentration of selected ions), as well as first results on microbial metabolic profiling. Our data has revealed an unexpected dynamic setting with a diversity of conditions and environmental niches, which is matched by differences in substrate use by the local microbial communities. This is the first step for further exploration of the biodiversity and biotechnological potential of the Anderton Park Brine Springs, which is currently under way [2,3].

[1] Norton et al. (1993). *Microbiology*, 139(5):1077-1081.

[2] Hardman et al. (2018). *Microbiology Society Annual Conference*.

[3] Kelbrick et al. (2018). *Microbiology Society Annual Conference*.

P034

Role of phosphate and magnesium in nisin biosynthesis in *Lactococcus lactis*

Athanasios Nikolaou, Claudio Avignone-Rossa, Jorge Gutierrez-Merino

University of Surrey, Guildford, United Kingdom

Abstract

Nisin is a post-translationally modified peptide of the lantibiotic family produced by the *Lactococcus lactis*. Nisin synthesis requires a functional cluster of 11 genes of which 7 are regulated by the presence of nisin. However, little is known about the regulation of the 4 other genes -constitutive-, whose expression is crucial to initiate nisin production and may be dependent on nutritional and environmental factors. **The aim of this work is focused on the understanding of the role of phosphate and magnesium in nisin biosynthesis by monitoring the expression of nisin constitutive genes in *Lactococcus lactis*.** Based on the experimental data generated by transcriptional analysis, we have observed that both inorganic phosphate (P_i) and magnesium (Mg^{2+}) have a critical regulatory effect upon the expression of the 4 constitutive genes. These results have been correlated with the antimicrobial phenotype since the level of expression of each gene is affected depending on the concentration of P_i and Mg^{2+} . Our results allow determining the dependence of the expression of each of these 4 genes on P_i and Mg^{2+} , providing key information to understand the importance of those nutrients for optimal regulation of nisin biosynthesis. We will show how the biosynthetic conditions affect the growth of *L. lactis*, and a model to understand the role of lantibiotics as growth regulators, which can be to other Gram positive species able to produce those peptides, including pathogens and antibiotic resistant bacteria.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P035

Increased tolerance to contact lens disinfecting solution in clinical isolates of *Pseudomonas aeruginosa* from keratitis patients

Yasmin Hilliam, Craig Winstanley, Stephen Kaye

University of Liverpool, Liverpool, United Kingdom

Abstract

Bacterial infections of the cornea are a major cause of vision loss worldwide, with approximately 6000 cases of bacterial keratitis per year in the UK alone. Through the UK Microbiology Ophthalmic Group (MOG), we have collected 658 isolates of *Pseudomonas aeruginosa* associated with keratitis (2003 – 2012), with linked clinical metadata including antibiotic MIC data and patient contact lens (CL) use.

Screening of the *P. aeruginosa* isolates indicated that some were more resistant to CL disinfection solution than others. In order to understand the mechanisms underlying this resistance, two susceptible clinical isolates and a laboratory strain control (PAO1) were subjected to growth in sub-inhibitory concentrations of CL disinfecting solution over a period of 21 days. The aim was to evolve resistant variants with enhanced resistance. Although we were unable to isolate variants exhibiting stable resistance (due to fixed mutations) to the CL disinfecting solution, the treatment allowed us to identify isolates with an increased tolerance to CL disinfecting solution following growth in sub-inhibitory concentrations of the solution. However, when these isolates were grown in nutrient rich media without the presence of disinfecting solution, their susceptibility to CL disinfectant was greater than the initial inoculating strain.

In order to elucidate the mechanisms of tolerance in these isolates, we will use RNAseq to investigate gene expression changes in the presence of the disinfecting solution.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P036

Differential amplicons for the evaluation of RNA integrity – adaptation to complex microbial communities

Fabien Cholet, Umer Zeeshan Ijaz, Cindy J. Smith

University of Glasgow, Glasgow, United Kingdom

Abstract

Reliability and reproducibility of transcriptomics-based studies are highly dependent on the integrity of RNA. Microfluidics-based techniques are now the best approaches to detect RNA degradation, although rarely used or reported in microbial ecology publications. Still, these techniques are ignorant of the nature of the RNA, and will only give an overview of the integrity of the sample. Since ribosomal RNAs constitute the majority of the total RNA pool, such integrity indexes are not specific to the meaningful part of the sample, the mRNAs. We therefore propose to adapt a recently developed integrity index that only targets mRNA, based on the differential amplification of RT-qPCR products with different lengths from the same reference transcript. We show that this index was a better predictor of the outcome of transcriptomic analysis of a functional gene (bacterial *amoA*) across a range of controlled-degraded RNA samples extracted from complex microbial communities. Such indexes could therefore be used as routine check points in complementation of microfluidics-based techniques.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P037

Public swabbing activity to look for environmental bacteria with antimicrobial properties

Alice Huxley¹, Adam Blanchard¹, Steve Hooton², Sabine Totemeyer¹

¹School of Veterinary Medicine and Science, University of Nottingham, Loughborough, United Kingdom.

²School of Biosciences, University of Nottingham, Loughborough, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a worldwide health concern, with multi-drug resistance on the rise. Methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant (MDR) *Escherichia coli* (*E. coli*) can cause serious, complicated infections that are becoming more difficult to treat.

As part of an outreach programme aimed to increased awareness, the general public were involved in collecting samples by swabbing for bacteria in the environment and the isolates were tested for antimicrobial properties against MRSA and MDR *E. coli*. The isolates were overlaid with the resistant bacteria. Of 635 individual isolates, 14 were found to produce antimicrobial agents inhibiting MRSA and/or MDR *E. coli*, isolated from diverse places such as shoes, piano keys, railings, coins, wood and stairs. Further characterisation is required to determine if this is due to any novel antibiotics.

A survey was taken alongside the practical activity. While knowledge about the effect of antibiotics against bacteria and viruses was good overall, there were still common misconceptions amongst the general public, such as assumptions that the body develops resistance to antibiotics. The practical activity was engaging and sparked discussion amongst participants, although the type of person who took part was more likely to have an interest in antibiotic resistance, and therefore already hold a positive attitude toward antibiotic use.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P038

Isolated bacterial strains with capabilities of aflatoxin B1 degradation and aflatoxigenic fungi inhibition

Hye-Eun Lee, Hong-Gyu Song

Kangwon National University, Chuncheon, Korea, Republic of

Abstract

Aflatoxin B₁ (AFB₁) produced mainly by *Aspergillus flavus* is a common contaminant on grains, posing health hazards to human and animal. The aim of this study is screening of bacteria that can degrade AFB₁ and inhibit aflatoxigenic *A. flavus*. Over 350 strains were isolated using coumarin medium and their AFB₁ degrading capability was measured by HPLC and inhibition of growth, spore formation and germination of *A. flavus* were examined. Isolates identified as *Bacillus subtilis* AF11, *Streptomyces panaciradicis* AF34, *S. thermoviolaceus* AF125 and *Rummeliibacillus pycnus* AF129 indicated antifungal and AFB₁ degradation activity. Some strains decreased up to 80% fungal colony radius and inhibited sporulation up to 99%. Reduction of spore germination was also observed (20 to 70%). Production of siderophore which selectively absorbs iron from environments, and chitinase and β -1,3-glucanase which break down fungal cell wall was part of the antifungal activity of these isolates. When the isolate and aflatoxigenic fungi were inoculated simultaneously in yeast extract sucrose medium, mycelial growth and AFB₁ concentration decreased in all experimental groups. Especially, AF11 strain decreased 97.1% of fungal growth and completely reduce AFB₁ at 30°C, 200 rpm and pH 5.5~7. Strains AF34, AF125 and AF129 degraded 100.0 μ g/L of AFB₁ to 0.0, 9.9 and 14.9 μ g/L, respectively, and more than 83% of degradation occurred by cell-free supernatant. These results suggest that these isolates can be used to protect crops against toxigenic fungi and treat mycotoxin contaminated grains, and therefore decrease economic damage in agriculture.

P039

Microbial Bioprospection in the Cheshire Salt District: Surveying Bioplastic-Producing Potential

Ruth Hardman¹, Matthew Kelbrick², Marta Filipa Simões², André Antunes²

¹School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom. ²Biology Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

The commercial and scientific development of bioplastics as natural environmentally-friendly and renewable alternatives to petroleum plastics, is an area of emerging development and growth. The current production of bioplastics or polyhydroxyalkanoates (PHAs) is underused due to high production and extraction costs, unsatisfactory yields, and lack of novel PHA-producing microorganisms [1]. In this regard, the exploration of microbes living in hypersaline environments (halophiles) for production of PHAs has gained considerable traction due to the resilience of these strains and expected reduction of costs [2,3].

Here we present the results of our study, focused on the bioprospection of novel halophilic microbial strains isolated from several poorly studied hypersaline environments in the Cheshire Salt District (brine springs, brine fields, and a salt mine) [4,5]. We have successfully isolated over 180 microbial strains, which were screened for the production of bioplastics using several staining methodologies (Nile Red, Nile Blue, Sudan Black B), and have identified 26 strains that tested positive for PHA production. Our results highlight the biotechnological relevance of these underexplored extreme sites, and further characterization of our isolates and their bioplastic production is expected to yield further advances in this field.

[1] Madison and Huisman (1999). *Microbiology and Molecular Biology Reviews* 63:21-53.

[2] Antunes et al. (2018). Bioprospecting. *Topics in Biodiversity and Conservation*, vol 16, pp. 81-112.

[3] Yin et al (2015). *Biotechnology Advances* 33(7):1433-1442.

[4] Gray et al. (2018). *Microbiology Society Annual Conference*.

[5] Kelbrick et al. (2018). *Microbiology Society Annual Conference*.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P040

The Cheshire Salt District: An Unexplored Source of Halophiles with Biotechnological Potential

Matthew Kelbrick, André Antunes

Biology Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

Despite recent developments and successes in biotechnology, wider use is frequently hindered by a combination of several factors. Among these, the mismatch between resilience of used microbial strains (and their biomolecules) and the harsh conditions associated with industrial processes, as well the high economic costs of maintaining a sterile environment and of extracting bioproducts, are seen as major limitations [1]. Many of these can be overcome by the use of halophiles, which explains the current boom in biotechnological research associated with these organisms (e.g. [1,2]).

Our study focuses on the bioprospection of novel halophilic microbial strains isolated from several hypersaline environments in the Cheshire salt district. The district has an impressive range of interesting high salinity environments, which remain mostly unstudied [3,4]. We have collected samples from brine springs, brine fields, and a salt mine and successfully isolated over 50 microbial isolates. All strains were screened for the production of specific enzymes relevant for biotechnology (amylase, proteases, lipases, alkane hydroxylases), and for antimicrobial activity. Our results are quite promising and highlight the enormous untapped potential of further exploration of these locations.

[1] Yin et al (2015). *Biotechnology Advances* 33(7):1433-1442.

[2] Antunes et al. (2018). Bioprospecting. *Topics in Biodiversity and Conservation*, vol 16, pp. 81-112.

[3] Gray et al. (2018). *Microbiology Society Annual Conference*.

[4] Hardman et al. (2018). *Microbiology Society Annual Conference*.

P041

Genomic-based Insights into the Production of CaCO₃ Biominerals by *Bacillus* and *Idiomarina*

Philippa Loftus, André Antunes

Biology Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

'Microbially Induced Calcite Precipitation' (MICP) has been reported in numerous species, yet remains poorly elucidated. This process offers a wide variety of current and potential applications, ranging from e.g. agricultural soil improvement, concrete strengthening and façade restoration, bio-remediation, to CO₂ sequestration [1-2]. The current literature seems to point to urease being the main pathway involved in the process, although a few additional enzymes have been identified in restricted groups of species [3-5].

In our study, we looked into a selected group of well-known CaCO₃ biomineral producing species in the genera *Idiomarina* and *Bacillus*. We produced a list of all genes currently recorded in the literature as being implicated in MICP, and used this to analyse whole-genome sequence data of our target microbes. Our approach allowed us to identify common traits and trends and we used these results to predict MICP capability in previously untested strains within these two genera. Our preliminary results point to clear differences in MICP-gene presence in both genera and question the relevance of urease in the species that we have analysed. Our study provides important insights into understanding the genomic basis for MICP and into the feasibility of using genomic-based prediction for quick identification of new strains with such capabilities.

[1] Anbu et al. (2016). *SpringerPlus*. 5:250.

[2] Rossi et al. (2015). *N Biotechnol* 32:109-120.

[3] Maciejewska et al. (2017). *Front Microbiol* 8:1181.

[4] Mobley and Hausinger (1989). *Microbiol Rev* 53(1):85-108.

[5] Zhu et al. (2017). *Front Microbiol* 8: 602.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P042

Plant growth promoting Burkholderia induce the Grapevine (*Vitis vinifera* L.) defense response against the grey mould caused by *Botrytis cinerea*

Qassim ESMAEEL, Mathilde Robineau, Stephan Dorey, Christophe Clément, Cédric Jacquard, Lisa Sanchez, Essaid Ait Barka

Laboratoire de Stress, Défenses et Reproduction des Plantes URVVC-EA 4707, UFR Sciences Exactes et Naturelles, University of Reims-Champagne-Ardenne, Reims, France

Abstract

Grey mould caused by the necrotrophic pathogen *Botrytis cinerea* is among the most important disease affecting production of Grapevine worldwide. The high economical lose each year has led producers to become more dependent on chemical pesticides for protection. However, environment impacts of the pesticide overuse have sparked crescent interest in developing alternative biocontrol method. The use of plant-associated bacteria has received many scientific and economic attentions as an alternative method of reducing the use of chemical pesticides in agriculture. Thirty-three strains isolated from the rhizosphere of corn in the Northeastern France were evaluated for their antagonistic effect. Seven strains were found to exhibit an antagonistic effect against phytopathogenic fungi including *Rhizoctonia solani*, *Fusarium oxysporum* and *Botrytis cinerea*. Phenotypic and molecular characterization showed that isolates belong to the genus *Burkholderia*. When the grapevine plants were infected with *Botrytis*, all plants associated with isolated strains showed a significant protection against *Botrytis* compared to non-bacterized plants. To understand the mechanisms contributing to the biocontrol effect of selected isolates, the production of reactive oxygen species and the induction of several defense genes were investigated. The maximum accumulation of H₂O₂ was detected in the inoculated cell suspension medium 30 min after the challenge with *B. cinerea*. Interestingly, no significant accumulation of H₂O₂ was detected in the cell suspension medium after challenge with isolated strains. After the pathogen challenge, results showed that grapevine cell culture inoculated with isolated strains exhibited significant expression of *PR* genes (*PR5* and *PR10*) in response to *B. cinerea*.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P043

Evaluating the most appropriate pooling ratio for EDTA blood samples to detect Bluetongue virus using real-time RT-PCR

John Flannery, Paulina Rajko-Nenow, Hayley Hicks, Holly Hill, Simon Gubbins, Carrie Batten

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Background: Bluetongue (BT) is a viral disease of ruminants caused by BT virus (BTV) that is transmitted by *Culicoides* biting midges. Numerous BTV serotypes are circulating in Europe resulting in animal movement restrictions. Ruminants imported to BTV-free areas (e.g. UK) from BTV-endemic areas must be tested using real-time RT-PCR. Pooling of EDTA blood samples can reduce the cost of analysis and increase throughput.

Methods: BTV-negative EDTA blood was spiked with three BTV serotypes (BTV-1, BTV-4 and BTV-8) to yield C_T values similar to those reported in BTV infection studies. These positive samples were then diluted in EDTA blood at pooling ratios of 1:2, 1:5, 1:10 and 1:20 and analysed using two group specific BTV assays (Hofmann *et al.*, 2008 and a commercial assay). A statistical model was used to determine the probability of detecting a single BTV positive sample in a pool.

Results: Both assays detected BTV in the unpooled samples, but the detection rate diminished with increased pooling ratio. BTV was detected at peak or late infection irrespective of the pooling ratio with a >80% probability of detecting BTV in a pool.

Conclusion: We determined the most appropriate pooling ratios to detect BTV based on a statistical model. Pooling at ratios of 1:10 or greater is appropriate for use in BTV-endemic countries e.g. during surveillance or declaration of freedom of disease. However, a ratio of 1:5 may be more appropriate for countries e.g. the UK where BTV is not endemic.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P044

Genomic mining to identify novel biosynthetic pathways encoding novel bioactive compounds in marine bacterial strains.

Sloane Patry, Lekha Margassery, Alan Dobson

University College Cork, Cork, Ireland

Abstract

Microbes associated with marine sponges are exposed to competitive environments both physiologically and nutritionally which is likely to promote the production of novel secondary metabolites. This study focuses on the identification of novel bioactive compounds from a number of bacterial strains that were isolated from marine sponges. Twenty two bacterial isolates were found to be active against a range of clinical pathogens in spot-plate overlay assays. Eighteen of the strains were confirmed as being *Streptomyces* strains following 16S rRNA sequence analysis. We then focused on three of these strains which displayed the best range of bioactivity, namely SM3, SM9 and B226SN104 and employed the OSMAC (one strain many compounds) approach involving the use of different nutrient sources, followed by well diffusion assays to verify the production of bioactive compounds. Cell free supernatants were chemically extracted using Liquid Liquid Extraction (LLE) with ethyl-acetate. Secondary metabolites from both aqueous and organic phases were tested using the NCCLS colorimetric broth micro-dilution assay to determine the Optimal-Nutrient-Source media. Both strains B226SN104 and SM9 produced antifungal bioactive compounds, while SM3 produced antibacterial bioactive compounds. M400 and M19 were the optimal media for production of anti-fungal compounds for B22SN104 and SM9. The genomes of these three strains have been sequenced and genome scanning approaches, coupled with chemical analysis will be employed to characterize the gene clusters encoding the observed bioactivities, coupled with chemical analysis to characterize the molecules involved.

Keywords: Marine bio-discovery, *Streptomyces* species, bioactive compounds, OSMAC.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P045

Antimicrobial activity of soil bacterial isolates against several human skin pathogens

Da-Sol Lee, Hong-Gyu Song

Kangwon National University, Chuncheon, Korea, Republic of

Abstract

Contaminating microorganisms in cosmetics may cause a spoilage of the products and represent a serious health risk for consumers. This study focuses to screen environment-friendly antimicrobial substances produced from bacteria to replace harmful chemical antiseptics such as parabens. Thousands of bacterial strains were isolated from soils and their antimicrobial activity against human skin pathogens, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* was examined. Isolated strains *Burkholderia gladioli* DS518, *Pseudomonas fluorescens* DS1386 and *Paenibacillus elgii* DS1515 showed 14 to 26 mm diameter of growth inhibition zone against all target organisms on agar plates. Antimicrobial substances in the culture supernatants of DS518 and DS1515 were extracted using ammonium sulfate precipitation method and freeze drying, and that of DS1386 was extracted with ethyl acetate. Purified compounds from each strains showed low minimum inhibitory concentration (MIC) against all target organisms (0.156-10 mg/ml). Time-kill assay with 1 MIC purified compounds indicated a significant antimicrobial effect on target organisms. Colonies of all target organisms were undetectable after 4-24 h incubation in the presence of purified compounds (1 MIC). Antimicrobial compounds from DS518 and DS1515 may be antimicrobial peptide and biosurfactant such as lipopeptide, respectively. Culture supernatant of DS1515 showed clear zone in oil spreading test and reduced surface tension of culture from 60 to 42 mN/m. DS1386 produced 0.087 mmol/ml of siderophore. These results suggest that isolates DS518, DS1515 and DS1386 may be utilized as an environment-friendly biocontrol agent against some important human skin pathogens in cosmetics.

P046

Evaluation of effect of urea concentration on CaCO₃ mineralization induced by ureolytic *Bacillus amyloliquefaciens* subs. *plantarum* U17

Merve Tepe, Nazime Mercan Doğan, Tamer Koralay

Pamukkale University, Denizli, Turkey

Abstract

Background: The aim of this research is to determine the effect of urea concentration on amount and structure of the produced calcium carbonate crystals by U17.

Methods: Strain U17 was inoculated in CPM which was comprised of 25 mM CaCl₂, 25 mM NaHCO₃ and 3 g/l nutrient broth at 37°C. The urea concentration was 25-350 mM. Precipitate were collected and analysed by SEM and EPMA.

Results: Urea concentration was detected to have significant effect on amount and structure of the crystals. Highest calcium carbonate production rates were observed in 25 mM urea concentration in 5th day (1863 mg CaCO₃/lt) 333 mM urea concentration in 5th day (1954 mg CaCO₃/lt). In other urea concentrations, production rates were detected to be lower. CaCO₃ minerals produced by U17 were detected to differ with regards to amount and structure in different urea concentrations. In 25 mM urea spherical vaterite crystals were observed predominantly. On the contrary trigonal calcite crystals were observed to dominate the mineral formation in 333 mM urea.

Conclusion: This feature may be utilised in various executions of biomineralization on a great deal of engineering applications.

Acknowledgements: We thank to PAUBAP, Project Number: 2017FEBE016.

Keywords: *Bacillus amyloliquefaciens* subs. *plantarum*, MICP, urea, calcium carbonate.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P047

What impact does cold air atmospheric plasma have on bacterial biofilms formed on hospital surfaces?

Muireann Fallon¹, Sarah Kennedy¹, Stephen Daniels², Hilary Humphreys^{1,3}

¹Royal College Of Surgeons in Ireland, Dublin, Ireland. ²Dublin City University, Dublin, Ireland. ³Beaumont Hospital, Dublin, Ireland

Abstract

Background: Healthcare-acquired infections are a growing concern the world over. The microorganisms responsible for these infections are capable of living on inanimate surfaces and these surfaces become a source of infection. A potentially exciting breakthrough in hospital decontamination methods has been the recent research into cold atmospheric air plasma systems.

Methods: Confocal microscopy was performed on *Staphylococcus* and *Escherichia coli* biofilms to assess the microbial viability after plasma treatment. Biofilms were grown on various hospital surfaces and the viability of the bacteria post treatment was evaluated using fluorescent staining. Scanning electron microscopy of biofilms showed the topology of treated bacterial cells.

Results: The work in this poster demonstrates the effective killing of *Staphylococcus* and *Escherichia coli* biofilms on several common hospital surfaces by cold atmospheric air plasma treatment. A decrease in microbial viability is seen in both Gram positive and Gram negative bacterial biofilms. Plasma treatment has been shown to be effective at reducing viability of these bacteria on both metal and non-metal surfaces commonly used in hospitals. The scanning electron microscopy images showed distortion of the bacteria after plasma treatment.

Conclusions: The antimicrobial properties of cold atmospheric air plasma systems make it an attractive new method in hospital decontamination. The use of these devices could reduce healthcare-acquired infections, particularly through the inactivation of bacterial biofilms on hospital surfaces.

P048

An Investigation into Avian Malaria in Humboldt Penguins at Twycross Zoo

Isabella Hannay, Eleanor Ransford

University of Nottingham, Nottingham, United Kingdom

Abstract

Avian Malaria is caused by Apicomplexa parasites, which infect a wide range of avian species, and is transmitted by a dipteran vector. During 2016, several UK zoological collections experienced high mortality rates in their Humboldt Penguin collections due to Avian Malaria. Twycross Zoo has had no recorded deaths from Avian Malaria. The purpose of this research project was to investigate whether Avian Malaria could be detected in insects and Humboldt Penguins at Twycross Zoo.

Insect trapping was conducted over a period of 6 weeks between June and August 2017. Species identification was conducted using a preliminary morphological examination and subsequent PCR. The PCR products were purified and underwent DNA sequencing by Sanger to give the species identification. Very few mosquitoes (all *Culex pipiens* species) were collected. One explanation for this could be the increased rainfall compared to the past 3 years.

A qPCR protocol was determined and a positive control for the Avian Malaria parasite was created using a plasmid. Bacterial transformation was used to amplify this plasmid.

DNA extraction was performed on surplus blood samples from 3 Humboldt Penguins and 2 Arctic Owls, which were part of the Twycross Zoo collection. None of the samples tested were positive for Avian Malaria.

Whilst these results do not conclusively prove that Avian Malaria is not present in insects and Humboldt penguins at Twycross Zoo, it does contribute to epidemiological data regarding Avian Malaria in UK Zoological Collections.

P049

Characterisation of inducible antibiotic production by streptomycetes isolated from hyper-arid environments

Tiago Santos, Jana Schniete, Paul Herron

University of Strathclyde, Glasgow, United Kingdom

Abstract

Extreme or untapped environments are being explored for holding antimicrobial producer strains with novel metabolic pathways. One example is the Atacama Desert in Chile, where lack of precipitation and a very high solar radiation makes it the ultimate arid desert on Earth. The main aim of this project is to isolate strains from this extreme environment, the Atacama Desert, and identify novel secondary metabolites and/or induce their production using elicitors.

Two different locations in the Atacama Desert were sampled for soil at the same altitude. In total, from both soils, seventeen isolates were identified based on 16S rRNA as belonging to the same species, *Streptomyces phaeoluteigriseus* DSM 41896. Bioactivity was found against the ESKAPE pathogens, when grown on different ISP media. The seventeen isolates showed a wide range of activity, mostly against *Staphylococcus aureus* (15/17) and *Enterococcus faecalis* (9/17). Additionally, 3 isolates showed activity against *Acinetobacter baumannii* and one against *Escherichia coli*. Furthermore, the isolate that inhibited *E. coli* could also inhibit the other four pathogens. Isolates that showed activity against *A. baumannii* shared some phenotypic similarities and were selected for whole genome sequencing that revealed some differences between isolates and the type strain. Several biosynthetic clusters for secondary metabolites were predicted by antiSMASH including NRP's and lanthipeptides. Interestingly, the average nucleotide identity obtained for the isolates, when compared with the type strain, was below the threshold considered as the same species.

We are currently investigating the differences between isolates from both locations, their elicitation potential and interactions.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P050

The diversity of microbiome composition of wastewater treatment plant effluent

Thi Thuy Do, Sarah Delaney, [Fiona Walsh](#)

Maynooth University, Maynooth, Ireland

Abstract

The microbiome composition of effluent from two wastewater treatment plants (WWTPs) in Ireland were analysed using 16S rRNA amplicon sequencing. DNA was extracted from samples collected on three consecutive days in triplicate in March and October 2015 and 2016. The phyla abundance analysis show that Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Tenericutes and Verrucomicrobia phyla dominated the effluent microbiomes in most sampling periods in both WWTP A and B effluents, which is similar to results from Hong Kong WWTP effluent. The abundances of the phyla Bacteroidetes and Firmicutes in WWTP effluent samples were consistent with that of the human microbiome. The α -diversities of microbiomes were analysed by Shannon Index rarefaction measure. The α -diversity of the phyla composition was significantly different between sampling periods and both WWTPs. The effluent microbiome was relatively stable in March but highly variable in October. The phyla composition of samples collected on the first two days (6 replicates) from both WTTTPs in October 2015 varied greatly. A major change in the bacterial composition was observed for five phyla Bacteroidetes, Actinobacteria, Spirochaetes, Chloroflexi and Planctomycetes. The reasons for the variability in bacterial composition are still unknown. Our results demonstrated the seasonal diversity in bacterial composition of the WWTP effluent and the bacterial profile of treated wastewater share a high similarity to human microbiome. This information provides the basis for understanding the structure and the fate of microbiome in the WWTP effluent.

P051

Characterisation of a colistin resistant plasmid isolated from the gastrointestinal tract of broiler chickens

Sarah Delaney^{1,2}, Richard Murphy², Fiona Walsh¹

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

Abstract

Antibiotic resistance is a major problem for the treatment of infectious diseases in animals and humans. Of particular concern is plasmid-mediated resistance, as many resistance plasmids have the ability to transfer between different bacterial species. Colistin is an antibiotic of last resort for multi-drug resistant gram-negative infections. We investigated the presence of plasmid-mediated colistin resistance in the caecum of broilers, which were raised for meat production. DNA was extracted directly from a broiler caecal sample and plasmid DNA amplified with phi29 DNA polymerase. DNA was transformed into *Escherichia coli* DH5 α and selected on tetracycline (16 mg/L). Antibiotic susceptibility testing was performed on the transformants using the agar dilution method according to the EUCAST guidelines. The transformants had a minimum inhibitory concentration to colistin of >128 mg/L. Conjugation was successfully performed between the transformants and a rifampicin resistant *Escherichia coli*, indicating that the plasmid is transferrable by conjugation. PCR was negative for the presence of the *mcr-1* gene. The plasmid will be sequenced using Illumina MiSeq, and analysis of the sequence may allow for the identification of a novel gene responsible for the colistin resistance phenotype. The identification of a plasmid conferring high-level colistin resistance is concerning, as it may have the ability to transfer to other pathogenic Enterobacteriaceae. This would further limit the available treatments for multi-drug resistant infections.

P052

Bioremediation of Hexavalent Chromium by Methane Oxidising Bacteria

Salaheldeen Enbaia

Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Background - Chromate (VI) is an oxidising pollutant that is harmful to humans and the environment. Previously it was shown that *Methylococcus capsulatus* (Bath), an example of a diverse group of methane-oxidising bacteria widespread in the environment, can reduce Cr (VI) to the less toxic Cr (III), although the cellular location and mechanism of the reaction had not been investigated.

Methods - *Mc. capsulatus* cultures were grown on methane (Bath) were broken and fractionated by differential centrifugation into cytoplasm, cell wall and cell membrane fractions. A Cr(VI)-removing activity was partially purified from the cytoplasm fraction via anion exchange and Blue Sepharose affinity chromatography. Chromium species were quantified via inductively coupled plasma-mass spectrometry (ICP-MS) connected to ion exchange HPLC.

Results - All cellular fractions reduced Cr (VI) to Cr (III); reduction activity was greatest in the cytoplasm fraction. The chromium (VI)-removing activity was partially purified from the cytoplasmic fraction. Its activity was enhanced by adding the electron donor NADH. Reduction of Cr (VI) (20 ppm) by *Mc. capsulatus* cultures was monitored over 144 h. Within 48 h most of the Cr (VI) within the culture supernatant was lost and Cr (III) appeared in the cellular cytoplasm+membranes fraction. When Cr (III) was added to separate cultures, this entered the cytoplasm+membranes fraction during a similar period.

Conclusion - These results are consistent with a model in which *Mc. capsulatus* cells accumulate the chromium concomitant with reducing it. This may be a useful property for bioremediation applications.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P053

One Yeast Strain to Rule Them All: Candidate Screening to Identify Industries Precious Production Strain.

Ainsley Beaton, Nicholas Tucker

University of Strathclyde, Glasgow, United Kingdom

Abstract

Industrial bio-processes require production strains that are highly optimised for very specific conditions and feedstocks. Strain improvement processes generally produce a collection of candidate strains from a specific high-throughput assay. Candidate strains must then be fully characterised for a variety of properties such as utilisation of diverse carbon sources, reduced stress responses and product yield. In this industrial collaboration, we are using phenotypic profiling to improve the pipeline for evaluation of engineered *Saccharomyces cerevisiae* strains for use in bio-ethanol production. We are using a combination of Omnilog:Biolog phenotypic arrays and next-generation sequencing technologies to characterise the effects of both random and targeted strain improvement strategies. Phenotypic analysis has shown small but measurable differences between industrial strains in growth conditions that are relevant to the industrial process. Our industrial partner is using both targeted and random chromosomal integration of transgenes in order to improve EtOH yield from specific feed stocks. Next generation sequencing is being applied to map the insertion sites of the transgenes that result from the random strain improvement method. Touch-down PCR, using one transposon specific primer and one variable primer allows for amplification of the flanking sequences of multiple random insertions in each strain. Barcoded sequencing allows the insertion sites of multiple strains to be sequenced simultaneously and mapped onto the chromosomes, giving not only the flanking sequence but also the details of multiple insertion sites.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P054

Antibiotic resistance profiles from gut microflora of Irish pigs

Aoife Joyce, Sophia Dix, Ciara Tyrrell, Sinéad Murphy, Fiona Walsh

Maynooth University, Maynooth, Co. Kildare, Ireland

Abstract

Antibiotic resistance (AR) is currently one of the greatest threats to animal and human health. It is crucial that all sources of AR are identified and controlled to minimize the transfer of resistance bacteria and/or genes within animals, and between animals and humans. AR is more frequently being identified in food animals. In this study we investigated the resistance profiles of the gut microflora of pigs to three critically important classes of antibiotics; polymyxins, aminoglycosides and carbapenems. Selective agars without/with antibiotics (amikacin, cefotaxime, colistin, imipenem and kanamycin) were used to isolate AR bacteria from pig faecal samples. The cultured isolates underwent antibiotic susceptibility testing. All isolates were speciated by 16S rRNA PCR and sequencing. A total of 476 bacterial strains were isolated from three faecal swab samples. The percentage of isolates resistant to each antibiotic was as follows: amikacin – 18%; cefotaxime – 27%; colistin – 10%; imipenem – 32%; kanamycin – 56%, while 3% of the isolates were multi-drug resistant (MDR). Resistant isolates were identified as *Alcaligenes*, *Carnobacterium*, *Citrobacter*, *Escherichia*, *Enterococcus*, *Klebsiella*, *Micrococcus* and *Pseudomonas*. The identification of resistant isolates to different antibiotic classes from pig faecal samples is concerning, especially when a small percentage of these isolates are MDR, which will be detrimental for the treatment of infections in humans and animals. This highlights the need to identify all potential reservoirs of AR bacteria in the gut microbiomes of animals.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P055

Can microbiocides improve the growth and resistance of forage varieties of *L. perenne* sown in Northern Ireland to *Drechslera*, a major grass fungal pathogen?

Juluri Rao^{1,2}, David Nelson¹, Colin Fleming^{1,3}, Thomas Fleming¹, Richard Salvage³, Christopher Selby¹, Trevor Martin¹

¹Agri-Food & Biosciences Institute (AFBI), Belfast, United Kingdom. ²University of Ulster, Coleraine, United Kingdom. ³Queen's University, Belfast, United Kingdom

Abstract

The UK climate change predictions project milder, wetter winters, and hotter, drier summers favouring grassland yield potential but equally conducive for grass diseases. UK records since 1980s suggest that dependent on overall climate, major grass fungal diseases are Crown rust, Mildew, *Drechslera* and *Rhynchosporium*. Pot trials were conducted on six varieties of *L. perenne* carrying a range of *Drechslera* resistances viz., Aberstar, GlenArm (highly susceptible), Boyne, Kilrea (intermediate resistance) and Fintona, Aston Energy (highly resistant) and varietal response was assessed against microbiocide treatments. Plastic, grey stacking boxes (400x300 x118 mm) filled with standard grass propagation (at 4 cm spacing, 6 x 8 grid 96 seeds per box) was accomplished in a compost soil mix. Treatments of microbiocides was replicated on six varieties of *L. perenne*, with 4 replicate blocks kept apart in three separate row plots, to prevent cross-contamination of the microbiocides. The row of plots were laid out on an open concrete space on black polythene sheets covered with capillary matting. Results from this experiment based on an image analyser was promising in that, either the native soil microorganisms (e.g. PP=Paenibacillus polymyxa isolated by AFBI) or potential candidate commercial microbiocide formulations (e.g. MP=Maxstim products) can be used as alternative fungicides. This preliminary study also suggests that microbiocides modify the growth and disease resistance of forage varieties of *Lolium perenne* and the potential of improving the resistance to *Drechslera*, a major fungal pathogen of *L. perenne* in the autumn in Northern Ireland, was of particular interest for grassland management.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P056

Targeted delivery system for colorectal cancer treatment using bacterial spores

Teja Sirec, Selvi Vickramathithan, Munehiro Asally

University of Warwick, Coventry, United Kingdom

Abstract

Bacterial spores have been used as platform for protein display; antigens, peptides and enzymes have been displayed on spores and used for oral vaccines, probiotics and food supplements. The system offers many advantages including the stability of displayed proteins and resistance to harsh conditions, which enables oral delivery of bioactive molecules to the gut. In this project, we aim to develop engineered *Bacillus subtilis* spores as a drug delivery system for the treatment of colorectal cancer (CRC), which we named SmartSpore. CRC is the third most common cancer with 450 thousand new cases diagnosed in Europe in 2012. Currently available treatments are invasive and carry risk of severe side-effects, smart drug delivery with spores could reduce the side-effects by locally increasing the dose of the therapeutic chemicals. Our SmartSpore is designed to bind specifically to the tumour cells with a germination control system. Firstly, we displayed on spores 4 ligands, which bind a CRC marker. Secondly, we displayed on spore surface 3 variants of an enzyme, which prevents undesired spore germination during the passage through the gut. Thirdly, we tested 4 cleavage sites for a CRC-specific protease, which would control spore germination upon binding to the tumour. The engineered spores carrying a CRC marker ligand and germination control system have been constructed and tested *in vitro*. To the best of our knowledge, this is a first example of a posttranscriptional regulatory circuit engineered on the spores and is a promising approach for targeted and controlled drug delivery for intestinal pathologies.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P057

Using novel iron-oxidising, halotolerant bacteria for energy production in microbial fuel cells

Patricia Linton¹, Carena Bell¹, Guadalupe Alvarez Gonzalez², Craig Banks¹, Robin Sen¹

¹Manchester Metropolitan University, Manchester, United Kingdom. ²University of Manchester, Manchester, United Kingdom

Abstract

Microbial fuel cell (MFC) technologies provide potential for a sustainable method of generating electricity to replace non-renewable sources of power. Strain and consortia selection is important in optimising the efficiency of such systems. The iron-oxidising bacteria are a largely overlooked group of microorganisms in MFC technology but have huge potential for power generation and may be able to provide further environmental benefit via bioremediation of metal contaminated environments such as acid mine drainage. This study aims to determine the power generation capacities of iron-oxidising bacteria in small-scale MFC systems. The performance of small scale MFCs were tested with pure cultures of *Acidithiobacillus ferrooxidans*, and three isolates of halotolerant iron-oxidising bacteria previously isolated from mine-tailings and contaminated estuarine sediments. The test strains were grown in ferrous iron medium with graphite felt as the electrode material and compared to power generation by *Saccharomyces cerevisiae* grown with glucose as the energy source and MFCs without bacteria. All strains produced a steady power output with the maximum power output for *A. ferrooxidans* being 160 mW/m², the three halotolerant isolates averaging 281mW/m², compared to 19.6mW/m² in control MFCs containing no bacteria and the yeast MFC producing maximum power of 79mW/m². This study highlights the potential of halotolerant iron-oxidising bacteria for bioelectrical generation of power. In coastal areas of acid mine drainage pollution and areas where mine tailings are contaminated with salt deposits these halotolerant bacteria could provide a potential method for both power generation and bioremediation.

P058

Environmental survival of *Shigella* species

Abigail Clements¹, Jayne Watson¹, Claire Jenkins²

¹Imperial College, London, United Kingdom. ²Public Health England, London, United Kingdom

Abstract

Shigella is a genus of gram-negative enteric pathogens comprised of four species that can all cause severe diarrhoea in humans. It is estimated that *Shigella* spp. cause 165 million infections and 120,000 deaths annually, accounting for 10% of deaths due to diarrheal disease worldwide. *S. flexneri* and *S. sonnei* are responsible for most of the infections, but the ratio of species dominance is highly dependent on the socio-economic conditions of the area. In fact, the frequency of *S. sonnei* isolation directly correlates with per capita GDP, although the underlying reason(s) for this association is not currently understood. One proposed explanation for the continued expansion of *S. sonnei* in areas with improved water quality is that it can survive in amoebae, which would provide a protective environment for the bacteria. We investigated this hypothesis but found both *S. flexneri* and *S. sonnei* were efficiently killed by amoebae. However long term culture of *Shigella* spp. in low nutrient conditions revealed a survival advantage for *S. sonnei* when compared to *S. flexneri*. The molecular basis of this observation is currently being investigated and could explain the different distribution of *Shigella* infections.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P059

Studies on the Source and Survival of *Listeria monocytogenes* in the Fresh Leafy Produce Supply Chain

Alva Smith, Clare Taylor, Ian Singleton

Edinburgh Napier University, Edinburgh, United Kingdom

Abstract

Listeria monocytogenes (*Lmo*) is an opportunistic bacterial pathogen and the causative agent of listeriosis, a disease which predominantly affects immunocompromised individuals and has a high mortality rate. The main cause of infection is the consumption of contaminated foodstuffs, specifically, foods which require no further preparation before consumption such as deli meats, cheese products and fresh fruit and vegetables (fresh produce). Fresh produce has been the cause of notable *Lmo* outbreaks in the past, therefore contamination of produce by this bacterium is of substantial concern to companies that operate in the horticultural industry. Research is urgently needed to understand how *Lmo* survives in the fresh leafy produce supply chain and contaminates produce.

Soil provides an ecological niche for this pathogen and we have investigated the effect of soil type, strain type and soil sterilisation on *Lmo* survival in soil. Additionally, phenotypic and molecular characterisation has been used to assess virulence potential and relatedness of *Lmo* isolates from the fresh produce supply chain. Results indicate that a wide variety of *Lmo* strains are recovered from the supply chain and that these strains are potentially pathogenic to humans. Interestingly, these strains survive poorly in typical horticultural soils used to grow leafy produce and populations decline to an undetectable level after 3-4 weeks due to the presence of indigenous soil microflora. Companies that operate in the supply chain can use this information to influence policy and take precautionary measures to reduce the risk to public health from *Lmo* infection and avoid costly product recall.

P060

INVESTIGATING THE MOLECULAR BASIS OF BIOREMEDIATION BY METHANE OXIDISING BACTERIA

Hania Aween

Sheffield Hallam University, sheffield, United Kingdom

Abstract

Background- Methanotrophic (methane-oxidising) bacteria are widespread in the environment and have a number of promising activities for bioremediation of organic and inorganic pollutants. There is a need for genetic systems to probe gene function in methanotrophs, although their development has proven challenging. This project aims to prepare a transposon library of the methanotroph *Methylosinus trichosporium* OB3b, with the initial intention of identifying genes involved in bioremediation of selenite.

Methods- A plasmid carrying the mariner transposon was transferred via conjugation from *Escherichia coli* SM10 λ pir to *Ms. trichosporium* OB3b. To locate the transposon within the genome of *Ms. trichosporium* OB3b, the genomic DNA from 10 of the exconjugants was sequenced.

Results- The conjugation was successful in transferring the plasmid from *E.coli* to *Ms. trichosporium* OB3b, as evidenced by resistance to kanamycin (conferred by the transposon) and the presence of the transposon sequence within the genome. Eight strains in which the transposon inserted in to the genome showed a total of only three different positions of the insertions. Based on the hypothesis that this lack of variation within the library is due to problems with the conjugation or growth of the exconjugants, the conjugation process is currently being optimised.

Conclusion- The insertion of the transposon into the methanotroph genome was successful, though sequencing results indicate that further development of the system is needed to produce a sufficiently large library of mutants.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P061

Investigating the ability of *Listeria monocytogenes* to form biofilm on surfaces relevant to the mushroom production environment

Lionel Kenneth Dygico^{1,2}, Helen Grogan¹, Cormac Gahan², Catherine Burgess¹

¹Teagasc, Dublin, Ireland. ²University College Cork, Cork, Ireland

Abstract

Listeria monocytogenes poses a threat to all fresh fruit and vegetables due to its ubiquitous presence in the natural environment. In this study, the biofilm formation potential of *L. monocytogenes* strains isolated from the mushroom production environment was investigated on surfaces and at temperatures relevant to mushroom production. Preliminary assessment of biofilm formation of 44 mushroom industry isolates was carried out using a crystal violet assay on polystyrene microtitre plates. Biofilm formation on different surfaces was then assessed using the CDC biofilm reactor. Stainless steel, aluminium, rubber, polypropylene and polycarbonate were all found to be able to support biofilm levels ranging \log_{10} 4-4.9 CFU/cm², for six different *L. monocytogenes* strains, with no significant difference ($p > 0.05$) between them. On the other hand, concrete had \log_{10} 7.7 CFU/cm² of biofilm from the same strains. These results indicate that *L. monocytogenes* can readily form biofilms on industry relevant surfaces, and additionally identifies areas of specific concern, where rigorous cleaning and disinfection is required.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P062

The experimental investigation of the relationship between bacterial adhesion upon a range of surfaces under different shear stresses within a microfluidic device.

Alexander Lai Man Chun¹, Ali Mosayyebi², Dario Carugo², Benjamin Griggs¹, Maria Salta¹

¹University of Portsmouth, Portsmouth, United Kingdom. ²University of Southampton, Southampton, United Kingdom

Abstract

Biofilms are intricate communities of microorganisms encapsulated within a self-produced matrix of extra-polymeric substances (EPS), creating complex three-dimensional structures allowing for liquid and nutrient flow through. These aggregations offer constituent microorganisms enhanced protection not only from environmental pressures like flow but are associated with higher resistance to antimicrobial compounds, providing a persistent cause of concern in relation to marine biofouling and medicinal infections. Bacterial attachment is affected by surface properties, such as hydrophobicity, as shown in previous research. Using an innovative microfluidic flow cell, we investigated the relationships between both shear stress and surface properties upon the biofilm formation of two biofilm forming species, *Cobetia marina* and *Pseudomonas aeruginosa*. In this study we investigated biofilm development on surfaces with varying degree of hydrophobicity, namely low-density polyethylene membranes (LDPE), permanox and glass slides. Biofilm development was measured using nucleic acid staining and end-point confocal laser scanning microscopy (CLSM). We have demonstrated that flow conditions affect biomass, maximum thickness and the surface area of biofilms, with higher shear stresses (5.6 Pa) resulting in thinner, more compact biofilms than lower shear stresses (0.2 Pa). Control experiments performed under static conditions have shown that an absence of flow creates much thinner, smaller biofilms suggesting a growth response to the surrounding flow. With respect to surface properties, initial biofilm formation was also impacted as hydrophilic surfaces resulted in thinner biofilms when compared to hydrophobic ones. Alongside this, we observed the formation of biofilm streamers under laminar flow conditions within straight, micro-channels for the first time.

P064

Metagenomics: revealing the antimicrobial potential of the cryosphere

Melanie Hay^{1,2}, Sara Rassner Edwards¹, Andrew Mitchell¹, Arwyn Edwards²

¹Department of Geography and Earth Sciences, Aberystwyth University, Aberystwyth, United Kingdom.

²Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Growing antimicrobial resistance poses a threat to human health and novel antibiotics are urgently needed. Antimicrobial secondary metabolites are often synthesised by bacteria as a defence against competitive species. Much of the biosynthetic potential in microorganisms may be found in the 99% of uncultivable bacteria or in silent, unexpressed biosynthetic gene clusters (BGCs). Sequence-based metagenomics can effectively identify BGCs that otherwise elude detection. Although marine and soil environments have been extensively investigated for antimicrobial compounds, the cryosphere remains under-explored.

This study screened metagenomes from seven glaciers around Ny-Ålesund, Svalbard for potential antimicrobial compounds. The metagenomes ranged in size from 29,711 reads to 4,443,964 reads. After assembly, contigs > 1000 bp in length were submitted to antiSMASH, PRISM and BAGEL3. AntiSMASH identified numerous putative BGCs including saccharides, terpenes, fatty acids, bacteriocins, bacteriocin-lantipeptides, nonribosomal peptide-synthetases (nrps), type III polyketide synthases and cyanobactins. Approximately 25 % of the BGCs did not belong to a known class. Four of the compounds had high similarity to gene clusters in the Minimum Information about a Biosynthetic Gene cluster (MIBiG) database. PRISM indicated the presence of ribosomally synthesised and post-translationally modified peptides (RiPPs), while BAGEL3 identified several lanthipeptides.

The cryosphere contains bacteria able to synthesise diverse secondary metabolites. Screening metagenomes to investigate BGCs from uncultivable bacteria and silent BGCs is a key step in developing strategies to heterologously express compounds with high antimicrobial potential.

P065

Investigations into *Apium graveolens* as a novel antimicrobial agent

Sophie A Hutchinson¹, Graham P Stafford², Thomas J Smith²

¹Sheffield Hallam Univeristy, Sheffield, United Kingdom. ²University of Sheffield, Sheffield, United Kingdom

Abstract

Background

Celery (*Apium graveolens*) seed has been widely used within traditional medicines. A number of antimicrobial compounds have been discovered in celery seed extract (CSE), including one termed CAH, which has potent antimicrobial activity against *Helicobacter pylori* but not *Escherichia coli* or *Campylobacter* spp. CSE also contains potent antifungal activity, which is of particular interest in view of the shortage of effective anti-fungal drugs.

Methods

CSE was extracted using petroleum ether and water and fractionated using column chromatography (silica gel 60) using hexane-ethyl acetate (95:5, 7:3), ethyl acetate and methanol as the mobile phases. Fractions were collected and analysed using TLC before being pooled into subfractions according to Rf values, before the solvent was removed by evaporation.

Disc diffusion and liquid MIC and MBC assays were conducted on CSE and each distinct subfraction using *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Staphylococcus aureus*, *Helicobacter pylori*, *Candida albicans*, *Fusarium solani* and *Trichophyton* spp.

Results

Thirteen distinct subfractions, as judged by their TLC profiles, were obtained from the CSE. Various subfractions showed antimicrobial activity against *S. aureus*, *H. pylori*, *A. niger*, *C. albicans*, *F. solani* and *Trichophyton* spp. (i.e. important Gram-positive and Gram-negative pathogens, together with a variety of pathogenic fungi). These results indicate four antimicrobial molecules in CSE. Among these, one is active only against *S. aureus* and three have both antibacterial and antifungal activity. These molecules are currently undergoing structural characterisation.

Conclusions

Four antimicrobial agents have been detected within CSE, including three molecules with both antibacterial and antifungal activity.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P066

INDUCING BIOFILM GROWTH WITH SYNTHETIC POLYMERS

Jose Luis Brioso, Francisco Fernandez-Trillo, Tim W Overton

University of Birmingham, Birmingham, United Kingdom

Abstract

About 99% of the world's population of bacteria are found in the form of a biofilm at various stages of growth. Biofilm could be described as a consortium of bacteria attached to a surface and embedded in a matrix of extracellular polymeric substance. In this stage, the bacterial community behaves more like a supra-cellular organism rather than a unicellular one, showing off traits that individuals lack.

Our aim is to synthesize polymers that induce biofilm formation in *Escherichia coli*. We chose to synthesize Poly(acryloyl-hydrazide), every monomer unit is easily modifiable with functional groups that carry ketones or aldehydes, these groups will be positively charged, or able to interact with FimH type1 receptors.

We were able to successfully develop the backbone and eight different modifications of it. Polymers were tested against *E. coli* PHL 644 and its effect measured via: spectrophotometry, flow cytometry, optic microscopy, and metabolic assays.

Three polymers were able to induce aggregation on *E. coli* 644: protonated Poly(acryloyl hydrazide), Poly(acryloyl-hydrazide-glucofuranose), Poly(acryloyl-hydrazide-mannopyranose). Clusters appear between the first 3 hours and aggregates can be seen with the naked eye, this aggregation is stable after vortexing, it can be measured as drop in OD600. OM pictures of bacterial samples inoculated with these polymers show aggregates of bacteria embedded into a mucus like substance.

We suspect that the protonated backbone induces clustering due to charge interaction with the negatively charged bacteria, and that the sugar functionalized polymers are recognized by FimH adhesins inducing biofilm formation.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P067

Microscopic and structural characterization of selenium nanoparticles produced by *Stenotrophomonas bentonitica*.

Miguel Angel Ruiz-Fresneda, Jaime Gomez-Bolivar, Victoria Fernandez-Cantos, Josemaria Delgado-Martin, Mohamed L. Merroun

University of Granada, Granada, Spain

Abstract

Deep geological repositories (DGR) have been proposed as one of the best option for the disposal of radioactive wastes in the near future. These repositories consist of the encapsulation of radioactive wastes in metal containers surrounded by natural and artificial barriers like bentonite clay formations. Specifically Spanish bentonites have been selected for their possible use as an engineered barrier in DGR because of their well-characterized mineralogical and geochemical properties. The recently described new species *Stenotrophomonas bentonitica*, isolated from Spanish bentonites, interact efficiently with elements relevant for DGR like selenium (Se). This strain is able to reduce selenite [Se(IV)] to less toxic forms [Se(0)] producing nanoparticles under aerobic conditions. Scanning Transmission Electron Microscopy (STEM) equipped with High-Angle Annular Dark Field (HAADF) detector combined with Fast Fourier Transform (FTT) demonstrated the production of Se NPs with 3 different morphologies (spheres, hexagons and nanowires) and distinct crystallographic properties. In addition, EDX (Energy Dispersive X-ray) along with elemental mapping confirmed the presence of selenium and sulphur in the nanoparticles. To the best of our knowledge this the first report describing the formation of biogenic Se NPs with 3 different morphologies and crystallographic properties. These results demonstrate the potential impact of microorganisms isolated from bentonites on the long-term safety of the DGR system.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P068

Arctic antimicrobials: Identifying novel antimicrobials from microbes in extreme environments.

Aliyah Debbonaire, Luis Mur, Arwyn Edwards

IBERS, Aberystwyth, United Kingdom

Abstract

Antimicrobial resistance (AMR) and climate change are two of the biggest threats to humanity. The global number of deaths from AMR is estimated to rise to 10 million by 2050, overtaking the current death rate from cancer. Therefore, bioprospecting in rapidly diminishing glacier environments is of critical importance to utilise this resource before perhaps hitherto undiscovered microorganisms with novel antimicrobial properties are lost forever.

Samples of cryoconite (Aeolian debris on glacier surfaces that has been colonised by microbes) have been collected over several years (2013-2017), from Sweden, the Alps and Svalbard. These have been inoculated onto several high and low nutrient media, and sub cultured until pure bacterial isolates have been obtained. Using an optimised phenol:chloroform:dH₂O extraction method, we have reliably extracted high molecular weight DNA from the isolates. Using 16S amplicon Sanger sequencing, we have characterised over 100 isolates.

These are currently being tested for both AMR and antimicrobial activity using the Hidex Sense microplate reader. The most promising isolates will then undergo whole genome sequencing. Assembled contigs from these will be run through a variety of tools to identify either biosynthetic gene clusters, AMR genes or antimicrobial peptides. Meanwhile, the compounds produced by the isolates will be fractionated and analysed until their structure has been elucidated, enabling mode of action studies to identify drug candidates.

Isolates are already showing encouraging activity: one strain has showed considerable inhibition of *Escherichia coli*, an important Gram-negative pathogen. This is particularly exciting given the lack of new drug candidates against Gram-negative organisms.

P069

DEVELOPMENT OF A CYANOBACTERIAL PLATFORM FOR THE PRODUCTION OF METHYL-BRANCHED LIPIDS

Julia Roulet¹, Arnaud Taton², Michael Burkart³, Ana Arabolaza¹, Hugo Gramajo¹

¹Institute of Molecular and Cell Biology of Rosario. IBR-CONICET-UNR., Rosario, Argentina. ²Division of Biological Sciences, University of California, San Diego, USA. ³Department of Chemistry and Biochemistry University of California, San Diego, USA

Abstract

Cyanobacteria are the only prokaryotes that perform plant-like oxygenic photosynthesis and convert sunlight-derived energy into

biomass. They possess several advantages as hosts for biotechnological applications, including simple growth requirements, ease of genetic manipulation and robust high-scale production. At present, many bioindustrial processes rely on the fermentation of heterotrophic bacteria to produce various fine chemicals. Nevertheless, the economic viability of these production schemes is limited by the cost of carbon substrates used in the fermentation processes. Cyanobacteria, endowed with photosynthesis system to fix carbon dioxide into reduced form, are ideal biosynthetic machinery for sustainable production of various chemicals and biofuels. In this context, we aim to develop a cyanobacterial platform for the production of polyketides of industrial interest.

For this purpose, and as a proof of concept, we designed and constructed recombinant cyanobacteria strains capable of producing methyl-branched chain lipids (MBL), which have interesting physicochemical properties for their application in the bio-lubricants, pharmaceuticals and/or cosmetics industries.

The heterologous pathways for the biosynthesis of the PKS substrates and the final products was performed using a combinatorial modular design. After the recombinant-cyanobacteria strains were obtained, the functionality of the system was assayed by bioconversion experiments, demonstrating that the cyanobacterial strains successfully produced MBL.

In summary, we have developed a cyanobacterial high-value-molecules production platform with a modular-functional design that allows a versatile, interchangeable and combinatorial construction of the desired cyanobacterial strain.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P070

Ancient Welsh Herbal Remedies from the Physicians of Myddfai: A Potential Source for New Antibiotics?

Rowena Jenkins¹, [Ivan Hutchinson](#)²

¹Swansea University, Swansea, United Kingdom. ²Cardiff Metropolitan Univeristy, Cardiff, United Kingdom

Abstract

In an attempt to find new antibiotics in a world with rising antimicrobial resistance, this study looked at ancient Welsh wound healing plant remedies as a source of potential new antibiotic compounds.

Two methods of water-based extraction were used, blended and infused. These methods were used for twelve different plants: eight plants that are native to the United Kingdom and four Mediterranean herbs that were purchased from a local supermarket. The resulting compounds were tested in zone of inhibition plate assays (ZOI), synergy plate assays and minimum inhibitory concentration assays (MIC).

The native plants that were assayed did not reveal any antimicrobial activity, suggesting that those specific plants in the ancient remedies were merely a filler substance of no medicinal value. The traditionally Mediterranean plants produced antibacterial activity and showed consistent activity against five bacteria: *S.aureus* 6571, *MRSA*, *S.pseudintermidis* GC15467, *S.epidermidis* and *S.pseudintermidis* GC011b. This activity was expressed in the ZOI plate assays, synergy plate assays and MIC assays. Rosemary was the most successful as it inhibited all bacteria assayed. Mint inhibited three of the five bacteria in blended extract form and inhibited *S.aureus* by the largest margin of all plants assayed.

The research shows that the method is appropriate and gives a good indication of antimicrobial activity for water-based extraction methods, however, of the twenty six plants listed in the ancient remedy book, only eight were assayed, suggesting that there are many more plants to explore in regards to new antibiotics.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P071

Anaerobic fungi as a source of novel enzymes and antimicrobials

Rebeca Evans

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of many herbivorous animals, and play an essential role in degrading food with a range of powerful hydrolytic enzymes. Whilst these enzymes are important to rumen efficiency, they can also be useful to biotechnological and biomedical industries. The rumen microbiome presents an underexplored source for novel microbial enzymes and metabolites, such as lipases and antimicrobial peptides, which are promising drug candidates to target ever-increasing antimicrobial resistance.

Following DNA extraction and Sanger sequencing on previously collected anaerobic fungi samples, the next step is whole genome sequencing to undertake comparative genomic studies with existing sequences (e.g. *Orpinomyces* and *Piromyces*). By using metagenomics and next-generation sequencing such as Ion Torrent and the MinION, it allows screening of enormous samples to yield potentially useful gene clusters. The goal is to discover and annotate any hydrolytic enzymes and antimicrobial genes, including within existing sequence data and functional genomic libraries, which will also allow evolutionary origin and genetic divergence analysis. These can then be transferred into suitable vectors in order to produce them *in vitro*, and study any antimicrobial or enzymatic expression and activity with high throughput screening methods and computational approaches.

Microbial composition and genetic variation in the rumen have been well studied in many animals, however it can be argued that functional variation such as enzymatic and antimicrobial activity is still unclear. Findings may indicate that the rumen microbiome, with reference to anaerobic fungi can provide alternative enzymes or antimicrobials for future therapeutic applications.

Forum: Environmental and Applied Microbiology
Zone A
Presentations: Thursday Evening and Friday Lunchtime

P072

A Potential Cholera Epidemic Source: Some Fresh Vegetables in Gombe

Musa Dahiru, Hafiz Sulaiman

Federal University Kashere, P.M.B. 0182, Gombe State, Nigeria, Gombe, Nigeria

Abstract

Most of the reported outbreaks of gastrointestinal disease are linked to the consumption of fresh produce contaminated by bacteria. It is in view of these, the research wishes to determine the presence *Vibrio cholerae*, in fresh vegetables sold and consumed in Gombe metropolis, Nigeria, and for that purpose isolate, characterize and identify various biotypes. A total of 184 vegetable samples consisting of 3 vegetable types; Cabbage (*Bressica oleracea* L.), Lettuce (*Lactuca sativa* L.) and Tomato (*Solanum lycopersicumm* Mill.) were collected from Shongo, Gombe Main and Gombe Old markets, during the month of August. Samples were grown on Thiosulfate Citrate Bile-salt Sucrose (TCBS) Agar and subjected to standard biochemical tests. Among sampled analyzed (184), 59.24 % of the samples showed growth on TCBS agar, out of which 73.39% had yellow colonial growth. 16.25% of the yellow colonies were confirmed *Vibrio cholerae*. The *Vibrio cholerae* identified were further screened, results showed 23.08% each of O139 and O1 Eltor biotypes, while other *Vibrios* biotypes were 53.85%. Lettuce sample had higher percentage of yellow growth on TCBS of 89.13%, while tomatoes sample had highest percentage of confirmed of (22.73%) *V. cholerae*. *Vibrio* isolates from cabbage were 50% each of O139 and O1 Eltor biotypes. The different biotypes observed among samples, indicating close association between the vegetables and these pathogenic specie and its biotypes, thereby posses the risk of cholera not only at sporadic cases but of epidemics capacity, in Gombe and to the entire people of North Eastern Nigeria.

Forum: Environmental and Applied Microbiology

Zone A

Presentations: Thursday Evening and Friday Lunchtime

P073

Canthaxanthin, Astaxanthin And Adonixanthin Production From A *Dactylococcus* Microalga In A New Flat Plate Airlift Photobioreactor.

Borhane Samir GRAMA¹, Samira CHADER², Clayton Jeffryes³

¹FSNV, Université d'Oum El Bouaghi, BP358, Oum El Bouaghi –04000, Algeria. ²Algerian Center of Renewable Energy (Centre de Développement des Energies Renouvelables), Unité de D'développement des Equipements Solaires (UDES), Bou-Ismaïl, 42415 W, Tipaza, Algeria. ³Dan F. Smith Department of Chemical Engineering, Lamar University, PO Box 10053, Beaumont,, Texas, USA

Abstract

In the current study we developed a two-stage, sustainable, semi-continuous bioprocess at the PBR scale for the production of carotenoids. Carotenogenesis began when a sterile solution which contained NaCl and no nitrate was added directly to the photobioreactor, to arrive at a final concentration of 7.5 g L⁻¹. Illumination was delivered from both surfaces of the FPAPBR and the initial mean light intensity within the reactor was 100 μE m⁻² s⁻¹ at a cell density of (4.4 ± 0.2)·10⁶ cells mL⁻¹. After 9 days of carotenogenesis cultivation the recovery process was initiated directly in the FPAPBR when 80 % of the cell culture was removed and NaCl free, nutrient replete medium was added. The recovery phase culture was conducted for 4 days. The current work showed that the strain *D. dissociatus* MT1 isolated from the Sahara Desert of Algeria was an efficient producer of canthaxanthin, astaxanthin and adonixanthin, the first report of an alga from the Sahara Desert to produce these three carotenoids. We observed that the carotenogenesis and growth phases were reversible and a bioprocess was developed to alternate between the two phases for the sustainable, semicontinuous production of high-valued carotenoids at the photobioreactor scale in a reliable and reproducible manner. The demonstrated bioprocess should be easily amenable to cultivations at the larger scale. Thus, our results represent a step towards developing algal strains suitable for secondary carotenoid production at the commercial level.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P074

Analysis of microbiological tests results for pork and pork products produced in the Central Region of the Russian Federation in 2012-2016.

Natalia Shadrova, Galina Skitovich, Olga Pruntova

FGBI "Federal Centre for Animal Health", Vladimir, Russian Federation

Abstract

The FGBI "ARRIAH" annually performs tests of raw food materials and ready-to-eat food products in the framework of National laboratory veterinary monitoring of banned and harmful substance residues in live animals, products of animal origin and feed in the Russian Federation territory. The study was aimed at analysis of the results of microbiological tests of pork and pork products produced in the Vladimir, Ivanovo and Kostroma Oblasts in 2012-2016. During this period 3,878 tests were carried out including 1,062 tests of pork (27.38%); 293 tests of offal (7.56%); 1,264 tests of pork fat (32.59%), 1,259 tests of pork preparations (32.46%). It was demonstrated that 5.57% of samples of raw meat and food products were non compliant with microbiological criteria laid down in hygienic standards. Therewith, the proportion of samples that were noncompliant with sanitary and hygienic requirements (total viable count and coliforms) and safety criteria (detection of *Listeria monocytogenes* and *Salmonella*) increased from 1.63% in 2012 r. up to 14.89% in 2016. Analysis of porcine products monitoring with microbiological tests (detection *Listeria monocytogenes* and *Salmonella* bacteria) for 5 years demonstrated an increase in their contamination with pathogens: *Salmonella* spp. detection rate increased from 2% in 2012 up to 5.7% in 2016; *L. monocytogenes* contamination detection rate increased from 0.66% in 2012 up to 14.77% in 2016.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P075

Deciphering the Evolutionary Significance of Maintaining Cryptic Genetic Systems by Bacteria.

Kartika Vashishtha, Subramony Mahadevan

Indian Institute of Science, Bangalore, India

Abstract

Cryptic genes are phenotypically silent DNA sequences that have the potential to code for a specific function upon activation by different genetic mechanisms. The *bgl* operon, which is involved in the utilization of aromatic β -glucosides salicin and arbutin that are plant secondary metabolites, has long served as a paradigm for cryptic genes. It has been observed earlier that wild type *Escherichia coli* shows antibiotic resistance when exposed to salicylate and related compounds by stimulating the expression of *marRAB* operon (multiple antibiotic resistance). Salicylate being structurally similar to salicyl alcohol and hydroquinone (aromatic part of salicin and arbutin respectively), suggests the possibility that bacteria having the functional form of the *bgl* operon and are actively metabolizing salicin, can exhibit a significant growth advantage under antibiotic stress conditions. Using *E. coli* strains carrying different *bgl* genotypes, we have observed that presence of both inducible, as well as the constitutive form of the *bgl* operon, imparts a growth advantage when exposed to Nalidixic Acid. Approximately 2-3 fold increase in Minimal Inhibitory Concentration was observed when salicin or arbutin was provided as a carbon source. Our observations strongly suggest a strong selective pressure for the retention of the *bgl* operon in the genome of bacteria in environments such as soil in which both aromatic β - glucosides as well as antibiotics are present.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P076

Investigating the differential virulence of *Salmonella enterica* serovars in livestock animals using quantitative proteomics

Imogen Johnston-Menzies, Prerna Vohra, Jo Stevens

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

Abstract

Salmonella enterica is a bacterial pathogen of global importance for both human and veterinary medicine. Farmed animals, as a source of food and environmental contamination, represent a critical reservoir for human non-typhoidal salmonellosis. For some serovars of *S. enterica*, the outcome of infection in different livestock species is dependent on host-specificity. For example, *S. Typhimurium* is a broad-spectrum pathogen and causes acute self-limiting enteritis in a range of hosts including cattle, chickens, and pigs, whereas *S. Choleraesuis* is host-adapted and causes systemic typhoid-like disease in pigs but enteritis in cattle. It has been previously hypothesised that the type III secretion system (T3SS), a critical virulence factor in *Salmonella*, is a major factor in host restriction.

We aimed to investigate this using quantitative proteomics to characterise the secretomes of two strains of well-defined virulence in livestock animals – *S. Typhimurium* ST4/74 and *S. Choleraesuis* SCSA50. We found that not only did the repertoire of secreted effector proteins differ between the two strains, but the amount of protein secreted was also different. This was information that could not have been deduced from the published genome sequences. We have validated the differences in secretion of several proteins by immunoblotting and are investigating possible differences in T3SS transcriptional regulation between the two serovars, with the ultimate goal of understanding how protein secretion could influence host restriction and predict the zoonotic potential of other serovars.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P077

Developing phage therapy for the treatment of E.coli infections in companion animals

Lucy Rhys-Davies, Arnoud van Vliet

University of Surrey, Guildford, United Kingdom

Abstract

Background: Antibiotic resistance is a global issue threatening the future of medicine. The emergence of resistance to "antibiotics of last resort", such as colistin via the MCR-1 gene, highlights the urgent need for identification or development of alternative antimicrobial strategies. The possibility of using bacteriophages for treating multi-drug resistant infections in veterinary practice needs exploring. *Escherichia coli* is associated with a wide range of infections with increasing numbers of multi-drug resistant strains isolated in small animal practice.

Results: Ten strains of *E. coli* were isolated from dogs in a veterinary referral hospital, all of which were implicated in surgical wound infections or UTI's. *E. coli* strains were found to be distributed across 4 phylogroups, including groups B2 and D, which are the major phylogroups associated with extra-intestinal infections. Seven strains were multi-resistant, showing *in vitro* resistance to 3 or more classes of antibiotics by disk diffusion testing, and all 10 genomes contained genes associated with antibiotic resistance, such as penicillins, sulphonamides, aminoglycosides or tetracyclines. Canine faecal samples and soil were obtained to detect and extract lytic phages, using standard plaque assays against a control strain of *E. coli* and the 10 canine isolates. Two lytic phages have been isolated from the first faecal sample and are being tested against the panel of canine isolates.

Future work: This work is part of an ongoing project and is in the early stages of obtaining suitable phages for further testing and development.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P078

Yeast cell wall mannan-rich fraction reduces growth and abundance of antibiotic resistant bacteria

Helen Smith, Richard Murphy

Alltech, Meath, Ireland

Abstract

Antibiotic resistance has become a major global health concern affecting both animal and human health. There is now political pressure worldwide to restrict the use of antibiotics in animals to therapeutic use only, resulting in increased pressure on livestock producers to use alternative strategies to reduce their antibiotic load. The focus of this research is to assess the role of mannan rich fraction (MRF) from the yeast cell wall of *Saccharomyces cerevisiae* in mitigating antibiotic resistance. The effect of MRF on transformed *E. coli* harbouring ampicillin resistance was assessed by monitoring microbial growth, in the presence and absence of MRF. Growth of transformed *E. coli* in the presence of ampicillin ($45 \mu\text{g mL}^{-1}$) and MRF (0.5%, w/v) was reduced by 43% ($p \leq 0.05$). Thereby, MRF was noted to enhance the sensitivity of the resistant strains to the relevant antibiotic. Additionally, RT-qPCR analysis revealed a reduction of up to 98% in plasmid copy number in samples treated with MRF ($p < 0.05$). MRF was noted to reduce growth, prevalence and potential transmission of multi-drug resistant bacteria. Given these observations MRF may be an ideal alternative to antibiotic growth promoters in livestock. These results support the search for alternative strategies to promote animal health without contributing to the growing issue of antimicrobial resistance.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P079

Understanding the diversity of Staphylococcus aureus Pathogenicity islands (SAPIs) and their virulence genes using whole genome sequencing

Cosmika Goswami¹, Stephen Fox¹, Alistair Leonard¹, Matthew Holden², Thomas Evans¹

¹University of Glasgow, Glasgow, United Kingdom. ²University of St. Andrews, St. Andrews, United Kingdom

Abstract

Background: Staphylococcus aureus pathogenicity islands (SaPIs) are mobile genetic elements carrying genes encoding virulence factors and superantigen toxins responsible for the toxic shock syndrome and other superantigen-related diseases. To study the evolution and diversity of SAPIs within and between different S. aureus sequence types, we performed whole genome sequencing of isolates from bacteremic patients.

Material/methods: 540 Blood culture bacteria isolates were collected from all 15 health boards of Scotland for the year 2015 and were sequenced using the Illumina Hi-Seq platform. Virulence genes, SAPIs and phage integrases were detected using an in-house database. Multilocus sequence typing was performed to identify sequence types.

Results: Within our isolates, 7 dominant sequence types were detected: ST22, ST15, ST45, ST30, ST5, ST8 and ST1 along with 43 non-dominant (<20 isolates) sequence types. The toxic shock syndrome toxin (TSST) genes were prevalent in ST30 (86%, n=60) and were carried in SAPI2. Staphylococcal enterotoxin (se) genes were present in different SAPIs. Seb genes were in ST59 (99%, n=16) carried by SAPI3. 43% (n=26) of ST45 have sec and sel virulence genes in SAPI Tokyo12381 whereas ST22 (24%, n=10) contain those genes in SAPI68111. Seq and sek were found in ST1, carried in phage Sa3. Associations between the sequence types, virulence genes and SAPIs or phages was analysed through correlation network analysis. Using enhanced surveillance data, transmission events of patient-to-patient phages/SAPIs were also studied.

Conclusions: Whole genome sequencing provides insight on relations between and within sequence types and different SAPIs and phages and effects on clinical outcomes.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P080

Sequencing of 10,000 *Salmonella* genomes: a worldwide effort to understand the epidemiology, transmission and virulence of invasive non-typhoidal Salmonellosis

Blanca Perez-Sepulveda¹, Caisey Pulford¹, Kate Baker¹, Neil Hall², Nicholas Feasey³, Jay Hinton¹

¹University of Liverpool, Liverpool, United Kingdom. ²The Earlham Institute, Norwich, United Kingdom.

³Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Non-typhoidal *Salmonella* (NTS) are typically associated with gastroenteritis, often related to the industrialisation of food production. In the developing world, however, NTS also causes invasive disease (iNTS) that kills an estimated 680,000 people each year worldwide, the majority of which occur in sub-Saharan Africa (sSA) (Ao *et al.* 2015, *Emerg. Infect. Dis*). The high prevalence of immunosuppressive conditions in sSA predisposes individuals to iNTS infections and accounts for the high case-fatality rate of 20 %. Limited genomic characterisation of the *Salmonella* pathogens has identified new clades of *S.* Typhimurium and *S.* Enteritidis, which are characterised by genomic degradation, distinct prophage repertoires and novel multidrug resistant plasmids.

To understand how these clades are contributing to the burden and severity of iNTS disease, it is crucial to expand the molecular surveillance of Salmonellae from Africa and other parts of the world, including isolates associated with invasive disease, gastroenteritis and from both animals and the environment. The “**10,000 *Salmonella* genomes**” project established collaborations with researchers from many African and Latin-American countries, assembling a diverse collection of clinical and environmental *Salmonella* isolates with associated metadata. This enormous dataset is generating information relevant to the epidemiology, drug resistance and virulence factors of Salmonellae using a whole-genome sequencing approach. The resulting genome sequence data will contribute to our understanding of the evolution of iNTS-associated *Salmonella* and the zoonotic or environmental reservoir of human disease, and could impact upon public-health control strategies in developing countries.

Bacterial Zoonoses: Ecology, Epidemiology and Evolution

Zone A

Presentations: Wednesday and Thursday Evening

P081

Investigation of transcriptional response of related *Salmonella* Typhimurium strains to Ciprofloxacin treatment

Sushmita Sridhar^{1,2}, Josefin Bartholdson Scott², Sally Forrest², Derek Pickard¹, Stephen Baker^{3,2}, Gordon Dougan^{2,1}

¹Wellcome Trust Sanger Institute, Hinxton, United Kingdom. ²Department of Medicine, University of Cambridge, Cambridge, United Kingdom. ³OUCRU, Ho Chi Minh City, Vietnam

Abstract

Antibiotic resistance (AMR) is an enormous challenge to the clinical treatment of infectious diseases. To tackle this problem, we need to understand how antimicrobials work against different bacterial species and to be able to predict whether/when AMR is likely to evolve. We are using RNA-Seq analysis of *Salmonella* Typhimurium treated with varying levels of ciprofloxacin to study how gene transcription changes when bacteria are treated with concentrations of ciprofloxacin below and above the minimum inhibitory concentration. In addition, we are interested in tracking how gene expression changes over multiple generations of bacteria, and to that end, we are collecting RNA over 24 hours to better assess prolonged bacteria-drug interactions. This research will contribute to our understanding of how bacteria respond to various ciprofloxacin dosage levels and the impact that has on bacterial fitness, growth, and antimicrobial resistance.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P082

Identification of a Staphylococcal Complement Inhibitor with broad host specificity in equid *S. aureus* strains

Manouk Vrieling^{1,2}, Nienke de Jong²

¹The Roslin Institute, Edinburgh, United Kingdom. ²University Medical Center Utrecht, Utrecht, Netherlands

Abstract

Staphylococcus aureus is a versatile pathogen capable of causing a broad range of diseases in many different hosts. *S. aureus* can adapt to its host through modification of its genome, e.g. by acquisition and exchange of mobile genetic elements that encode host-specific virulence factors. Recently the prophage Φ Saeq1 was discovered in *S. aureus* strains from six different clonal lineages almost exclusively isolated from equids. Within this phage we discovered a novel variant of Staphylococcal Complement Inhibitor (SCIN), a secreted protein that interferes with activation of the human complement system, an important line of host defense. We here show that this equine variant of SCIN, eqSCIN, is a potent blocker of equine complement system activation and subsequent phagocytosis of bacteria by phagocytes. Mechanistic studies indicate that eqSCIN blocks equine complement activation by specific inhibition of the C3 convertase enzyme (C3bBb). Whereas SCIN-A from human *S. aureus* isolates exclusively inhibits human complement, eqSCIN represents the first animal-adapted SCIN variant that functions in a broader range of hosts (horses, humans and pigs). Binding analyses suggest that the human-specific activity of SCIN-A is related to amino acid differences on both sides of the SCIN-C3b interface. These data suggest that modification of this phage-encoded complement inhibitor plays a role in the host adaptation of *S. aureus* and are important to understand how this pathogen transfers between different hosts.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P083

The diversity and mobility of toxin antitoxin systems in a large dataset of *Klebsiella* spp.

Gal Horesh^{1,2}, Eva Heinz¹, Nicholas Robert Thomson^{1,3}

¹Wellcome Trust Sanger Institute, Cambridge, United Kingdom. ²University of Cambridge, Cambridge, United Kingdom. ³London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Toxin antitoxin (TA) systems are bicistronic operons which encode for a toxin that inhibits a cellular process such as translation or replication, and its cognate antitoxin. TA systems were first described as a mechanism of plasmid maintenance via post-segregational killing. Subsequently, they have been found to be ubiquitous in bacterial chromosomes, suggestive of a larger role than originally thought. For example, they have been implicated to play a role in stress response, persistence and protection against phages, however, their precise function is still not well understood. We developed POSH, Prokaryotic Operon Search using HMMs, a command line tool for the annotation of operons in large datasets of bacterial genomes. We used POSH to describe for the first time the diversity of TA systems in a large dataset of 259 *Klebsiella* spp. genomes. *Klebsiella* spp. is a commensal pathogen which has become a major threat to public health in recent years due to the emergence of multidrug resistant strains. Our analysis identifies groups of TA systems which present different patterns of inheritance within the *Klebsiella* spp. phylogeny. By examining the operon structure of each system, we gain insight on distinct classes of toxins which present different levels of antitoxin diversity and ability to be horizontally transmitted. The importance of this analysis is not limited to TA systems, and could be expanded to understand the diversity and mobility of other important operons.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P084

Investigation of anti-bacterial strategies deployed by a clinical isolate of *Serratia marcescens*

Connor Bowen, Sarah Coulthurst

University of Dundee, Dundee, United Kingdom

Abstract

Serratia marcescens can be isolated from a large number of diverse environments, including the soil, water courses and clinical infections. To allow itself to flourish in these environments, strains of *S. marcescens* have developed a number of strategies to effectively compete with other organisms. Two primary examples are the deployment of a Type VI secretion system (T6SS) and the production of diffusible antimicrobial compounds. In this work, the nature and the molecular basis of both of these strategies are being investigated in a multi-drug resistant clinical isolate of *S. marcescens*. Type VI secretion involves the deployment of a proteinaceous nanoweapon that can deliver toxic effector proteins directly into target cells. Here we show that a clinical isolate of *S. marcescens* can deploy a T6SS to actively compete with other bacterial species and block their proliferation and present initial findings regarding the regulation of this system. Additionally, a second antibacterial strategy has been identified in the clinical *S. marcescens* isolate, namely the production of diffusible antimicrobial molecule(s). The antimicrobial molecule(s) block growth of Gram-negative bacteria synergistically with the T6SS. Complementary genetic approaches have been adopted to identify the genes required for the production of the molecule(s). Understanding the apparent synergistic deployment of the contact-dependent T6SS with contact-independent antimicrobial activity, allows a more complete picture of the competitive strategies adopted by this opportunistic pathogen to be formed.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P085

THE IMPACT OF LIVE ATTENUATED INFLUENZA VACCINE (LAIV) ON PNEUMOCOCCAL CARRIAGE

Jessica Jones¹, Denise Morris¹, Rebecca Gladstone², Johanna M. Jefferies¹, David W. Cleary^{1,3,4}, Stephen D. Bentley², Saul N. Faust^{1,4,5}, Stuart C. Clarke^{1,3,4,5,6}

¹Faculty of Medicine, University of Southampton, Southampton, United Kingdom. ²Infection Genomics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom. ³Institute for Life Sciences, University of Southampton, Southampton, United Kingdom. ⁴NIHR, Biomedical Research Centre, University Hospital Southampton Foundation NHS Trust, Southampton, United Kingdom. ⁵NIHR Southampton Clinical Research Facility, University Hospital Southampton Foundation NHS Trust, Southampton, United Kingdom. ⁶Global Health Research Institute, University of Southampton, Southampton, United Kingdom

Abstract

Background: *Streptococcus pneumoniae* is an opportunistic pathogen that inhabits the human nasopharynx. Within this ecological niche, pneumococci may reside alone or co-colonise alongside other bacterial, viral and fungal species. Administration of the Live Attenuated Influenza Vaccine (LAIV) introduces a live virus into this niche and has the potential to affect pneumococcal carriage. The burden of pneumococcal carriage is greatest in children and it is this age group who are the main recipients of LAIV.

Method: The Southampton paediatric pneumococcal carriage study started in 2006 during the introduction of the pneumococcal conjugate vaccine in the UK. Swabs are taken each winter from children aged 4 years and under to monitor circulating pneumococcal serotypes in the community. During winters 2014/15, 2015/16 and 2016/17 we collected vaccine data from 1,312 children who participated in the carriage study. Pneumococcal carriage rates of LAIV recipients were compared to LAIV non-recipients.

Results: The overall pneumococcal carriage rate for children aged 4 years and under was 36.1%. Pneumococcal carriage was higher in LAIV recipients than in non-recipients for each of the three winters. LAIV recipients had a significantly higher overall pneumococcal carriage rate of 41.5% while carriage in non-recipients was 33.4% ($p = 0.02$).

Conclusion: Our study indicates a higher pneumococcal carriage in LAIV recipients. Further work is required to further understand the dynamics of LAIV immunisation and pneumococcal carriage.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P086

Pneumococcus is smart enough to sense temperature shift

Ozcan Gazioglu, Peter Andrew, Hasan Yesilkaya

Infection, Immunity and Inflammation, Leicester, United Kingdom

Abstract

Streptococcus pneumoniae is a commensal of the human nasopharynx that becomes pathogenic after moving to other niches. During progression from colonization to disease, *S. pneumoniae* must contend with temperature changes in different parts of the body. The aim of this work is to identify how temperature effects pneumococcal phenotype associated with virulence and survival, and determine the genetic basis of thermal adaptation.

To understand pneumococcal strategies in adapting to different temperatures, transcriptional gene expression was determined in mid- and late-exponential growth phase at 34°C and 40°C relative to 37°C. SOEing PCR was used for gene mutation, and the mutant was analysed by growth studies, biochemical assays, biofilm formation, pH adaptation, and antibiotic tolerance assays.

At different temperatures, the pneumococcus displayed differences in growth, and production of glycosidases and haemolytic activity, which are important for colonization and invasive disease. A large array of pneumococcal genes of diverse gene classes were differentially expressed at 34°C (491 genes) and 40°C (189 genes) relative to 37°C. One of the differentially expressed genes was annotated as glutamate dehydrogenase (gdh). The gdh mutant was less able to grow at 40°C, but the mutation had no significant impact on pneumococcal growth at 34- and 37°C.

Transcriptional profile of *S. pneumoniae* is highly sensitive to temperature fluctuations which have a significant impact on pneumococcal phenotype. The highest number of differentially expressed genes belongs to the hypothetical genes suggesting much remains to be investigated. It was also found that nitrogen metabolism is an important component for pneumococcal thermal adaptation.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P087

New inhibitors of ExsA: disabling virulence in *Pseudomonas aeruginosa*

Rory Triniman

Cambridge University, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa is globally recognised as a serious threat to population health, appearing second on the WHO's list of highest priority bacteria in need of new treatments, published in February 2017. It is an opportunistic and prevalent Gram-negative bacterium that accounts for ~10% of all hospital acquired infections within the UK and the main problem leading to its high mortality is rapid emergence of antibiotic resistance. The prevalent demographic to suffer both acute and chronic *P. aeruginosa* infections are cystic fibrosis patients where this pathogen is able to survive and impair lung function under difficult environmental conditions, such as low oxygen and iron.

Using a combination of structure and ligand based *in silico* methods for selection and characterisation using pPcrV-luciferase reporter system assays and biophysical methods, I have identified new potential inhibitors of ExsA, the master transcriptional factor for the major virulence system in *P. aeruginosa*, the Type III Secretion System (T3SS). This is a needle like structure protruding extracellularly and is capable of piercing host cell membranes and injecting cytotoxic effector proteins directly into the cytoplasm. Around 40 genes encode the components of the T3SS and all are under regulation by ExsA. Development of ExsA inhibitors have the potential to be used as adjunctive therapies to antibiotics, targeting virulence and in essence disarming *P. aeruginosa*. So far, only one chemical class (N-hydroxybenzimidazole) has emerged as an ExsA inhibitor prior to those presented here.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P088

Investigating epigenetic regulation by Type I Restriction Modification in a historic collection of *Staphylococcus aureus* isolates.

Rebecca Mekler¹, Simon Harris², Matthew Holden¹

¹University of St Andrews, St Andrews, United Kingdom. ²Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

Abstract

Staphylococcus aureus is a well-characterized opportunistic pathogen, which is the leading causative agent of health-care associated infections. A facet of the *S. aureus* genome, which remains scantily studied, is the methylome. In prokaryotes, Restriction-Modification (RM) systems are responsible for facilitating the methylation of nucleic acids through methyltransferase proteins. RM systems function as part of the cellular defence, but their role in the regulation of gene expression is not known.

The main RM system present in *S. aureus* is *Sau1*, a type I system. *Sau1* is usually seen in two sets, consisting of a grouped methyltransferase (*hsdM*) and specificity unit (*hsdS*), typically found in separate genomic islands (*vSaα* & *vSaβ*), working with a single restriction endonuclease (*hsdR*) located elsewhere in the core genome. Bioinformatics and comparative genomic analysis have been conducted on PacBio sequenced (Wellcome Trust Sanger Institute) genomes, from a historically and phylogenetically varied collection of *S. aureus* isolates from the National Collection of Type Cultures (NCTC) of Public Health England. This technology is used to investigate the diversity and variability of RM systems in 131 isolates, allowing the identification of the modification states, methylation motifs and overall methylome profile of these isolates.

Initial characterisation of the genetic relatedness of *Sau1* within the collection has shown intra-species variation in the distribution of the type I RM coding complexes, however further investigation of *hsdS* show lineage specific clustering. The relative diversity of *Sau1 hsdS*, and resulting methylomes of the isolates, may uncover the potential epigenetic effects of DNA methylation in *S. aureus*.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P089

Functional characterisation of (p)ppGpp synthetases: enzymes required for bacterial stress adaptation and survival

Sophie Irving, Rebecca Corrigan

University of Sheffield, Sheffield, United Kingdom

Abstract

In the host, *Staphylococcus aureus* encounters nutrient stresses which trigger the activation of the stringent response. This response is a conserved mechanism by which bacteria adapt to environmental changes such as amino acid starvation. It is mediated by two nucleotides, ppGpp and pppGpp, collectively known as (p)ppGpp. These small alarmones have many binding targets ultimately causing the cells to enter a slow growing state. In *S. aureus* (p)ppGpp is synthesised by three members of the RSH superfamily: RSH, RelP and RelQ. RSH is a bifunctional enzyme with both synthetase and hydrolase activities and a C-terminal regulatory region. RelP and RelQ are both small monofunctional synthetases with no regulatory domain. The aim of this project is to research how these synthetases are regulated transcriptionally, translationally and post-translationally. Altogether, this work aims to provide insights into the synthesis of (p)ppGpp by these enzymes and thus contribute to the mechanistic understanding of the pathogenesis of *S. aureus*.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P090

Host Generated Inhibitory Antibodies to *Pseudomonas aeruginosa* in Bronchiectasis Patients Aids Infection

Anna Schager¹, Emma Sheehan¹, Tim Wells², Ian Henderson¹

¹Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom. ²The University of Queensland Diamantina Institute, University of Queensland, Queensland, Australia

Abstract

Chronic *Pseudomonas aeruginosa* lung infections is a significant problem in patients suffering from bronchiectasis and is associated with declining lung function as well as increased morbidity and mortality. Recently, in approximately 20 % of bronchiectasis patients chronically infected with *P. aeruginosa*, it was found that an excess of IgG2 directed against the bacterial O-Ag was responsible for actively blocking *in vitro* serum killing. This is in contrast to the effect normally associated with antibodies and their mediated serum killing. Furthermore, this occurrence was linked with worse clinical diagnosis. Based on these findings, two critically ill patients were subjected to plasmapheresis where blocking antibody was removed from the serum. This intervention resulted in immediate health benefit for both patients, suggesting the occurrence of blocking antibodies are an important contributor to the severity of disease, yet still majorly overlooked. Occurrence of blocking antibodies to other Gram-negative bacteria than *P. aeruginosa* has also previously been described in the literature. However, little is known about the relative importance and significance the two parts in the host-pathogen interaction contributes to the occurrence. Here we are focusing on the host, investigating different characteristics of the inhibitory antibodies. Preliminary studies suggest that it is not only the amount of antibodies directed at the O-Ag that are prerequisite for inhibition, but that other aspects, such as their affinity and avidity, comes in to play. These results will aid the understanding of the mechanism behind inhibitory antibodies.

Breaking Bad: Factors Affecting the Commensal to Pathogen Switch

Zone A

Presentations: Tuesday and Wednesday Evening

P091

***Pseudomonas aeruginosa* persistence in the bronchiectasis lung – Inhibitory antibodies and serum resistance**

Emma Sheehan¹, Anna Schager¹, Timothy Wells², Adam Cunningham¹, Ian Henderson¹

¹University of Birmingham, Birmingham, United Kingdom. ²University of Queensland, Queensland, Australia

Abstract

Pseudomonas aeruginosa is a Gram negative, opportunistic human pathogen, which is beginning to pose a major challenge in healthcare settings. *P. aeruginosa* is frequently found to be a causative agent of pneumonia and urinary tract infections in hospitalised patients and commonly infects immunocompromised individuals, including those suffering from burn wounds, cystic fibrosis (CF) and bronchiectasis.

Chronic colonisation with this bacterium has been associated with more severe disease in patients suffering from non-CF bronchiectasis. It has recently been shown that ~20% of these patients overproduce IgG2 antibodies (inhibitory antibodies) specific to their colonising strain. Production of these antibodies has been shown to protect the bacteria from serum-mediated killing and correlates with an increase in morbidity and mortality.

We recently reported the use plasmapheresis as a potential novel treatment option for patients with bronchiectasis, chronic *P. aeruginosa* infection and inhibitory antibodies. Analysis of the serum resistance profiles and LPS specific antibody binding patterns of longitudinal isolates obtained from these patients identified differences between isolates which may provide further insight into the mechanism of inhibitory antibodies.

Fully understanding the mechanism by which inhibitory antibodies protect the bacteria from serum mediated killing has the potential to identify novel therapeutic targets and is fundamental for the improvement of current diagnostic and treatment options.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P092

The role of ActA in peptidoglycan remodelling in *Listeria monocytogenes*

Ohoud .S Alhumaidan, Nino Iakochvili, Peter W Andrew, Galina V. Mukamolova

Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom

Abstract

Listeria monocytogenes is a food-borne bacterial pathogen. It may cause abortion in pregnant women, septicaemia and meningitis in immunocompromised patients.

L. monocytogenes has many virulence factors that enable its replication in macrophages and the escape from the phagolysosome to the cytoplasm. One of these virulence factors is the actin-assembly inducing protein (ActA). Recently, the ActA protein has been shown to regulate peptidoglycan (PG) biosynthesis during *L. monocytogenes* replication in macrophages. However, the exact mechanism for this phenomenon is unknown. The central hypothesis of the present study is that ActA possesses PG hydrolysing activity and belongs to the family of lytic transglycosylases (LTGs) that are known to control PG biosynthesis and remodelling in other bacteria.

To address this hypothesis, three His-tagged forms of ActA have been expressed in *Escherichia coli*. These forms included a full-length ActA protein and two versions of the N-terminal domain (ActAN1 and ActAN2). ActAN1 and ActAN2 versions have been successfully purified and their identity has been confirmed. PG hydrolysing activity of these proteins has been assessed. Both versions showed significant peptidoglycan-hydrolysing activity as judged by zymography and digestion of FITC-labelled PG. Candidate catalytic residues are currently being identified by application of bioinformatics and their function will be verified by site directed mutagenesis. Future experiments such as analysis of muropeptides released from PG by ActA, complementation studies and pull-down assays will shed light on the function of this protein in PG remodelling.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P094

The cyanobacterial rhomboid protease is a regulator of the CCM

Iskander Ibrahim^{1,2}, Modesta Blunskyte¹, Dale Harrison¹, Elinor Thompson¹

¹University of Greenwich, Chatham Maritime, United Kingdom. ²Purdue University, Lafayette, USA

Abstract

Cyanobacteria are aquatic photosynthetic bacteria and useful models for study of the chloroplast and photosynthesis. We are studying a 'rhomboid' membrane-located proteases in *Synechocystis* sp. PCC 6803, which appears to function as a previously undiscovered regulator of the carbon concentrating mechanism (CCM) of this phototroph.

Rhomboids are almost ubiquitous across evolution, and are known to activate diverse cellular processes via proteolysis of their specific, membrane-sequestered substrates. Although this well-conserved family has solved crystal structures of bacterial enzymes such as *Escherichia coli* GlpG, ironically, most work has been carried out on eukaryotic representatives. Following our study of the *Arabidopsis thaliana* chloroplast RBL10 protease, we identified cyanobacterial orthologues with the aim of discovering if roles might be conserved between these and organellar rhomboids. Molecular biology and reverse-genetics studies were made on *slr1461*, a mutant in the single rhomboid protease of *Synechocystis*. When photosynthetic parameters were investigated, it could be seen that inactivation of *slr1461* did not affect nonphotochemical quenching, unlike the chloroplast *rb10* mutant, but Slr1461 was required for reduction of photosynthetic activity in mixotrophic conditions. This reduction allows cyanobacteria to avoid expending energy on the uptake of CO₂ when an organic carbon source can be utilised: as might be expected, therefore, Slr1461 transcription was linked with downregulation of genes encoding proteins facilitating high-affinity CO₂ import under high CO₂ and mixotrophic conditions. Quantitative RT-PCR of CCM network genes suggested that Slr1461 is located upstream of known regulators, including another membrane protease, the Slr0228 FtsH, and a central, controlling transcription factor NdhR.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P095

The influence of strain background and variation in protein structure on the roles of pneumolysin in infectious diseases due to *Streptococcus pneumoniae*

EMAD MOHAMED, Peter Andrew, Hasan Yesilkaya

University of Leicester, Leicester, United Kingdom

Abstract

Emad Essa Mohamed, Hasan Yesilkaya and Peter W. Andrew

Department of Infection, Immunity and Inflammation, University of Leicester

Background: *Streptococcus pneumoniae* is a major cause of pneumonia, bacteremia, meningitis and otitis media. Pneumolysin (PLY) is the major toxin of the bacterium, and it has many adverse effects on immune cell functions. PLY has a generally conserved sequence but recently 20 different PLY variants have been described among clinical pneumococcal isolates (Jefferies *et al.*, 2007, Jefferies *et al.*, 2010). These variants have differences in cytotoxic activity but the impact of these differences on virulence is hard to ascertain because they are being made in different strain backgrounds.

Method: To study the significance of polymorphisms on PLY function in a single genetic background, unmarked mutations were introduced into the *ply* in the type 2 D39 strain background using pORI280. The selected PLY variants were expressed and purified using affinity chromatography and gel filtration. Haemolytic and complement activation assays were done as before.

Results: The results showed that of 8 naturally occurring single nucleotide polymorphisms (SNP), three of them reduced the haemolytic activity of PLY significantly compared to the D39 PLY. and the other five alleles showed the same haemolytic activity of D39 PLY.

Conclusion: The strain background has an impact on PLY activity, comparing the results with reference strain D39 and the published data, there are some mutations were showed reduction in PLY haemolytic activity, while the others were showed approximately same lytic activity.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P096

Involvement of organic acids and amino acids in ameliorating Ni(II) toxicity induced cell cycle dysregulation in *Caulobacter crescentus*: a metabolomics analysis

ABHISHEK JAIN^{1,2}, CHEN WEI NING³

¹Interdisciplinary Graduate School, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, SINGAPORE, Singapore. ²Advanced Environmental Biotechnology Centre, Nanyang Environment & Water Research Institute, Nanyang Technological University, 1 CleanTech Loop, Singapore 637141, SINGAPORE, Singapore. ³School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, SINGAPORE, Singapore

Abstract

Nickel toxicity is a routine physiological concern of bacteria but bacterial responses to nickel stress are still unclear. We studied the effect of nickel on cell proliferation of α -proteobacterium *Caulobacter crescentus*. Next, we showed the mechanism that allows *C. crescentus* to survive in nickel stress condition. Our results revealed that exposure to nickel severely affected the growth of the bacterium, 0.003 mM slightly affected the growth, 0.008 mM reduced the growth by 50% and growth was completely inhibited at 0.015 mM. Fluorescence microscopy analysis further showed that nickel toxicity induced mislocalization of major regulatory proteins such as MipZ, FtsZ, ParB, and MreB, resulting in dysregulation of the cell cycle. A time dependent GC-MS metabolomic profiling of nickel stressed *C. crescentus* showed an increased level of nine important metabolites including TCA cycle intermediates and amino acids. This indicates that changes in central carbon metabolism and nitrogen metabolism are linked with the disruption of cell division. Addition of malic acid, citric acid, alanine, proline and glutamine to 0.015 mM nickel treated *C. crescentus* restored its growth. Thus, the present work shows a protective effect of these organic acids and amino acids on nickel toxicity. Metabolic stimulation through the PutA/GlnA pathway, accelerated degradation of CtrA, and Ni-chelation by organic acids or amino acids are the mechanisms suggested in enhancing *C. crescentus*'s nickel tolerance. A deep understanding of *C. crescentus*'s nickel stress response may be useful in bioremediation strategies and synthetic biology applications such as the development of whole cell biosensor.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P097

Adaptation of lysozyme to a deacetylation specific variant aids host cell exit by the intraperiplasmic predator *Bdellovibrio bacteriovorus*

Chris Harding¹, Hannah Somers², Carey Lambert², Rob Till², Patrick Moynihan¹, R. Liz Sockett², Andrew Lovering¹

¹University of Birmingham, Birmingham, United Kingdom. ²University of Nottingham, Nottingham, United Kingdom

Abstract

Bdellovibrio bacteriovorus is a predatory bacterium that preys on other Gram-negative bacteria by invading the prey periplasm, wherein they grow and replicate. During predation the prey peptidoglycan is modified to create an osmotically stable bdelloplast. One such modification involves N-deacetylation, acting as a “self vs prey” signal, marking the host cell wall for destruction at a later stage. To complete the predatory lifecycle, *Bdellovibrio* must exit from the bdelloplast. This step requires an enzyme that possesses activity towards the modified prey cell peptidoglycan. Here, we present Bd0314 as the enzyme that facilitates prey cell lysis at the end of the predatory lifecycle. We show that Bd0314 possesses specific activity towards a deacetylated peptidoglycan substrate (generated from a reaction of cell wall material with the previously identified *Bdellovibrio* peptidoglycan N-Acetylglucosamine deacetylases). To uncover the mechanism of substrate selection and cleavage, we have solved the structure of Bd0314 to 1.25Å, revealing an adapted lysozyme fold. We observe that the placement of Bd0314 catalytic residues is similar to chitosanases, which act on a similar partly deacetylated polymer. Further to this, two adaptations are identified that would putatively block enzyme action on a fully acetylated cell wall substrate. This work builds on the importance of cell wall modification during predation, providing yet another example of diversification of known folds for new predatory roles and reveals how *Bdellovibrio* achieves exit from the bdelloplast. Our identification of a new type of lysozyme may have implications that extend into other bacteria that appear to possess similar enzymes.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P098

Diet as an evolutionary driver in the microbiota genus *Bifidobacterium*

Magdalena Kujawska¹, Charlotte Leclaire¹, Raymond Kiu¹, Shabhonam Caim¹, Jennifer Ketskemety¹, Sara Goatcher², Trevor Lawley³, Lindsay J Hall¹

¹Quadram Institute Bioscience, Norwich, United Kingdom. ²Banham Zoo, Banham, United Kingdom.

³Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Abstract

Background: Bifidobacteria are a prominent member of the gut microbiota, and have been associated with improved host health through production of beneficial metabolites, maintenance of the intestinal barrier, and colonisation resistance. Notably, the genus *Bifidobacterium* has a wide host range, including mammals, birds, reptiles, and insects. The diverse host range may be linked to the ability to ferment complex host- and diet-derived polysaccharides, which suggests co-evolution of bacteria-host and diet. Currently, the pan-genome and phylogenetic relationship of the genus *Bifidobacterium* remain unresolved. Thus, here we explore the evolutionary diversity of bifidobacteria isolated from a wide range of animal species in order to study the host-diet adaptations of members of this group and the molecular basis behind their beneficial properties.

Methods: We have assembled a significant collection of *Bifidobacterium* species and strains that represent geographical and host range diversity, together with corresponding clinical and veterinary metadata. Using bioinformatics tools, we have mined the genomic data to determine evolutionary relationships, pan-genome, and functional attributes.

Results: Our analysis indicates several major phylogenetic groups, which cover a diverse host range. Functional analysis (i.e. glycosyl hydrolases) indicates a link with host diet and *Bifidobacterium* species/strains, and significant variability of encoded beneficial traits (e.g. bacteriocins). Finally, our analysis indicates the isolation of potential new bifidobacterial species from certain hosts, including birds and primates.

Conclusions: These data highlight the significant host diversity of the genus *Bifidobacterium*, linked to metabolism, and beneficial properties, which could be translated into novel microbiota therapies to improve human and animal health.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P099

The influence of strain background and variation in protein structure on the roles of pneumolysin in infectious diseases due to *Streptococcus pneumoniae*

Emad Mohameed, Peter Anderw, Hasan Yesilkaya

University of Leicester, Leicester, United Kingdom

Abstract

Emad Essa Mohamed, Hasan Yesilkaya and Peter W. Andrew

Department of Infection, Immunity and Inflammation, University of Leicester

Background: *Streptococcus pneumoniae* is a major cause of pneumonia, bacteremia, meningitis and otitis media. Pneumolysin (PLY) is the major toxin of the bacterium, and it has many adverse effects on immune cell functions. PLY has a generally conserved sequence but recently 20 different PLY variants have been described among clinical pneumococcal isolates (Jefferies *et al.*, 2007, Jefferies *et al.*, 2010). These variants have differences in cytotoxic activity but the impact of these differences on virulence is hard to ascertain because they are being made in different strain backgrounds.

Method: To study the significance of polymorphisms on PLY function in a single genetic background, unmarked mutations were introduced into the *ply* in the type 2 D39 strain background using pORI280. The selected PLY variants were expressed and purified using affinity chromatography and gel filtration. Haemolytic and complement activation assays were done as before.

Results: The results showed that of 8 naturally occurring single nucleotide polymorphisms (SNP), three of them reduced the haemolytic activity of PLY significantly compared to the D39 PLY. and the other five alleles showed the same haemolytic activity of D39 PLY.

Conclusion: The strain background has an impact on PLY activity, comparing the results with reference strain D39 and the published data, there are some mutations were showed reduction in PLY haemolytic activity, while the others were showed approximately same lytic activity.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P100

Development of Defined *Clostridium difficile* Growth Media for Physiological and Metabolic Studies

Hanson Ogbu^{1,2}, David Barrett², Klaus Winzer²

¹University of Port Harcourt, Port Harcourt, Nigeria. ²University of Nottingham, Nottingham, United Kingdom

Abstract

Developing a media that is suited for the physiological studies of *Clostridium difficile* in order to determine amino acid requirements, extent and order of amino acid utilisation (as carbon and energy sources), virulence and pathogenesis seems to be somewhat challenging. This is possibly due to the complexity of cellular metabolism and the attendant variations amongst the different strains. Although a number of *C. difficile* strains, have been successfully cultivated on defined media, but that these media provide excess of nutrients, particularly in terms of amino acid provision, resulting in undesirable background growth in the absence of glucose. This study presents three variants of a defined medium (DMaa1mM, DMaa10mM, DMMaa1mM) that allows good growth of representative strains of the *C. difficile* pathogens (*C. difficile* 630 Δ erm, DH 196, R20291, EK 15, EK 28, R12801, L26, O17 Serotype F) in the presence of a carbon and energy source such as glucose. These media are useful for different purposes in the physiological and metabolic analysis of this and other toxinogenic strains. All strains tested grew on this fully defined medium, however, growth profiles were different in terms of lag phase, growth rate, and maximum OD reached. This medium therefore represent a significant step forward in determining the roles of nutritional and metabolic factors in the control of virulence in this bacterium.

Keywords: Defined media, *Clostridium difficile*, amino acids, optimisation, casamino acids, growth profiles.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P101

An Environmental Response Regulator Links Fundamental Cell Growth and Metabolism with Virulence in *Bordetella pertussis*.

Thomas Belcher^{1,2}, Iain MacArthur¹, Andrew Preston¹

¹University of Bath, Bath, United Kingdom. ²Institute Pasteur Lille, Lille, France

Abstract

The *B. pertussis* Bvg two component system is well characterised as a regulator of virulence gene expression and thus its role in determining the virulence state of *B. pertussis* is well understood. Here we reveal that Bvg regulates much wider aspects of *B. pertussis* physiology including metabolism and cell growth and division.

We used Bvg- knockout mutants and chemical modulation of Bvg activity to show that *B. pertussis* growth rate is Bvg-dependent. Interestingly, high Bvg activity produced slow growth and lower final biomass of cultures than when Bvg was inactive, and yet more carbon source was consumed per unit of biomass; identifying a Bvg-regulated switch in metabolism. Metabolomics is being used to identify specific pathways regulated by Bvg activity. Tra-DIS mediated identification of genes essential for growth under these two Bvg-regulated growth states revealed that key cell wall biosynthesis genes (for example, the entire *mre/mrd* operon) are conditionally essential, dependent on Bvg-activity, and that fundamental processes such as cell wall synthesis operate differently, dependent on Bvg.

While it is well known that bacterial metabolism and growth rate is regulated in response to environmental conditions, this is often in response to changes in nutrients or other metabolites or changes in environmental conditions such as temperature. Here we demonstrate that a single environmental response regulator coordinates the expression of virulence genes with switches in the modes of metabolism and growth and division; providing a key opportunity to explore the interplay between fundamental bacterial physiology and virulence.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P102

Effect of Trace Metals on the Production of Specialised Metabolites of Marine Microalgae

Alison H. Hughes¹, Douglas McKenzie², Katherine R. Duncan¹

¹University of Strathclyde, Glasgow, United Kingdom. ²Xanthella Ltd, Oban, Argyll, United Kingdom

Abstract

Microalgal primary metabolism has been increasingly studied due to the relevance of their lipids for biofuel applications. However, their secondary (i.e. specialised) metabolism remains under explored. In nature, microalgae respond to stressors (nutrition, temperature, salinity) in their environment by producing defensive specialised metabolites. Because of their ecological function, these small molecules hold great potential for use as antimicrobial agents against the life-threatening clinical pathogens, such as *Staphylococcus aureus*. Microalgae require trace metals as a source of nutrition and these are believed to act as co-factors in secondary metabolism. The work presented investigates the effect of eliminating trace metals, namely Fe, Co, Cu, Zn, Mn, and Mo, on the production of specialised metabolites. A panel of ten marine microalgal strains, from British waters, were chosen to cover a wide phylogenetic spectrum. These are *Chlorella*, *Dunaliella*, *Tetraselmis*, *Rhodella*, *Colaconema*, *Nannochloropsis*, *Rhodomonas*, *Phaeodactylum*, *Pavlova*, and *Prorocentrum* spp. Liquid cultures of each strain (grown in f/2 medium missing a single trace metal), as well as controls, were extracted using ethyl acetate and subjected to bioassay and LC-MS/MS analysis. A disc diffusion assay against the ESKAPE pathogens evaluated the antibiotic activity of the extracts, whilst molecular networks of the LC-MS/MS data were used to compare the chemical space and the effect of the culture conditions on each strain. Prioritised culture conditions of chemical interest will then be upscaled using photobioreactors (microPharos™) designed by Xanthella Ltd., Scotland. These systems allow greater control over light and temperature parameters compared to traditional culturing methods.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P103

Identification, cloning and characterization of high affinity zinc transporter gene (PiZRT1) from endophytic fungus *Piriformospora indica*.

Uzair Ahmad, Shazia Shaheen Mir, Asif Anas, Tanzeel ., Nimisha Patel, Sukhwinder Kaur, Arif Ali

Jamia Millia Islamia, New Delhi, India

Abstract

Zinc is an essential micronutrient required by plants and animals affecting more than 300 proteins, and required for maintaining immunity. It is an immobilized nutrient; plants need to send their root to absorb zinc from the different parts of soil via a specific zinc transporter present in the plasma membrane of plant root cells. In adverse condition, plants interact with the mycorrhizal fungi also known as the custodian of plants where fungus helps plant to cope with stress condition ranging from nutrient deficiency, high salt stress and protection against pathogen. In this study, we identify and characterize the high affinity zinc transporter gene (PiZRT1) from the endophytic fungus *Piriformospora indica*. In silico analysis shows PiZRT1 from the *Piriformospora indica* belongs to the ZIP (ZRT,IRT-like Protein) family having eight transmembrane domain. Heterologous expression of PiZRT1 in *zrt1zrt2* mutant strain of *Saccharomyces cerevisiae* shows mutant strain acquired the wild type phenotype after transformation. To our knowledge, our group is among the first to characterize the high affinity zinc transporter gene (PiZRT1) from endophytic fungus and this study will provide further insight into the molecular level details for the functioning of zinc transportation by the endophytic fungus to the plants.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P104

Understanding new ways to inhibit bacterial growth through structural studies of toxin-antitoxin systems

Ben Usher, Izaak Beck, Tim Blower

Durham University, Durham, United Kingdom

Abstract

Mycobacterium tuberculosis remains one of the world's deadliest pathogens, infecting a third of the global population and resulting in 1.7 million deaths in 2016 alone. Treatment is often complicated by the increasing prevalence of multi-drug-resistant infections; as a result, novel approaches to control *M. tuberculosis* infections require exploring. Toxin-antitoxin (TA) systems are ubiquitous in bacteria, with at least eighty identified in *M. tuberculosis*. These systems are involved in phage resistance, response to stress, and implicated in mediating antibiotic tolerance by regulating bacterial growth. Typically, TA systems function through reversible bacteriostasis. Once activated by stress the toxin stalls growth; when stress is removed the antitoxin inhibits toxin activity, autoregulates expression of the TA operon and restores growth. In this study, we have functionally characterised three putative Type IV TA systems identified in *M. tuberculosis*, assessing the phenotypic effects of toxin and antitoxin expression in *Escherichia coli* via toxicity assays, growth curves and microscopy. Furthermore, we have begun protein purification to carry out biochemical characterisation of our systems; assessing the DNA binding properties of antitoxins, determining toxin targets, and carrying out structural and functional studies through X-ray crystallography. TA systems essentially function as bacteria-borne antibiotics: consequently, elucidating the manner by which these systems regulate bacterial growth will potentially reveal a host of new drug targets, help inform future drug design, and present a conserved mechanism to tackle a range of bacterial species.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P105

Identification of key transcriptomic differences between global and African sequence types of *Salmonella* Typhimurium

Rocío Canals¹, Disa Hammarlöf², Carsten Kröger³, Siân V. Owen¹, Wai Yee Fong¹, Lizeth Lacharme-Lora¹, Will P. M. Rowe¹, Jay C. D. Hinton¹

¹Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom. ²Dpt. Cell and Molecular Biology /Microbiology, Uppsala University, Uppsala, Sweden. ³Moyne Institute of Preventive Medicine, Trinity College Dublin, Dublin, Ireland

Abstract

Salmonella Typhimurium sequence-type ST313 is associated with an emerging invasive nontyphoidal *Salmonella* (iNTS) disease in sub-Saharan Africa that targets susceptible HIV⁺, malarial or malnourished individuals. A genomic comparison between an ST313 isolate, D23580, and the well-characterized isolate 4/74 (ST19), that causes gastroenteritis across the globe, showed that the two strains share 96% of coding genes. Genetic differences included 1000 SNPs, two D23580-specific prophages, a different plasmid repertoire, and the presence of pseudogenes.

To investigate the hypothesis that altered gene expression patterns reflect virulence mechanisms that differ between ST19 and ST313, RNA-seq-based transcriptomic data were obtained for the two strains, 4/74 and D23580, grown under sixteen infection-relevant *in vitro* conditions and during infection of murine macrophages. Five conditions were selected for differential expression analysis: ESP (Early Stationary Phase), anaerobic growth, *Salmonella* pathogenicity island 2 (SPI2)-inducing and SPI2-non-inducing conditions, and intracellular growth in murine macrophages.

The comparative gene expression analysis revealed that 14% of genes/sRNAs were differentially-expressed in at least one of the five conditions. An average of 160 genes were differentially-expressed in each condition. In ESP, a condition that induces the SPI1-encoded invasion system, 4% of genes/sRNAs showed differential expression. Label-free LC-MS/MS proteomic analysis confirmed that 7% of the differentially-expressed genes showed altered expression at the protein level.

Differences observed in gene expression of virulence-associated genes under specific environmental conditions reflect the distinct pathogenic mechanisms of these two *S. Typhimurium* strains. We will present the latest results of our comparative transcriptomic approach.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P106

Autoregulation by type IV toxin-antitoxin systems of *Mycobacterium tuberculosis*

Izaak Beck, Ben Usher, Tim Blower

University of Durham, Durham, United Kingdom

Abstract

Toxin-antitoxin systems are ubiquitous in bacteria, most notably in *Mycobacterium tuberculosis*, the causative agent of tuberculosis responsible for around 1.7 million deaths a year, which boasts more than eighty. Their functions vary greatly, ranging from plasmid maintenance and control of capsule synthesis to altruistic cell death and phage abortion. Our current work focuses on three putative type IV toxin-antitoxin systems in *M. tuberculosis* that are upregulated during macrophage infection. Complementary to this, we are studying a bacteriophage abortive infection system from *Streptococcus agalactiae*, a homologue of one of the *M. tuberculosis* systems. The type IV systems are comprised of two non-interacting proteins and our aim is to validate these systems and to elucidate the molecular targets of the protein toxins and antitoxins. Recently, we have successfully purified and crystallized AbiEi, the antitoxin from our single *S. agalactiae* system. We are using fluorescence anisotropy and gel-based methods to study the binding of this protein to the *abiE* promoter, in order to determine how the antitoxin regulates expression of the *abiE* operon. This work will set the precedent for replicating these studies in the *M. tuberculosis* systems in order to better understand toxin-antitoxin system autoregulation and their protein-DNA interactions.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P107

Making vital connections: probing the molecular interactions underpinning the segregation of a multidrug resistance plasmid

Cecilia Pennica¹, Madhuri Barge², Andrew Leech¹, Daniela Barillà¹

¹University of York, YORK, United Kingdom. ²University of Glasgow, Glasgow, United Kingdom

Abstract

Accurate segregation of newly replicated plasmids is essential for genetic stability throughout bacterial generations. The conjugative plasmid TP228 can confer bacteria resistance to several antibiotics and it is segregated during cell division thanks to the self-encoded ParFGH partition system. This is composed of two *trans*-acting proteins, ParF and ParG, and a centromeric region, *parH*. Interactions between the three components are essential for plasmid maintenance and are the main focus of this work.

ParG is a dimeric protein composed of a folded domain and two unstructured tails. Alanine-scanning mutagenesis of the ParG tail has highlighted a number of residues, whose change impairs plasmid partition *in vivo*. These flexible regions were shown to play a fundamental role in binding to the partner protein ParF as well as conferring specificity to the interaction with the DNA. A combination of biochemical and biophysical techniques has been used to address which regions in the ParG tails are essential for the interaction with ParF and which play different roles. Surface plasmon resonance together with Microscale Thermophoresis allowed to quantify the change in binding between ParF and ParG mutant proteins. These findings also allowed to speculate that particular positions in the tail may be required for binding to the DNA and structure the tail at the initial stage of segrosome formation. Circular dichroism and electrophoretic mobility shift assay validated this hypothesis. Finally, a tandem affinity purification approach gave new insights into the interaction network of the system. A model for plasmid segregation will be presented and discussed.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P108

The role of Lsr2 phosphorylation in mycobacterial growth and dormancy

kawther ALQaseer¹, Galina Mukamolova¹, Obolbek Turapov¹, Pavithra Krishnan¹, Iona Bartek², Martin Voskuil², Andrew Bottrill¹, Paul Ajuh³, Martin Cohen-Gonsaud⁴, Simon Waddell⁵, Heena Jagatia⁶

¹University of Leicester, Leicester, United Kingdom. ²University of Colorado, Aurora, USA. ³Gemini Biosciences, Liverpool, United Kingdom. ⁴Centre de Biochimie Structurale, Montpellier, France.

⁵University of Sussex, Falmer, United Kingdom. ⁶University of Sussex, Brighton, United Kingdom

Abstract

Two billion people are estimated to have latent tuberculosis infection associated with dormant *Mycobacterium tuberculosis (Mtb)*. Lsr2 is a global transcriptional regulator which has nearly 600 DNA binding sites and controls gene expression in mycobacteria during growth, dormancy and resuscitation. A deletion of *lsr2* in slow-growing *Mtb* resulted in a dramatic growth defect on solid medium, the inability to survive in hypoxia and impaired persistence in animals (1). However, *lsr2* inactivation in fast-growing *Mycobacterium smegmatis* did not affect growth and was important for colony morphology, production of specific lipids and biofilm formation (2). Our phosphoproteomic studies and *in vitro* phosphorylation assays established that Lsr2 was phosphorylated by an essential protein kinase B (PknB) on several threonines and phosphorylation on threonine 112 was critical for the functions of Lsr2 in *Mtb* and *M. smegmatis*. Furthermore Lsr2 phosphorylation influenced its DNA binding ability and mobility of DNA binding domain. Our current CHIP-sequencing experiments will identify DNA binding sites for phosphorylated and non-phosphorylated forms of Lsr2 and provide further insights into the role of Lsr2 phosphorylation in mycobacterial growth and dormancy.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P109

Mechanistic and Phenotypic Characterisation of the Rgg/SHP Quorum Sensing System in *Streptococcus pneumoniae*

Iman Abdullah, Russell Wallis, Peter Andrew, Hasan Yesilkaya

University of Leicester, Leicester, United Kingdom

Abstract

The Rgg (Regulator Gene of Glucosyltransferase) regulators with their short hydrophobic peptides SHP form part of quorum sensing system in Gram positive bacteria. They play an important role in stress response, sugar metabolism, and virulence, but their function and mechanism of action remain unclear in the important human pathogen *Streptococcus pneumoniae*. The pneumococcal type 2 D39 has five homologues of Rggs, and two of them (SPD_0144 and SPD_0939) are associated with putative *shp* genes encoding for SHP peptides, which regulate their own expression, and are required for Rgg activation. This study was designed to identify optimum length of SHP144 to stimulate Rgg144-mediated transcription, and quantify functional importance of each selected SHP144 residue for Rgg144's activation and phenotypic manifestation.

Several synthetic peptides representing C-terminal end of SHP144 were synthesised to identify active SHP144 using reporter strains *Pshp144::lacZ-wt* and *Pshp144::lacZ-Δshp144* ('P'-promoter). Site directed mutagenesis was used for substitution of selected residues of SHP144 with alanine, and effect of each amino acid replacement was studied using transcriptional reporter assay. The phenotypic impacts of mutations were determined by H₂O₂ resistance, and by growth assays in chemically defined medium supplemented with different sugars.

The 12 and 13 amino acid long synthetic peptides representing C-terminal end of SHP144 are sufficient to stimulate *Pshp144::lacZ*. Furthermore, most of C13 residues are required for *Pshp144* driven LacZ activity, indicating the importance of selected residues in transcriptional activation. Finally, mutations of selected SHP144 residues decrease pneumococcal resistance to H₂O₂, and diminish its growth in CDM mannose.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P110

Systematics and bioactivities of thermotolerant antibiotics-producing *Streptomyces* sp. TM32 isolated from turmeric rhizosphere soil

Nareeluk Nakaew¹, Nitra Nuengchamnon², Shuhei Yabe³, William T. Sloan⁴, Rungroch Sungthong⁴

¹Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand. ²Science Laboratory Centre, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand. ³Graduate School of Agricultural Sciences, Tohoku University, Miyagi 981-8555, Japan. ⁴Infrastructure and Environmental Research Division, School of Engineering, University of Glasgow, Glasgow G12 8LT, United Kingdom

Abstract

With the framework for discovering novel drugs from novel microbes, we isolated an actinobacterium isolate TM32 from rhizosphere soil of turmeric grown at Nong Bua Lamphu Province, Thailand. Isolate TM32 has morphological characteristics of the genus *Streptomyces*, and its 16S rDNA sequence-based phylogenetic relationship was closest to *Streptomyces sioyaensis* NRRL B-5408^T with the gene sequence similarity of 99.23%. Despite DNA-DNA relatedness value between isolate TM32 and *S. sioyaensis* (79.2%) was higher than the threshold (70%) for determining the difference of genomic species, they exhibited distinct bioactivities under the same growing conditions. Isolate TM32 produced chitinase (enzyme activity at 0.093 U mg⁻¹) and did not produce β -1,3-glucanase, which was contrary to those tested with *S. sioyaensis*. Cell-free culture broths (CFCBs) of isolate TM32 and *S. sioyaensis* showed similar antimicrobial activities (i.e., anti-Gram-positive bacteria including antibiotic-resistant *Staphylococcus* spp. and anti-phytopathogenic fungi). However, the thermotolerant assays using their autoclaved CFCBs revealed differently, whereas the antimicrobial activities of isolate TM32's one remained the same, while the one of *S. sioyaensis* lost antifungal activities. A comparative study by liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometer also supported the different profiles of metabolites in CFCB crude extracts of isolate TM32 and *S. sioyaensis*. Further studies for the complete systematics of isolate TM32 and structural elucidation of its thermotolerant antimicrobials are essential and would be a promising mean to serve the global call for combating the antimicrobial resistance problem with the expanded discovery of novel drugs.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P111

Investigating the action mechanism of TprA/PhrA mediated virulence in *Streptococcus pneumoniae*

Banaz Kareem, Peter Andrew, Hasan Yesilkaya

University of Leicester, Leicester, United Kingdom

Abstract

Streptococcus pneumoniae, is one of the leading cause of death among children and elderly. It is predicted that several regulatory pathways control crucial microbial functions such as virulence. TprA/PhrA quorum sensing system is one of a new family of regulatory systems shown to be involved in virulence, galactose utilization, and neuraminidase production, and its potential utility as an antiinfective target has been demonstrated by our research group. However, it is not known in detail how TprA/PhrA contributes to the pneumococcal virulence, therefore, the goal of this study is to reveal the mechanism of TprA/PhrA mediated virulence in *S. pneumoniae*. Mutations were introduced into the selected genes shown to be regulated by TprA/PhrA QS system by splicing overlap extension. Pneumococcal strains were tested by growth studies using chemically defined medium (CDM) supplemented with different sugars (Motib et al., 2017). LacZ transcriptional reporter assays were used to determine the expression of selected genes in selected environmental conditions and to understand regulatory interactions (Al-Bayati et al., 2017). The results showed that the strains lacking *spd1517* or *spd1947* utilize galactose and mucin less efficiently than the wild type, and the mutant strains had lower neuraminidase activity than the wild type. In addition, the reporter assays showed that TprA is an activator of tagatose pathway while this regulator has no significant effect on expression of *galk*, a key gene of Leloir pathway. TprA mediates pneumococcal virulence through its impact on complex regulatory cascade controlling galactose catabolism

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P112

Functional Studies of Bacteriophage Exclusion (BREX) Systems

David Picton¹, Jay Hinton², Tim Blower¹

¹Durham University, Durham, United Kingdom. ²University of Liverpool, Liverpool, United Kingdom

Abstract

Bacteriophages (phages) are viral predators of bacteria that outnumber their prey by a factor of 10. Phages are the most abundant organisms on the planet, and are a major driving force in bacterial evolution. As obligate intracellular parasites, phages are reliant on their bacterial host for propagation, but bacteria have evolved means to prevent phage infections. BREX is a novel phage-resistance system that confers resistance to a wide array of phages, functioning independently of restriction modification, CRISPR-Cas and abortive infection mechanisms. It is present in ~10% of bacterial and archaeal genomes, including pathogenic strains such as non-typhoidal invasive *Salmonella* Typhimurium ST313 and multidrug resistant *Escherichia fergusonii*. The discovery of other phage resistance mechanisms led to vast biotechnological advances in genetic manipulation, and there is potential for BREX to be developed in a similar manner.

We have characterised the BREX locus of *E. fergusonii* and developed expression systems for the eight genes. The functionality of the pure proteins is diverse and includes; a DNA-binding transcriptional regulator, a Walker-box containing ATPase, a methyltransferase, an alkaline phosphatase and a Lon-like protease. Biochemical and structural analyses of these proteins are underway to pinpoint their likely roles in the BREX mechanism and to characterise the resulting protein-protein complexes. From these studies we will better understand how BREX works, ways in which phages might co-evolve to avoid BREX and finally, the potential biotechnological applications of BREX.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P113

Secretion of the *Clostridium difficile* S-layer

Peter Oatley, Joseph Kirk, Robert Fagan

University of Sheffield, Sheffield, United Kingdom

Abstract

C. difficile is a gram positive, anaerobic bacterium [\[RF1\]](#) that resides in the gut. Antibiotic mediated dysbiosis of the gut can lead to multi-drug resistant *C. difficile* infection (CDI) that can cause diarrhoea, colitis and even death. To allow the reestablishment of a normal gut microbiota during CDI, novel *C. difficile* specific antibiotic targets are being sought. *C. difficile* are surrounded by protective, proteinaceous Surface layer (S-layer) that is essential for its virulence. For S-layer components to function correctly they need to be transported across the cytosolic membrane and correctly processed. Identifying components that are involved in S-layer secretion and quality control may reveal new ways of combating CDI. Most secretory proteins are translocated across membranes by the universally conserved and essential secretory (Sec) system via a hetero-trimeric SecYEG complex. In bacteria, most proteins are secreted post-translationally with the aid of the cytosolic ATPase, SecA. *C. difficile* possess two SecA homologues, SecA1 and SecA2. Previous work from our lab revealed that S-layer secretion particularly requires the activity of the accessory secretory chaperone, SecA2. Using proteomics, we are now identifying candidates that may be important for S-layer secretion and factors that assist in the secretion of SecA1 and SecA2 specific substrates. Molecular and cellular techniques are now being applied to validate the function of these candidates and how they interact with S-layer proteins and the SecA homologues.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P114

Bacterial microcompartment generation using components of the propanediol utilisation metabolosome from *Geobacillus thermoglucosidasius* in *Bacillus subtilis*.

Yana Wade^{1,2}, Richard Daniel²

¹Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom. ²Centre for Bacterial Cell Biology, Newcastle University, Newcastle-upon-Tyne, United Kingdom

Abstract

Bacterial microcompartments (BMCs) are sub-cellular proteinaceous organelles whose one of purpose appears to be to sequester highly reactive pathway intermediates. The ability to control the assembly of BMCs and their biochemical cargo offers biotechnological opportunities not yet achieved in thermophiles.

The pdu operon consisting of 19 genes in the genome of thermophilic *G. thermoglucosidasius* NCIMB 11955 was studied. The five genes, *pduA*, *pduBB'*, *pduJ*, *pduK* and *pduN*, encoding proteins that form the shell of BMCs were amplified. The empty BMCs were assembled by expression of the *pduABB'JKN* proteins in *B. subtilis* 168CA. The ability to express heterologous proteins was confirmed by the examination of a green fluorescent protein (sfGFP) fusion to each individual gene at the C-terminus using both the fluorescent microscopy and western immunoblotting. Circular structures with clear boundaries were visualised in thin-sectioned cells by transmission electron microscopy. The sfGFP fused to the sequences of Propanediol utilisation protein (PduP), equivalent to the first 24 and 461 amino acids was incorporated into the lumen of the BMCs. Time-lapse imaging showed an ability to assemble BMCs after 20 minutes.

These results show for the first time the capacity to generate synthetic protein compartments derived from the shell proteins, *PduABB'JKN*, of the thermophile, *G. thermoglucosidasius*. Subsequent inclusion of heterologous protein into the interior of the compartment depends on its fusion to the N-terminus of PduP equivalent to a minimum of 24 amino acids. The current *Bacillus*-based system will assist in future construction of empty compartments for targeting proteins in *Geobacillus* spp.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P115

A novel sulfur oxidation pathway revealed in dimethyl sulfide-degrading *Hyphomicrobium denitrificans*

Tobias Koch, [Christiane Dahl](#)

Institute for Microbiology & Biotechnology, University of Bonn, Bonn, Germany

Abstract

While many sulfur-oxidizing bacteria operate the well-established Dsr (dissimilatory sulfite reductase) pathway, other bacterial and archaeal sulfur oxidizers lack this pathway. Bioinformatic analyses have indicated an alternative metabolic route involving a heterodisulfide reductase (Hdr)-like protein complex in these organisms [reviewed in 1], but direct genetic evidence for this suggestion has not been available. Here, this major knowledge gap was addressed using *Hyphomicrobium denitrificans* (DSM 1869^T). This Alphaproteobacterium typically grows on C1-compounds like dimethylamine but also uses the volatile organic sulfur compound dimethyl sulfide (DMS) as sole carbon and energy source during aerobic respiration. DMS is degraded to methanethiol from which formaldehyde and sulfide are released [2]. The latter is then fully oxidized to sulfate by wildtype *H. denitrificans*. In contrast, a knockout strain lacking a viable Hdr-like system was completely incapable of growth on DMS. Complementation with a plasmid containing the complete *hdr*-like genes under a constitutive promoter rescued the phenotype. Furthermore, we show that novel lipopeptide-binding proteins are key players in the Hdr-like sulfur oxidation pathway. Comparative proteomic as well as immunological data showed that the Hdr-like complex is specifically induced by DMS, thus further strengthening the notion that this complex catalyzes a key step in sulfur oxidation not only in *H. denitrificans* but also in a wide range of other environmentally important sulfur oxidizers.

[1] Venceslau et al 2014 BBA 1837, 1148

[2] Eyice et al 2017 ISME J, doi:10.1038/ismej.2017.148

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P116

New Understanding of Quinolone Resistance in *Pseudomonas aeruginosa* (core physiology rather than mere drug target changes)

Wedad Nageeb^{1,2}, Arshad Khan¹, Nigel Saunders¹

¹Brunel University London, London, United Kingdom. ²Suez Canal University, Ismailia, Egypt

Abstract

Background: Most previous studies have linked quinolone resistance to antibiotic target enzyme mutations. Although this commonly reported mechanism can be necessary for replication fork arrest, it may prove to be insufficient to cause the net behavior of resistance. Other system background elements can be of equal importance for determining the fate of the cell on exposure to quinolones.

Methods: In this study, comparative behavioral genomic approach was applied to analyze the whole genome sequence information of 87 genetically diverse clinical isolates considering both statistical significance and effect sizes for observed elements.

Results: Different genes and nucleotide changes appear to co-contribute to analyzed behavior. Those were mainly classified under different Central intermediary and energy metabolism pathways. Nucleotide polymorphism in Genes related to sub pathways of Sulphur compound metabolism and hydrogen sulfide biosynthesis were observed. These can be responsible for cellular defense by decreasing antibiotic related oxidative stress. In a similar way, genes and SNPs in Glutathione metabolism pathway were also found and can be acting on the balance between reactive oxygen species and cellular antioxidants. Importantly observed was the pathway of oxidative phosphorylation including NADH dehydrogenases and several members of flavoprotein family of azoreductases that neutralize toxic quinones.

Conclusion: *Pseudomonas aeruginosa* is one of the ESKAPE organisms declared as most resistant, however it is not considered as host adapted pathogen. This makes it necessary to consider physiology and genomic background structure of the species when investigating behavior of antibiotic resistance and this is important to make the most optimal related clinical decisions.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P117

Global suppressors are frequent in the histidine biosynthetic enzyme HisA

Hind Abdalaal, Joakim Nasväll

Uppsala University, Uppsala, Sweden

Abstract

Understanding how a specialized enzyme can evolve from a progenitor enzyme with promiscuous activity, is important for explaining the evolution of novel phenotype.

The project aims to generate a library of laboratory evolved orthologous genes for studying evolvability, the *hisA* gene from *Salmonella enterica* was taken through alternating rounds of weak selection (simulated by random mutagenesis and screens for partial loss of function) followed by strong selection (simulated by additional random mutagenesis and selections for restored function).

After the first few rounds of mutagenesis and screening/selection, we have found twenty deleterious and twelve compensatory mutations. Surprisingly, after performing the third round of mutagenesis and screening for loss of activity, three lineages reverted the compensating mutation rather than acquiring a second deleterious mutation. This could indicate that these compensating mutations are able to mask the effects of several deleterious mutations, and that they could be global suppressors that make the protein more robust, e.g. by making the structure more stable or by improving folding. Such mutations would be able to compensate against a wide variety of deleterious mutations, and thus make the protein more evolvable.

By combining these compensating mutations with other deleterious mutations and testing the function of the resulting HisA enzymes, we confirmed that these mutations are global suppressors. Further experiments will test the effects on evolvability, defined as the ability to accommodate additional mutations and/or acquire a new function (TrpF enzymatic activity).

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P118

A Novel Substrate of the *Staphylococcus aureus* Type VII Protein Secretion System

Fatima Ulhug¹, Zhen Cao¹, Holger Kneuper¹, Manman Guo², Matthias Trost³, James Chalmers⁴, Tracy Palmer¹

¹Division of Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee, United Kingdom. ²Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, United Kingdom. ³Cell and Molecular Biosciences, Newcastle University, Newcastle, United Kingdom. ⁴Division of Molecular & Clinical Medicine, School of Medicine, University of Dundee, Dundee, United Kingdom

Abstract

The Gram-positive bacterium, *Staphylococcus aureus*, is a major opportunistic pathogen of humans and animals. Virulence factors that directly interact with the host must be secreted from the bacterial cytoplasm where they are made, and protein secretion systems are therefore critical to the success of bacterial pathogens. The Type VII secretion system (T7SS) in *S. aureus* is a protein export system found in many Gram-positive bacteria. In *S. aureus* this secretion system is essential for virulence, in particular for the persistence of abscesses in murine models of infection. In addition, more recently some strains of *S. aureus* have been shown to secrete a T7-dependent nuclease, EsaD, which has been shown to play an important role in intra-species competition *in vitro*. To date, very little substrates of the T7SS from *S. aureus* have been identified. Using quantitative proteomic analysis we have identified a novel T7-secreted substrate, TspA. Genetic and biochemical approaches have been used to validate TspA as a T7-dependent substrate and understand its mode of action on target cells. My latest results will be presented.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P119

Analysis of post-translational modifications of proteins secreted by *Pseudomonas aeruginosa*

Suzie Forrest, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

Pseudomonas aeruginosa is a multi-drug resistant opportunistic pathogen that has recently been classified as a “critical threat” by the WHO. In order to launch an attack on the host, *P. aeruginosa* secretes a wealth of proteinaceous virulence factors, which target the host tissue for degradation. Some of these factors are potent proteases, so it is relevant to ask “what protects the secreted *P. aeruginosa* proteins from auto-digestion?”. We postulated that post-translational modifications (PTMs) of secreted effectors may prove protective, and that such modifications may account for the variable charge states of secreted proteins resolved by 2D-PAGE. Several consecutive spots associated with such “charge trains” were therefore analysed to determine whether the proteins exhibit PTMs, and whether any obvious patterns emerge from the spots comprising each charge train. We found a strong correlation between the position of a spot within a charge train and the number of PTMs associated. The higher the pI, the more PTMs. Additionally, when the PTMs were modelled onto the 3D structure of each protein, all modified residues were found to be surface-exposed. Moreover, in many cases, there is a clear bias towards PTMs mapping to one surface of the protein. Our data suggest that secreted *P. aeruginosa* proteins are likely to be modified by PTM post-folding, and possibly during the export process itself. We do not yet know whether these modifications really do protect secreted proteins against auto-digestion by self-produced or host proteases or what carries out these PTMs; these are questions currently being addressed.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P120

Characterisation of *Rhizopus delemarspore* germination, using RNA sequencing, live cell imaging and flow cytometry

Poppy Sephton Clark

University of Birmingham, Birmingham, United Kingdom

Abstract

Rhizopus delemar, a fungus belonging to the mucorales order, is saprophytic and found ubiquitously. Mucorales species are responsible for causing Mucormycosis, an emerging infection that has proven difficult to treat, presenting with unacceptably high mortality rates in immunocompromised individuals. Disease depends upon spore germination and hyphal growth leading to angioinvasion and subsequent vessel thrombosis and necrosis in the host. However, we currently have a very limited understanding on the genetic network underpinning the regulation of mucormycete spore germination.

We have determined the phenotypic changes that occur during germination of *Rhizopus delemar* RA99-880 spores by defining spore swelling, hyphal growth patterns and changes in cell wall composition by live-cell imaging and flow cytometry. We combined our phenotypic analysis with a high-resolution transcriptomics time course. We will present our phenotypic data in correlation with RNA-Seq data identifying differential gene expression during the germinating of *Rhizopus oryzae* spores. This is the first detailed study of the genetic regulation of mucormycete spore germination.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P121

Development Of *Streptomyces* To Utilise Sustainable Feedstock In Fermentations

Anna Birke¹, Steve Kendrew², Paul Herron¹, Ben Huckle², Iain Hunter¹, Paul Hoskisson¹

¹University of Strathclyde, Glasgow, United Kingdom. ²GSK, Worthing, United Kingdom

Abstract

Members of the *Streptomyces* genus have been used in industry for decades to produce bioactive specialised metabolites. Primary metabolic intermediates are required as precursors for the biosynthesis of these compounds that have found utility in medicine such as antibiotics. The carbon source available during the production phase of the fermentation has been shown to have a profound impact on antibiotic production via carbon source-dependent regulatory mechanisms, such as carbon catabolite repression. Since bacteria usually exhibit preferences of one carbon source over others with the former often being glucose, the range of carbon sources that can be utilised in fermentation media is often key for fermentation performance. We are interested in broadening the use of carbon sources throughout industrial fermentations in industrially relevant and model *Streptomyces*. Ultimately, the longterm goal is to optimise the catabolic capabilities of those strains to allow the usage of sustainable feedstocks in fermentation media such as those from waste food. Using a combination of bioinformatics and molecular genetics we have identified and compared actinobacterial carbon uptake and catabolic systems using a range of tools, followed by the construction of integrating vectors for the heterologous expression of either a sugar permease or sugar kinase from a range of *Streptomyces* species under the control of an inducible promoter in *S. coelicolor* and *S. clavuligerus*. These constructs allowed enhanced expression of the chosen genes in these strains and a detailed characterisation of the growth and antibiotic production phenotypes was undertaken in these modified strains.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P122

“Cell surface hydrophobicity and the transmission of *Mycobacterium abscessus*”

Enass Al-Mkhadhree, Michael . (Prof.) Barer, Helen O'Hare, Mutlaq Alshamri

Leicester university, leicester, United Kingdom

Abstract

M. abscessus is a rapid growing mycobacterium that is recognised as an emerging opportunistic pathogen causing life threatening infections in patients with pre-existing lung damage. Although non-tuberculous mycobacteria are generally considered to be transmitted from environmental sources, recent studies have provided evidence that person to person transmission may occur, particularly amongst patients with cystic fibrosis. The mechanisms underpinning this likely airborne transmission are not understood. This project addresses the hypothesis that bacterial surface hydrophobicity is a key factor determining the propensity of cells to enter aerosols and that alterations in this property may influence the transmission of *M. abscessus*.

Macroscopically *M. abscessus* shows two colony morphotypes, rough and smooth. Hexadecane partitioning and Congo Red binding assays were used to measure the cell surface hydrophobicity. Lipid body profiling was also performed as this is associated cellular buoyancy, another property potentially contributing to aerosolisation.

Multiple assays demonstrated that the rough phenotype is significantly more hydrophobic compared to smooth. Moreover, the rough variant showed presence of more lipid bodies. As hydrophobic microorganisms have more ability to adhere to abiotic surfaces, this feature was confirmed using biofilm assay. Result showed that rough phenotypes were quick biofilm formers compared to smooth. We suggest these properties contribute to the recent extensive spread and discuss how smooth to rough transition may contribute to the current increase in *M. abscessus* infections.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P123

Characterisation of *Vibrio cholerae* chromosome II segregation proteins

Sat Chodha, Ling Chin Hwang

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): Increasing concentrations of ParA2 (or ParB2) were incubated for 30 minutes in reaction buffer with Cy5-labelled nonspecific DNA (or *parS2* DNA) and ATP analogues, and analysed by native PAGE;

Thin layer chromatography (TLC): ParA2 was incubated with ParB2, DNA, and [$\alpha^{32}\text{P}$]-ATP. Reaction was spotted onto TLC plates and mobile phase developed. Plate was exposed to a phosphor screen for quantification.

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 ATP γ S. 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.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P124

The SOS-response controls intrinsic cephalosporin resistance in Enterococci

Laura Carrilero¹, Stephanie Matrat², Daniel Thomas-Lopez², Ingolf Nes³, Rob Willems⁴, Bruno Gonzalez-Zorn²

¹University of Sheffield, Sheffield, United Kingdom. ²UCM, Madrid, Spain. ³Norwegian University of Life Sciences, Ås, Norway. ⁴UMC Utrecht, Utrecht, Netherlands

Abstract

Background

Enterococcus faecalis is a bacterium well known for causing multidrug resistant nosocomial infections. The SOS response has been shown to be involved in both antimicrobial resistance and virulence of different bacterial species.

Methods

Several mutants for the SOS response in two different genetic backgrounds were constructed by interrupting target genes by a thermosensitive plasmid. MIC were determined using EUCAST methodology for the constructed mutants. A transposon mutant library to identify the genetic determinants involved in the observed sensitization to cephalosporins was constructed and the implicated genes identified.

Results

E. faecalis has a functional SOS response to cope with damaged DNA and the LexA box is conserved in this species. The SOS response and its induction leads to cephalosporin sensitization of *E. faecalis* both in vivo and in vitro. Within our transposon mutant library, we identified two glutamine transporters and the *cop* operon as components possibly implicated in decreasing the MIC of *E. faecalis* to cephalosporins. One of the glutamine transporters identified in our library is under the regulation of CroR/S, which has been previously described to play a role in cephalosporin resistance. Additionally, we found that the presence of copper also sensitizes *E. faecalis* to cephalosporins.

Conclusions

We discovered that *E. faecalis* has a functional SOS response and appeared to be involved in the sensitization of *E. faecalis* to methoxyimino cephalosporins. Glutamine transporters and the *cop* operon play a key role in decreasing the MIC of *E. faecalis* to cephalosporins.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P125

AMINO ACID SUBSTITUTIONS IN OUTER MEMBRANE PROTEINS SEQUENCE AMONG ADHERENT- INVASIVE ESCHERICHIA COLI (AIEC) AND NON-AIEC STRAINS FROM THE HUMAN INTESTINE

Carla Camprubí-Font¹, Belén Ruiz del Castillo^{2,3}, Luis Martínez-Martínez^{4,5,6}, Margarita Martínez-Medina¹

¹Universitat de Girona, Girona, Spain. ²Service of Microbiology, University Hospital Marques de Valdecilla-IDIVAL, Santander, Spain. ³Department of Molecular Biology, University of Cantabria, Santander, Spain. ⁴Microbiology Unit, University Hospital Reina Sofía, Cordoba, Spain. ⁵Department of Microbiology, University of Cordoba, Cordoba, Spain. ⁶IMIBIC, Cordoba, Spain

Abstract

Point mutations in Outer Membrane Proteins (OMPs) sequence or differential expression have been related with antibiotic resistance. A role of OmpA and OmpC in adherent-invasive *Escherichia coli* (AIEC) pathogenicity has been suggested but scarcely investigated. Our aim was to determine possible associations between particular amino acid (aa) substitutions in OMPs and antibiotic resistance or AIEC pathogenicity.

The genes of *ompA*, *ompC* and *ompF* were sequenced from a collection of 22 AIEC and 28 non-AIEC strains. The strains were tested for resistance to 27 antimicrobials. Aa mutations were analysed statistically according to AIEC pathotype, phylogroup origin and antibiotic resistance using the χ^2 test and to invasion index using the U-Mann Whitney.

Strains clustered in 15 variants for OmpA, 32 for OmpC and 11 for OmpF. The distribution of aa substitutions was similar between AIEC and non-AIEC. Despite not reaching statistical significance, higher invasion indices were observed in strains with specific aa changes. Aa substitutions correlated with phylogroup origin, being A phylogroup the one resembling more to B1 and B2 to D ($p < 0.05$). Resistance to aminoglycosides and doxycycline was observed for strains presenting 5 OmpA substitutions ($p = 0.024$), β -lactams, ampicillin and fluoroquinolones resistance associated with strains showing several OmpC mutations ($p = 0.010$) and tetracycline resistance with strain with one OmpF mutation ($p < 0.05$).

In conclusion, no particular aa changes in OMPs were associated with the AIEC pathotype. Some variants were associated with particular antibiotic resistances. Additional molecular studies are needed to corroborate if these mutations are directly implicated in the resistance phenotype or not.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P126

Mechanisms of inhibition of ribosome-associated GTPases by (p)ppGpp in *Staphylococcus aureus*

Daniel Bennison, Alison Wood, John Rafferty, Rebecca Corrigan

University of Sheffield, Sheffield, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of invasive human infection, including bacteremia and a variety of pulmonary infections. During host colonisation, the ability of *S. aureus* to adapt to stressful conditions is paramount to survival and the continuation of infection. During conditions of nutrient limitation, the alarmones guanosine pentaphosphate and tetraphosphate ((p)ppGpp collectively) are produced as the effector of the stringent response, and are known to be involved in cellular adaptation to nutrient limitation and potentially entry into stationary phase. Genome-wide screening has identified four ribosome-associated GTPases (RA-GTPases) to which (p)ppGpp binds, each of which have been implicated as a cofactor to facilitate 70s ribosome assembly. Further study has revealed that when associated with the 70s ribosome, GTPase activity is increased dramatically. However upon (p)ppGpp binding to these RA-GTPases, their activity is inhibited – negatively impacting ribosome assembly and bacterial growth. This mechanism of inhibition of RA-GTPases therefore has great potential regarding the design of novel bacteriostatic antimicrobials. Here, the contribution of each of these RA-GTPases to the survival of *S. aureus in vivo* is currently being assessed, in combination with *in vitro* site-directed mutagenesis, biochemical assays and X-ray crystallography to determine the molecular mechanism of inhibition by (p)ppGpp.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P127

Two similar V5virus environmental coliphages escape a Type III toxin-antitoxin/abortive infection system through defects in a viral locus associated with RNA metabolism

Ray Chai, Nathalie Goeders, Jessica Bergman, George Salmond

University of Cambridge, Cambridge, United Kingdom

Abstract

Bacteria are constantly under threat by their viral parasites, the bacteriophages (phages) and as such have evolved numerous anti-phage systems. Abortive infection (Abi) is one such anti-phage system that is unique as it does not protect the individual cell infected with the phage. When phages infect bacterial cells carrying Abi systems, these systems may be activated, leading to the premature death of the infected cell. While the infected cell dies, precocious death prevents any potential progeny phages from being released and so protects the rest of the clonal population. Previous studies have shown that Type III toxin-antitoxin (TA) systems are able to cause Abi, however, the mechanism(s) by which these systems are activated during phage infection is still poorly understood. In this study we show that the Type III TA system, ToxIN_{pa}, from the phytopathogen *Pectobacterium atrosepticum*, when transferred to *Escherichia coli* is able to confer phage resistance against newly isolated coliphages from the environment. Furthermore, we show that two of these coliphages, ΦCHA18 and Φ607 are not only aborted by the ToxIN_{pa} system but also able to evolve rare escape mutants. Analysis of the wild type and escape phages has shown that both phages are highly similar V5viruses and all escape mutants have mutations in a similar locus encoding proteins involved in RNA metabolism. Deletion of this locus has a fitness cost; escape phages having extended eclipse periods and smaller burst size compared with the wild type. These findings suggest an interesting dynamic between bacteria and phage evolution.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P128

Sequential interactions of subunits and chaperones with the bacterial flagellar export machinery

Owain Bryant, Gillian Fraser

University of Cambridge, Cambridge, United Kingdom

Abstract

The flagellum is a large macromolecular complex that extends far beyond the cell surface. Flagella are assembled from thousands of protein subunits that are unfolded and exported into a narrow 20 Å channel in the centre of each flagellum, via a specialized type III secretion system. Unfolded subunits must transit the length of the channel to the flagellum tip up to ten cell lengths away, where they fold and assemble into the nascent structure. 'Late' filament structural subunits (FlgK, FlgL, FliD and FliC) are delivered from their site of synthesis in the cytoplasm to the flagellar export machinery by their subunit-specific chaperones. These chaperones initially dock at the FliI component of the membrane associated ATPase complex. They are then thought to interact with FlhA_c, followed by FliJ. However, the order of chaperone binding events after FliI has not been confirmed.

Chaperone variants defective for flagellar export were assessed by affinity co-purification for binding to cognate subunits and the flagellar export components FliJ and FlhA_c. All chaperone variants were found to be defective in binding one or more export components. In particular, one chaperone variant could bind subunits and FliJ but was unable to bind FlhA_c. An *in vivo* FliJ pull-down assay revealed that although some chaperone variants were able to bind FliJ *in vitro*, there was a significant reduction in binding to FliJ, *in vivo*. The data indicate the order of chaperone binding events at the membrane export machinery during flagellum assembly.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P129

Length regulation and ordered export in the bacterial flagellum

Paul Bergen¹, Lowri Williams¹, Owain Bryant¹, Katie Kemplen², Gillian Fraser¹

¹Department of Pathology, University of Cambridge, Cambridge, United Kingdom. ²University of Copenhagen, Copenhagen, Denmark

Abstract

To enable swimming and population swarming motility, bacteria build long, complex rotary 'nanomachines', called flagella, on their cell surface. Their sequential assembly requires export of thousands of structural subunits across the cell membrane and this is achieved by dedicated type III export machinery located at each flagellum base. Subunits then transit up to 20 cell lengths through a narrow channel in the external flagellum to reach the assembly site at the tip of the nascent structure.

Flagellum assembly is biphasic, with subunits for the long cell-surface filament only being exported once the cell-proximal rod and hook structures are complete. The order of subunit export is imposed by the FlhB¹ membrane export gate and by the intermittently exported molecular ruler, FliK², which monitors the length of the growing rod/hook and transmits this information back into the cell to the cytoplasmic domain of FlhB. This export machinery 'specificity switch' is a critical, yet unexplained, event in the sequential assembly of flagella.

Here, we will describe molecular, biochemical and biophysical data that indicate how FliK triggers the export specificity switch by promoting a radical conformational change in the FlhB export gate.

References

1. G.M. Fraser, T. Hirano, H.U. Ferris, L. Devgan, M. Kihara & R.M. Macnab, *Mol Microbiol*, 2003, **48**, 1043.
2. M. Erhardt, H.M. Singer, D.H. Wee, J.P. Keener & K.T. Hughes. *EMBO J*, 2011, **30**, 2948

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P130

Structural and functional analysis of the *Mycobacterium tuberculosis* transcriptional regulator FasR

Julia Lara¹, Lautaro Diacovich¹, Nicole Larrieux², Alejandro Busciazzo², Gabriela Gago¹, Hugo Gramajo¹

¹Institute of Molecular and Cell Biology of Rosario. IBR-CONICET-UNR., Rosario, Argentina. ²Institute Pasteur of Montevideo, Montevideo, Uruguay

Abstract

Mycobacteria have two fatty acid synthases (FAS I and FAS II) which work in concert to synthesize fatty acids and mycolic acids. We identified a transcriptional regulator essential for mycobacterial viability: FasR, which binds the *fas* promoter and controls the *de novo* fatty acid biosynthesis. The main purpose of our studies was to understand at the molecular level how mycobacteria exert a fine control over the biosynthesis of their membrane. The characterization of long chain acyl-CoAs that modulates the affinity of FasR for its target DNA was studied using electrophoretic mobility shift assay, SPR and *in vitro* transcription.

Crystallization screenings using a 40 aa N-terminal mutant of FasR we identified two different sets of conditions producing crystals of FasR alone and in complex with C20-CoA, both of which diffracted X rays at better than 1.8 Å resolution.

Electrophoretic mobility shift experiments using FasR mutants generated to prevent the binding of the ligand into the effector domain, designed from the structural analysis of FasR: C20-CoA complex, confirm the functionality and key role of the ligand in FasR-DNA interaction. In summary, we show that long-chain acyl-CoAs are key effector molecules that coordinate the expression of FAS system, by binding to FasR.

The structural and functional characterization of this novel transcriptional regulator will allow us to gain new insights into the transcriptional regulation to the fatty biosynthesis pathways in *M. tuberculosis*, and will help as determine if this protein could represent an attractive target for the development of new antituberculosis drugs.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P131

Transcriptional regulation by a complex ECF σ factor - anti- σ factor system in *Bacillus subtilis*

Maria Davis, Shawn MacLellan

University of New Brunswick, Fredericton, Canada

Abstract

Extracytoplasmic function (ECF) σ factor – anti- σ factor systems constitute a mechanism by which bacteria sense and respond to changes in the external environment. These systems respond by regulating transcription to ensure survival of cells in variable environments. This work investigated the structure and function of RsiO, an integral membrane protein and ECF anti- σ factor of the *Bacillus subtilis* two-subunit σ factor SigO-RsoA. The SigO-RsoA-RsiO regulatory system allows the cell to sense and respond to certain cues including acidic pH growth conditions. Protein-protein interaction assays demonstrated that RsiO negatively regulates the activity of SigO-RsoA by sequestering SigO from its co- σ factor RsoA and from RNA polymerase core enzyme. Characterization of RsiO function using systematic mutagenesis identified two highly conserved charged residues in RsiO are involved in SigO sequestration. We have also experimentally mapped the topology of RsiO as a N_{in} C_{out} polytopic integral membrane protein with three transmembrane segments - an usually complex topology for a membrane embedded anti- σ factor. Ongoing experiments are testing whether RsiO itself is the sensor of hydronium ion concentrations that result in the induction of the two-subunit σ factor SigO-RsoA.

132

Engineering *Cupriavidus Necator* for the Production of 3-Hydroxypropionic Acid

Jessica Locker, Christian Arenas, Katalin Kovacs, Nigel Minton, Klaus Winzer

University of Nottingham, Nottingham, United Kingdom

Abstract

3-hydroxypropionic acid (3-HP) is regarded as a significant precursor for renewable bioplastics and industrial chemicals. It can be reduced, esterified, dehydrated and oxidized to useful products for the production of adhesives and textiles. Potential commercial routes for chemical synthesis are impeded by unwanted by-products, high start-up costs or unacceptable environmental consequences.

Several enzymatic pathways for biological synthesis of renewable 3-HP have been demonstrated in a range of microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Saccharomyces cerevisiae*. However, all of these require substrates that compete with existing food supplies.

Cupriavidus necator is a facultative chemolithoautotrophic proteobacterium that can use H₂ and CO₂ as sole sources of carbon and energy. It has evolved the ability to survive in both aerobic and anaerobic conditions, due to its natural environment of soil and freshwater where oxygen levels can be variable. Its metabolic versatility and ability to grow to high cell densities have made it a prime chassis for metabolic engineering.

By inserting the 2-step malonyl-CoA pathway into *C. necator*, biodegradable 3-HP could be produced from waste gas rather than non-renewable resources. The first reaction of this pathway is the conversion of the central metabolite acetyl-CoA to malonyl-CoA, which is catalysed by acetyl-CoA carboxylase. The second step is the reduction of malonyl-CoA to 3-hydroxypropanoic acid, catalysed by a malonyl-CoA reductase. Here we report our recent progress in the implementation of this pathway.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P133

Staphylococcus Aureus Biofilm Specific Affimers as a Potential Diagnostic Tool for Infectious Endocarditis

Fayez Alsulaimani¹, Lia De Faveri¹, Thomas Taylor¹, Christian Teide¹, John Colyer¹, Jonathan Sandoe Sandoe^{1,2}, Paul Millner¹, Darren Tomlinson¹, Michael McPherson¹, Kenneth McDowall¹

¹University of leeds, Leeds, United Kingdom. ²Leeds teaching hospital, leeds, United Kingdom

Abstract

Staphylococcus aureus is an important cause of infective endocarditis – infection of the lining of the heart or devices inside the heart such as prosthetic valves/pacemakers. These infections can be debilitating, even life-threatening, and are often difficult to diagnose clinically. *S. aureus* grows on the surfaces of medical devices and natural tissues as a biofilm. Bacteria in biofilms can be difficult to eradicate as they are less susceptible to antibiotics. Thus, biofilms associated with medical devices or chronic infections often require surgical removal or debridement, respectively. Thus, it would be clinically advantageous to be able to not only detect the presence of bacteria when a patient presents with clear signs of infection, but also to determine the source by a non-invasive method. Affimers were selected against *S. aureus* biofilm, protein A (PA) and clumping factor A (ClfA) using phage display. Two out of eleven anti-biofilm Affimers screened against three *S. aureus* strains biofilms (SH1000, USA300 and UAMS-1) recognized a target present on all three strains. Affimers binding to protein A and CLFA were confirmed and binding affinity was measured by surface plasmon resonance (SPR). Affimer binding to PA and ClfA on *S. aureus* biofilms grown on the surface of medical devices such as catheters will be investigated. The ongoing characterisation of these Affimers with regard to the breadth of their specificity and their future engineering to allow sites of *S.aureus* infection in patients to be detected will be described.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P134

Characterization of the host-specificity of *Staphylococcus pseudintermedius* surface protein L (SpsL) and its role in the pathogenesis of skin disease

Amy Richards¹, Marie O'Shea¹, Brandon Garcia², Timothy J Foster³, Joan Geoghegan³, Magnus Hook⁴, Andreas Lengeling⁵, J. Ross Fitzgerald¹

¹Roslin Institute, Edinburgh, United Kingdom. ²Kansas State University, Manhattan, USA. ³Trinity College Dublin, Dublin, Ireland. ⁴Texas A&M, Houston, USA. ⁵Max-Planck, Munich, Germany

Abstract

Staphylococcus pseudintermedius is a major canine skin pathogen and a zoonotic pathogen of humans. Due to the high levels of multidrug resistance, treating recurrent canine pyoderma is becoming increasingly difficult. SpsL is a cell wall-associated protein of *S. pseudintermedius* with affinity for both fibrinogen and fibronectin. Notably, SpsL has a higher affinity for canine versus human fibrinogen. The construction of gene deletion mutants of *S. pseudintermedius* lacking fibrinogen-binding activity confirmed this host-specific binding interaction of SpsL. ELISA and surface plasmon resonance analyses of recombinant truncated derivatives of SpsL indicate that the predicted ligand-binding N2N3 subdomains are not sufficient for high-affinity host-specific interactions. However, the SpsL N2N3 subdomains presented on the bacterial cell surface are sufficient for host-specific fibrinogen-binding, illustrating an inconsistency between cell wall-associated and recombinant SpsL truncates. SpsL specifically adheres to the alpha chain of fibrinogen and site-directed mutagenesis is being used to identify the host-specific fibrinogen-binding site of SpsL. Mice subcutaneously infected with the *spsL* gene deletion mutant displayed increased length of surface lesions in comparison to the isogenic wild type and an *spsL* repaired strain of *S. pseudintermedius*. Upon histopathology analysis it was identified that mice infected with the *spsL* deletion mutant were more likely to develop a regionally extensive inflammation, akin to cellulitis, rather than a localised focal abscess. This demonstrates that SpsL is required for the development of skin abscesses in this infection model. Overall these studies are providing new insights into the role of cell wall-associated proteins in staphylococcal pathogenesis and host-specificity.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P135

Extensive polyP formation in *Methanosarcina mazei* in response to overplus stimulus.

Fabiana Paula^{1,2}, Jason Chin³, Anna Schnurer², Bettina Muller², Panagiotis Manesiotis⁴, Jasmine Connolly¹, Florence Abram¹, John McGrath³, Vincent O'Flaherty¹

¹Department of Microbiology, National University of Ireland Galway, Galway, Ireland. ²Department of Molecular Sciences, Biocenter, Swedish University of Agricultural Sciences, Uppsala, Sweden. ³School of Biological Sciences and the Institute for Global Food Security, The Queen's University of Belfast, Belfast, United Kingdom. ⁴School of Chemistry and Chemical Engineering, The Queen's University of Belfast, Belfast, United Kingdom

Abstract

Polyphosphate (polyP) metabolism is poorly explored in Archaea. Previous scarce reports indicated the presence of polyP in methanogens, but no information regarding the related functions, environmental triggers, and associated gene regulation are available. We investigated whether polyP accumulation could be triggered in methanogenic Archaea by applying extreme changes in P availability, also known as P-starvation/overplus method. For that end, *Methanosarcina mazei* cultures underwent a period of phosphate depletion, which was followed by the addition of phosphate in excess. Under P depletion conditions, the genes for alkaline phosphatase and for the high affinity P transport complex (PstSCAB-PhoU) were overexpressed, indicating that the cells were starving in phosphate. Upon P addition, we observed an up-regulation of the gene coding for the enzyme polyphosphate kinase 1 (*ppk1*), which is responsible for polyP chain elongation. Simultaneously, a 200% increase in P cellular content, and the production of polyP-like intracellular granules were observed. Our results demonstrate that polyP production can be induced in *M. mazei* by extreme changes in P availability. This function may provide advantage under unfavourable environmental conditions, and it might represent a significant process for P cycling in anaerobic environments. Furthermore, these findings may have important biotechnological implications for phosphate recovery in anaerobic systems.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P136

Perturbation of PQS biosynthesis in *P. aeruginosa* results in up-regulation of genes involved in anthranilate degradation

Frances Smith

University of Nottingham, Nottingham, United Kingdom

Abstract

Anthranilate sits at the metabolic junction between tryptophan biosynthesis, the tri-carboxylic acid (TCA) cycle, and biosynthesis of the *Pseudomonas* quinolone signal (PQS) in *P.aeruginosa*. PQS-dependent quorum sensing is a chemical messaging system employed by *P.aeruginosa* to influence biofilm formation and virulence. Anthranilate is a substrate for PqsA, the protein product of the first gene in the PQS biosynthetic operon. In addition, anthranilate is an intermediate in the degradation pathway that breaks down aromatic compounds and feeds them into the TCA cycle. The anthranilate degradation pathway begins with two gene operons: *antABC* and *catBCA*. Their expression is controlled by the regulators AntA and CatR respectively. Since mutation of *pqsA* is known to impact on extracellular anthranilate accumulate, we investigated the impact of PQS signalling on the expression of the *ant* and *cat* genes.

MiniCTX-*lux* transcriptional reporters were constructed to use bioluminescence as a measure of expression. The promoter regions of the genes *antR*, *antA*, *catB* and *catR* were fused upstream of the *luxCDABE* operon on a miniCTX-*lux* vector and inserted into the bacterial chromosome. These promoter fusions were used to measure expression of genes in both a wild-type PAO1 strain, and a *pqsA* mutant strain.

The results show that deletion of *pqsA* significantly increases expression from the *antA*, *antR*, and *catB* promoters. When grown in the presence of anthranilic acid, the differences in expression were augmented. Further work is underway to determine the impact of the observed changes in gene expression on intra- and extra- cellular anthranilate accumulation.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P137

Anti-inflammatory properties of plant-derived polysaccharides for prevention of peri-implantitis

Katarzyna Gurzawska¹, Mieszkowska Anna², Justyna Folkert², Bernard Burke³

¹University of Birmingham, Birmingham, United Kingdom. ²Environmental Biotechnology Department, Faculty of Energy and Environmental Engineering, Silesian University of Technology, Gliwice, Poland.

³University of Coventry Faculty of Health & Life Sciences, Coventry, United Kingdom

Abstract

Titanium implants have been used with success for bone anchoring of dental implants. However, when implant surfaces are exposed to the oral environment, the progression of peri-implantitis triggered by specific oral bacteria has been reported. A new strategy to improve implant biocompatibility and prevent peri-implantitis is to develop surface nanocoatings with pectins. These plant-derived polysaccharides are promising candidates for surface nanocoatings due to their osteogenic and anti-inflammatory properties. Therefore, the aim of the study was to evaluate the *in vitro* effect of nanocoating with plant-derived Rhamnogalacturonan-I (RG-I) on pro- and anti-inflammatory responses of human monocyte-derived macrophages (HMDMs) induced by *Escherichia coli* LPS and *Porphyromonas gingivalis* bacteria. *E. coli* and *P. gingivalis* are common species isolated from inflamed peri-implant tissues. In the present study, two different types of surface materials, tissue culture polystyrene (TCPS) plates and titanium (Ti) discs, coated with potato unmodified RG-I (PU) and potato dearabinanated RG-I (PA), have been examined. Uncoated TCPS and Ti were used as controls. The inflammatory responses of HMDMs after *E. coli* LPS/*P. gingivalis* stimulation were investigated through gene expression. The results showed that PU and PA decreased expression of the proinflammatory genes tumour necrosis factor-alpha (*TNFA*), interleukin-1 beta (*IL1B*) and interleukin-8 (*IL8*) in activated HMDMs cultured on TCPS/Ti surfaces. In contrast, the effects on anti-inflammatory interleukin-10 (*IL10*) gene expression were not significant. The results indicate that RG-Is should be considered as a candidate for organic nanocoatings of titanium implant surfaces in order to limit host proinflammatory responses.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P138

A conserved pleiotropic regulator of virulence factors, antibiotic production and quorum sensing in different enterobacteria

Rita Monson¹, Neil Williamson¹, Josh Ramsay^{1,2}, George Salmond¹

¹University of Cambridge, Cambridge, United Kingdom. ²Curtin University, Perth, Australia

Abstract

During infections, bacteria can produce secondary metabolites important for virulence. Regulating production of these compounds can be important as precocious expression of some virulence determinants can alert a host organism to pathogen presence and allow the host immune response to combat the bacteria. *Serratia* sp. ATCC39006 (S39006) is a Gram-negative enterobacterium that is pathogenic to both plants (e.g. *Solanum*) and animals (e.g. *C. elegans*). S39006 produces the red pigment 2-methyl-3-pentyl-6-methoxyprodigiosin (prodigiosin), the β -lactam antibiotic 1-carbapen-2-em-3-carboxylic acid (a carbapenem) and a suite of plant cell wall degrading enzymes (PCWDEs). The regulatory hierarchy controlling production of these secondary metabolites includes an acyl-homoserine lactone quorum sensing (QS) system, SmaIR, and other regulators such as the DNA-binding protein, PigP. Mutation of *pigP* causes decreased prodigiosin and carbapenem production, decreased virulence in *C. elegans* and diminished production of PCWDEs, though this regulation is independent of the SmaIR QS system. Using bioinformatics and the defined PigP₃₉₀₀₆ binding site, we identified PigP orthologues in other bacteria and investigated phenotypes predicted to be regulated by PigP. In the plant pathogen *Dickeya solani*, PigP affected production of PCWDEs through modulation of the QS system. PigP also modulated swimming motility by impacting transcription of the flagellum biosynthetic operon. In the opportunistic human pathogen, *Serratia marcescens* Db11, an organism without an acyl-homoserine lactone QS system, PigP modulated production of protease virulence determinants. These results demonstrate that PigP, is a pleiotropic regulator of virulence determinants across three pathogenic bacterial species.

Forum: Microbial Physiology, Metabolism and Molecular Biology

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P139

Investigating the biological substrates of ApeE, the *Salmonella* GDSL lipase autotransporter

Jessica Rooke^{1,2}, Jack Bryant¹, Richard Strugnell², Daniel Slade³, Ian Henderson¹

¹Univeristy of Birmingham, Birmingham, United Kingdom. ²University of Melbourne, Melbourne, Australia. ³Virginia Tech, Virginia, USA

Abstract

ApeE is an autotransporter protein that is conserved amongst all currently sequenced *Salmonella*. Previous work showed that ApeE is a GDSL lipase autotransporter protein that is able to cleave naphthyl esters and expression of ApeE is induced upon phosphate limiting conditions. Autotransporter proteins, also known as type 5 secretion, have 3 functional domains; an N-terminal sec dependent signal sequence, secreted effector (passenger) domain and a β -barrel translocation domain. Evidence suggests that many autotransporters are important outer membrane proteins during Gram-negative pathogenesis. A recent study linked the phospholipase activity of an ApeE homolog to a potential role in virulence. As ApeE is upregulated in phosphate limited conditions, we wanted to investigate whether ApeE has phospholipase activity because to date, a biological substrate for ApeE has not been identified. Here, we use *in vitro* recombinant protein system to determine biologically relevant substrates of ApeE and the Michaelis–Menten kinetics for these substrates. We show that ApeE is required for the growth of *Salmonella enterica* serovar Typhimurium in minimal growth medium with phospholipid as the sole carbon source, indicating that ApeE could be important for the growth of *Salmonella* in nutrient limiting conditions.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P140

A Novel Angle on the Microbiome – The Gut Phageome in Cystic Fibrosis; Structure, Diversity, and Antibiotic Resistance Gene Carriage.

Enrique González-Tortuero¹, Justine K Rudkin^{1,2}, Gwendoline Deslyper^{1,3}, Norbert Ács¹, Adrian R Fernandes¹, Fiona Fouhy⁴, Barry J. Plant^{1,5,6,7}, Catherine Stanton^{1,4}, R Paul Ross^{1,8}, Colin Hill^{1,2}

¹APC Microbiome Institute, University College Cork, Cork, Ireland. ²School of Microbiology, University College Cork, Cork, Ireland. ³School of Natural Sciences, Trinity College Dublin, Dublin, Ireland. ⁴Teagasc Food Research Centre, Moorepark, Ireland. ⁵HRB Clinical Research Facility, University College Cork, Cork, Ireland. ⁶Cork Cystic Fibrosis Centre, University College Cork-Cork University Hospital, Cork, Ireland. ⁷Department of Medicine, University College Cork, Cork, Ireland. ⁸College of Science, Engineering and Food Science (SEFS), University College Cork, Cork, Ireland

Abstract

Phages are relevant to ecosystems shaping bacterial communities, driving microbial evolution and diversification, and transferring genetic information including antibiotic resistance genes (ARGs). Surprisingly, little is known of the phage composition of the human gut microbiome. The aim of this study is to describe the gut “phageome” of cystic fibrosis (CF) and non-CF faecal samples, to determine whether phage populations are distinct between these different cohorts. Phage DNA was isolated from faecal samples of 38 CF and 38 non-CF individuals, and RT-PCR was performed to capture RNA viruses. DNA samples were sequenced using Illumina HiSeq platform. Reads were assembled using IDBA-UD, and filtered using VirSorter and VirFinder to retrieve all putative viral sequences. Bowtie2 was used to align the reads against these contigs. To classify all putative viruses and detect ARGs, BLASTn was launched against an in-house viral database and the CARD database, respectively. This study is the first to look at the gut phageome of CF patients. Comparing the phageome of CF and non-CF samples we have determined that gut phage populations are distinct between these cohorts. CF samples were defined by crAss-like phages, whilst unknown viruses from the Earth Virome Project were defining in non-CF samples. Firmicutes prophage, and unclassified viruses (Minot et al, 2013 PNAS), were more abundant in non-CF samples. For all alpha indexes, viral diversity was higher in non-CF samples. Finally, ARGs were more abundant in CF samples.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P141

Mycoviruses enhance the virulence of entomopathogenic fungi

Ioly Kotta-Loizou¹, Charalampos Filippou^{1,2}, John Daudu², Unnati Shah², Robert Coutts²

¹Imperial College London, London, United Kingdom. ²University of Hertfordshire, Hatfield, United Kingdom

Abstract

The current world population is increasing exponentially and so are our nutritional requirements. Chemical insecticides are used to protect the crops from damage and increase agricultural output; however, they are considered one of the top six global threats greatly impacting the biodiversity of the environment and putting human health at risk. An environmentally friendly alternative to traditional insecticides are biocontrol agents, including commercially available entomopathogenic fungi whose growth and pathogenicity may be enhanced by mycoviruses. The aim of the project is to investigate mycovirus-induced hypervirulence in entomopathogenic fungi. To this end panels of *Beauveria bassiana*, *Metarizhium anisopliae*, *Lecanicillium muscarium* and other entomopathogenic fungi were screened for the presence of double-stranded (ds) RNA elements, which were subsequently sequenced and classified. Among mycoviruses discovered, members of the novel virus family Polymycoviridae naturally confer mild hypervirulence to their hosts, are not conventionally encapsidated and are infectious as naked dsRNA facilitating genetic manipulations. Construction of virus-free and virus-infected isogenic lines followed by assessment of fungal growth revealed that a virus-infected *B. bassiana* isolate grows significantly faster than its virus-free counterpart and the commercially available biocontrol agents ATCC 74040 and GHA. Host-virus protein-protein interactions *via* Eukaryotic Linear Motifs and Gene Ontology analysis suggest that the observed hypervirulence may be attributed to virus proteins interfering with the host's cell cycle. Further investigations are in progress since knowledge of the mechanisms controlling mycovirus-induced hypervirulence would facilitate genetic engineering of mycoviruses to increase hypervirulence. In conclusion, this study discusses the potential of mycoviruses as enhancers of biocontrol agents.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P142

Novel genomics-led approaches to characterise viral diseases in Atlantic salmon

Michael Gallagher¹, Iveta Matejusova², Daniel Macqueen¹

¹University Of Aberdeen, Aberdeen, United Kingdom. ²Marine Science Scotland, Aberdeen, United Kingdom

Abstract

Global farmed production of salmonid fishes is worth >£8 billion annually, accounting for ~15% of total traded farmed fish. However, a major bottleneck limiting growth of this industry is loss caused by infectious viral diseases, which can have devastating economic impacts, with few effective therapeutics or preventative vaccines available. Genome-wide surveillance is currently lacking for salmonid viruses, despite the fact that such approaches have shown great promise for understanding pathogen dynamics and evolution, which can be applied to monitor and control disease outbreaks. This presentation reports my investigations into the usefulness of current-generation sequencing technologies to achieve accurate and economically-feasible whole genome sequencing of two distinct viruses: salmonid alphavirus and infectious salmonid anaemia virus. Long-range PCR and targeted sequence capture have been performed before sequencing using the MinION and Illumina platforms, respectively. These approaches have enabled us to recover full-length genomes for each virus, including ultra-deep coverage via the sequence capture approach. My talk will provide insights into our current downstream analyses of these genome-wide datasets, which aim to better understand viral evolution, phylogeography and population dynamics. We hope that application of such data within the aquaculture industry will ultimately help control the spread of devastating diseases and contribute to economic and food security.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P143

High degree of genetic variability in respiratory syncytial virus strains circulating in Ireland

Emer O'Byrne, [Allison Waters](#), Cillian De Gascun

National Virus Reference Laboratory, Dublin 4, Ireland

Abstract

BACKGROUND: Human respiratory syncytial virus (RSV) is the leading cause of severe lower respiratory tract infections in infants, young children, and, increasingly, the elderly. The impending introduction of RSV surveillance programmes by WHO and ECDC, coupled with the number of RSV vaccines and monoclonal-antibody prophylaxis treatments in various phases of clinical trials, means a deeper understanding of the circulating RSV strains is imperative. At present, the seasonal genetic diversity of RSV remains unmonitored by the majority of European countries.

METHODS: A proportion of RSV positive specimens, received for diagnostic testing from symptomatic patients by the National Virus Reference Laboratory (NVRL), Ireland, was selected for analyses (n=347). Real-time RT-PCR and sequencing analysis were used to determine the RSV subtypes and genotypes circulating between 2015 and 2017, respectively. Genotyping was based on partial coding sequences of the glycoprotein (G) gene.

RESULTS: RSV A (n=199) and RSV B (n=142) were co-circulating during each season. In addition, a small number of patients co-infected with RSV A and RSV B was identified (n=6/347). Phylogenetic analysis detected a high degree of variation between all circulating RSV strains, both at the nucleotide and amino acid level. As expected, the ON1 insertion was detected in all RSV A strains.

CONCLUSIONS: The present study provides baseline genotypic surveillance data of RSV in Ireland for the first-time, thereby facilitating the introduction of an RSV genotypic surveillance programme at the NVRL. RSV typing data is essential to inform future RSV surveillance, both pre- and post- vaccination introduction.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P144

Molecular characterisation of epizootic haemorrhagic disease virus serotype-6 in Trinidad, West Indies

Honorata Ropiak¹, Paulina Rajko-Nenow¹, John Flannery¹, Tamiko Brown-Joseph², Carrie Batten¹, Chris Oura²

¹The Pirbright Institute, Pirbright, United Kingdom. ²University of the West Indies, St. Augustine, Trinidad and Tobago

Abstract

Background: Epizootic haemorrhagic disease (EHD) is an infectious, non-contagious viral disease transmitted by *Culicoides* midges that affects deer and cattle. Seven EHD virus (EHDV) serotypes (based on VP2) have been identified and in recent years EHDV has spread to previously virus-free areas. This is likely due to climate change and the consequent introduction of competent EHDV vectors into new areas.

Methods: Naïve cattle (60) were imported from the USA to Trinidad in 2013, from which monthly blood samples were tested using real-time RT-PCR and ELISA over a 6 month period. Real-time RT-PCR positive samples were subjected to virus propagation in KC (*C. sonorensis*) cells. EHDV RNA was extracted using TRIZOL from cell pellets. Libraries were prepared using Nextera XT DNA kit and run on a Miseq instrument.

Results: After 6 months, all cattle seroconverted to EHDV. VP2 phylogenetic analysis of a single EHDV isolate identified EHDV serotype 6 belonging to the eastern topotype. The VP2 from the Trinidad EHDV strain was closely related to EHDV-6e strains from Guadeloupe, Martinique, the USA and Australia. Full genome analysis of the Trinidad strain identified a reassortment event with segments 1-7, 9 & 10 originating from EHDV-6 and segment-8 originating from a New Jersey, USA strain of EHDV-1.

Conclusion: A unique reassorted EHDV-6 virus is circulating asymptotically in domestic cattle in Trinidad that is different to other EHDV-6 viruses previously identified in the region. Reassortment events like this may increase the risk of more virulent strains of EHDV emerging.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P145

NCPV500: An Electronic Resource for Mining Viral Genomes

Karen Buttigieg¹, Sarah Alexander¹, Lidia Liberty¹, Joseph Taylor¹, Nicholas Grayson², Edward Burnett¹, Julian Parkhill², Julie E. Russell¹

¹Public Health England, Porton Down, United Kingdom. ²Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Abstract

Next Generation Sequencing is an emerging technology that is becoming more widely used in pathogen microbiology. Applications have become more diverse, including real-time epidemiology, clinical diagnosis, microbe discovery, taxonomic classification, quality control of vaccines, tracking adaptation and evolution, and understanding the roles of viral genes in infection. The National Collection of Pathogenic Viruses (NCPV) curates and supplies authenticated human pathogenic viruses for the research community.

Annotating the sequences of individual genomes helps to catalogue the genes encoded in a particular strain and is a vital step for in-depth characterisation studies. Sequencing of multiple isolates, strains or species enables understanding of the factors responsible for varying virulence using comparative genomics.

Viral DNA was extracted from virus strains within NCPV, using a Maxwell 16 automated system. Whole genome sequencing was performed on the Illumina platform by the Wellcome Trust Sanger Institute. Assembled and annotated genome sequences will be freely publicly accessible via a comprehensive web-based Biological Resource Information Centre (BRIC).

Nucleic acids from 196 ACDP Hazard Group 2 viruses have been extracted and 104 sequenced. Future work will focus on sequencing a further 304 viruses, including Hazard Group 3 organisms.

Online availability of the viral genomes, coupled with the biological availability of the virus strains from NCPV, will enable investigation of the interactions between genotype and phenotype of known, emerging and novel viral pathogens.

Researchers are invited to deposit newly isolated virus strains into NCPV free of charge, and have the viral genomes sequenced and added to the BRIC.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P146

A Comparative Investigation of Methods for Sequencing the Complete Fusion Gene of Avian Avulavirus type 1 (AAvV-1): Improving Epidemiological Understanding of Newcastle Disease

Skinner Paul, Mayers Jo, Mansfield Karen, Sutton David, Brookes Sharon, Brown Ian

Animal and Plant Health Agency, Weybridge, United Kingdom

Abstract

Avian avulavirus serotype-1 (AAvV-1) is a widespread paramyxovirus capable of infecting wild and domesticated avian species. Its virulent form, termed Newcastle disease virus (NDV), has a significant impact on global poultry production. The pathogenicity of the virus is determined through sequencing the cleavage site contained within the fusion protein gene (F-gene), where specific amino acid motifs define whether the virus is virulent or avirulent. Current sequencing methods typically use a partial F-gene fragment of 374bp, which incorporates the cleavage site.

An investigation was conducted to compare the full F-gene against the partial sequence, to determine if this could improve our understanding of AAvV-1 genetic diversity and origins of potential disease incursions.

Three protocols for sequencing the full F-gene were evaluated. A panel of viral isolates obtained from the International Reference Laboratory at APHA-Weybridge was sequenced using two Sanger sequencing protocols, alongside whole genome sequencing (WGS). MAFFT and MEGA software were used to create and compare maximum likelihood trees of partial versus full F-gene sequences. A cost-benefit analysis was undertaken to determine the practicality and cost effectiveness for each method, to help inform APHA's approach to AAvV-1 sequencing processes and phylogenetic analyses.

Whilst the distribution of the lineages/genotypes are similar between the full and partial trees, the full F-gene tree deliver stronger bootstrap values. This allows for greater confidence in the relational distances, yielding a more accurate picture of disease epidemiology, but at greater financial cost, with no significant effect on notifiable avian disease diagnosis.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P147

Collaborative Research and Resource: The European Virus Archive goes Global (EVAg).

Mehnaz Qureshi, Paulina Rajko Nenow, Honorata Ropiak, Amanda Corla, Carrie Batten

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Background:

EVAg is one of the largest international collections of viruses. It is an infrastructure project financed by the European Commission, working as a consortium dedicated to the conservation, production and distribution of viruses/other products from the high calibre international laboratories.

Methods:

The idea of EVAg is to meet the needs of scientists worldwide by generating a carefully authenticated virus collection which is readily available to all laboratories that meet approved ethical, safety and security standards. The ultimate goal is to provide global access to standardised diagnostic, therapeutic and research tools that will significantly enhance our efforts to control diseases and improve health.

Results:

To date, it is the largest active virtual virus collection worldwide with more than 2000 products distributed globally, with features like metadata related to the viruses including full genome sequence, certificate of origin, and high standard quality certification. In addition to providing complete databases related to virus-derived products for the development of molecular & serological diagnostic tests, EVAg also offers an access to high containment facilities and to high risk pathogen laboratories in addition to teaching/training courses. EVAg have been acknowledged in more than 100 peer-reviewed publications.

Conclusion:

We present this as an example of unparalleled integrative scientific collaboration, which will initiate more ventures in the future to make a real difference at the global level in terms of animal and human health.

Virology Workshop: The Scope, Causes and Consequences of Viral Diversity

Zone B

Presentations: Thursday Evening and Friday Lunchtime

P148

Distinct features of intrinsic disorder in viral proteins inferred from a high-throughput in silico analysis

Michal Barski

Imperial College London, London, United Kingdom

Abstract

Intrinsic disorder is an important structural and functional feature of proteins from all kingdoms of life. In recent years, the seminal role of intrinsic disorder in the multitude of functions and interactions played by viral proteins has been gradually brought to light. Although the distribution of intrinsic disorder in viruses has been studied in the context of whole viral families and genomes, there is no comprehensive, comparative data gathered at the individual protein level. The latter is particularly complex in the case of viruses, which often express multiple proteins from a single open reading frame in the form of polyproteins.

Computation of intrinsic disorder in all available viral protein sequences which have been proteolytically-processed confirms previous observations at the genomic scale, but reveals novel trends within viral families and between viral protein classes. Most notably, *herpesviridae* emerge as the family encoding the most highly-disordered proteins. I also show a strong function-specific distribution of intrinsic disorder among viral proteins and highlight the importance of disordered N- and/or C-terminal regions in certain protein classes, most notably in nucleoproteins. Unexpectedly, non-structural proteins, commonly considered as very flexible, showed an average medium to low degree of intrinsic disorder. Overall, the results of this study shine a new light on the functions and patterns of structural flexibility encoded by viruses.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P149

Pathogenic Bacteria Profile and Antimicrobial Susceptibility Patterns of Ear Infection at Bahir Dar Regional Health Research Laboratory Center Ethiopia

Derese Chekol

Amhara Public Health Institute, Bahir Dar, Ethiopia, Ethiopia

Abstract

Abstract

Ear infection linked with frequent antibiotic prescription, hearing impairment, severe disability and death is a public health threat in developing countries. However, there is scarcity of documented data in the study area. Therefore, this study aimed at determining bacterial etiologic agents and their antimicrobial susceptibility patterns among patients of all age groups referred to Bahir Dar Regional Health Research Laboratory Center. Retrospective data recorded on culture and antimicrobial susceptibility profile were retrieved for analysis. Pus swabs from discharging ears collected and processed for aerobic bacteria culture and susceptibility testing. Of the total 368 pus swab samples processed,

296 (80.4 %) were culture positive. Of which, 289 (97.6 %) were bacteria and 7 (2.4 %) were yeast cells. The proportion of ear infection was higher in males (92.7 %) than females (65 %) ($P = 0.014$). The frequency of ear infection below

21 years of age was 65.2 %. The predominant isolate was *Pseudomonas aeruginosa* (29.7 %) followed by *Staphylococcus*

aureus (26.3 %) and *Proteus* spp. (21.9 %). High level of antimicrobial resistance rates were observed for amoxicillin/clavulanic acid, ampicillin and penicillin whereas ciprofloxacin, ceftriaxone, chloramphenicol, cotrimoxazole, gentamicin and amikacin were found effective against the isolated bacteria. Aerobic bacterial otitis media linked with high levels of resistance against amoxicillin/clavulanic acid and ampicillin is major health problem in the study area. Moreover, considerable level of oxacillin resistant *S. aureus* suggests the diffusion of methicillin resistant *S. aureus* in the community.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P150

Antibiogram of Gram-negative human pathogens isolated in India.

Ritesh Thakare, Sidharth Chopra

CSIR-CDRI, Lucknow, India

Abstract

WHO has declared antimicrobial resistance (AMR) as an urgent global health emergency and has categorized these deadly bugs in critical, high and medium category in their “priority pathogen” list. While *Acinetobacter baumannii*, carbapenem-resistant *Enterobacteriaceae*, fluoroquinolone-resistant *Salmonellae* have been included in the critical, critical and high category respectively. In this study, we have investigated the present status of resistance patterns being exhibited by isolates of *A. baumannii*, *Escherichia coli* and *Salmonella enterica* from India.

We isolated *A. baumannii* (n=52), *E. coli* (n=20) and *S. enterica* (n=100) from the clinical specimen. All the isolates were confirmed by various biochemical, microbiological and molecular techniques. The MIC of the isolates against an antibiotic panel was determined by CLSI broth microdilution and confirmed at the molecular level. Multiple drug resistance (MDR) has been observed in 94.2% *A. baumannii* isolates with 86.5% isolates found to be resistant to 5 or more different class of antibiotics. We have found significant correlation between increased MIC and presence of antibiotic resistance marker gene in *A. baumannii* isolates. In *S. enterica*, 100% isolates were resistant to nalidixic acid, Decreased ciprofloxacin susceptibility (DCS) was seen in 85% and 10% were completely resistant to ciprofloxacin. Presence of mutations in the conserved regions is significantly correlated with the DCS. Results of the present study revealed the increasing resistance to all the antibiotics including recently approved tigecycline is a serious concern for healthcare. Taken together, the antibiogram of bacterial isolates clearly reflects the misuse of antibiotics.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P151

Confirmatory Assays for Detection of *Neisseria Gonorrhoeae* using *porA* pseudogene Real-Time PCR Base Methods

EDOBOR PETER KENNETH IMARENEZOR

FEDERAL UNIVERSITY, WUKARI, Nigeria

Abstract

BACKGROUND: Since the advent of molecular techniques, diagnosis of *Neisseria gonorrhoeae* has been ruined by false positive results due to cross-reaction of nucleic acid amplification test (NAAT).

OBJECTIVES: This research aims to compare the sensitivity and specificity of *N. gonorrhoeae* real-time assays targeting the *porA* pseudogene. *METHODS:* Gonococci (156) and non-gonococci (30) culture specimens were used. Optimization of the *PorA* pseudogene real-time PCR was done by varying

concentration of magnesium chloride between 19.08 (4.31) and 23.27 (17.57), 17.18 (1.15) and 22.01 (16.43), 21.71 (2.20), 27.33 (15.27) and forward and reverse primers concentration as 50mM, 300mM and 900mM for both. *RESULTS:* This show high specificity assays for gonococci specimens, whilst non-gonococci specimens were negative. This shows that *PorA* pseudogene real time PCR is a suitable assay

for the confirmation of *N. gonorrhoeae*, particularly where biochemical and immunology tests failed. The potential of *PorA* pseudogene real-time PCR to detect *N. gonorrhoeae* specific DNA directly from clinical samples was evaluated using the addition of a primer and probe set which acted as an internal control, it was determined that the internal control did not compromise the sensitivity of the *PorA*

pseudogene real-time PCR and could be used as assay inhibition. The *PorA* pseudogene real-time PCR was used to examine clinical specimens previously examined using different commercial *N. gonorrhoeae* NAAT platforms. Results show a high specificity. *CONCLUSION:* The study succeeded in establishing *PorA* pseudogene real-time PCR is valuable assay in detection and confirmation of *N. gonorrhoeae* specific DNA.

P152

EFFICACY OF COTRIMOXAZOLE IN CONTROLLING MALARIA PARASITAEMIA AND BACTERIA INFECTIONS IN HIV-INFECTED PREGNANT WOMEN IN JOS, NIGERIA

Ali, M.A¹., Agbo, E.B²., Suleiman, M.M²., ¹Department of Microbiology, Faculty of Natural Sciences, University of Jos, ²Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria. alimurna@yahoo.com 08036914138

MURNA ALI¹, Ediga Agbo², Maryam Suleiman²

¹University of Jos, Jos, Nigeria. ²Abubakar Tafawa Balewa University, Bauchi, Nigeria

Abstract

Although Cotrimoxazole is being used for antimalarial prophylaxis among HIV-infected individuals, there is insufficient data on its dual effectiveness and safety in HIV-infected pregnant women in Nigeria. The efficacy of Cotrimoxazole (CTX) on malaria parasitaemia and bacterial infections in 235 HIV-infected pregnant women in Jos was studied. Study population was divided into 2 groups; Group A took CTX while group B took SP. Peripheral blood samples were examined for malaria parasitaemia using the thick and thin blood smears. CD4+ count and PCV were determined using flow cytometry and capillary tube methods respectively. Bacteria were isolated using cultural methods and biochemical tests. Cotrimoxazole reduced malaria parasitaemia by 89.4%, while SP gave 79.01% reduction. Mean CD4+ count (cells/ μ l) was significantly ($P < 0.05$) increased from 223.55 to 300.54, and decreased from 570 to 534.4 with CTX and SP respectively. Mean PCV was significantly increased from 33.09% to 33.20% after prophylaxis with CTX, but insignificantly ($P = 0.154$) reduced from 33.11% to 32.90% with SP. Placental malaria was 5.9% among group A and 7.5% among group B. Enteric bacteria isolated include *Salmonella sp*, *Shigella sp*, *Proteus sp*, *Enterobacter sp*, *Klebsiella sp*, *Citrobacter sp* and *E. coli* while respiratory tract bacteria include *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Proteus species*. All the *Shigella*, and *Salmonella sp* isolated were resistant to Cotrimoxazole,. Prophylaxis with Cotrimoxazole is associated with more reduction in malaria parasitaemia with reduced effect on associated respiratory tract and enteric bacteria compared to standard SP.

P153

Virulence of the cystic fibrosis pathogen *Burkholderia cenocepacia* is reduced in the presence of manuka honey

Leanne Fisher¹, Helen Louise Brown², Rowena Jenkins³

¹Cardiff Metropolitan University, Cardiff, United Kingdom. ²Cardiff University, Cardiff, United Kingdom.

³Swansea University, Swansea, United Kingdom

Abstract

Background: Airways of cystic fibrosis patients are often colonised with highly antibiotic resistant pathogens such as, *Burkholderia cenocepacia*. Virulence factors produced by *B. cenocepacia* may also contribute to pathogenicity within the CF lung and the infective process. Manuka honey possesses antimicrobial ability across a broad range of pathogens and has been shown to be able to restore antibiotic susceptibility. The objective of this study was to investigate whether sub-inhibitory concentrations of manuka honey could reduce virulence expression in *B. cenocepacia*.

Methods: The minimum inhibitory concentration of manuka honey against 26 *B. cenocepacia* isolates was determined using an adapted EUCAST microbroth dilution technique. The ability of the isolates to produce protease, lipase, gelatinase, DNase and haemolysin in the presence and absence of sub-lethal concentrations of manuka honey using agar incorporation and zone of inhibition assays, and the effect of manuka honey on established biofilms was determined.

Results: The MIC of isolates was between 8 and 16% w/v. Virulence factor expression was found to be isolate specific. At half MIC, all virulence factors were down regulated as depicted by a decrease in zone of inhibition size ($p < 0.05$). Both the formation and disruption of an established biofilm was also reduced with honey treatment.

Conclusion: These results could suggest a role for manuka honey as an adjuvant to antibiotics when treating *B. cenocepacia*. To determine whether this could potentially be used to reduce bacterial load and virulence in CF patients, further testing in *ex vivo* and *in vivo* studies is needed.

P154

Antibiotic Susceptibility Pattern of Salmonella Isolated from Enteric Fever Suspected Patients

Bijayata Shrestha^{1,2}, Gita Sayami¹, Sarita Manandhar², Santosh Khanal²

¹HAMS Hospital, kathmandu, Nepal. ²National 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 Amoxicillin, 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

P155

MOLECULAR CHARACTERISATION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING GRAM-NEGATIVE UROPATHOGENS AT A SPECIALIST HOSPITAL IN NORTH WESTERN NIGERIA

Tanko Nuhu¹, Bolaji R.O², Olayinka B.O²

¹Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria. ²Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

Abstract

Background:

Extended Spectrum Beta-Lactamase (ESBL)-producing enterobacteriaceae are fast becoming the leading aetiological agents of antibiotic resistant infections. The study determined the prevalence and genetic profiles of ESBL-producing from Gram-negative uropathogens.

Methods:

A total of 365 mid-stream urine sample collected over a period of 4 months were studied. Isolates were identified using Microgen Identification Kit, GN-ID. Susceptibility testing against panel of 7 antibiotics was performed using the modified Kirby-Bauer method, and results were interpreted according to Clinical Laboratory Standards Institute (CLSI). Molecular characterization of the ESBL positive isolates were confirmed by PCR method.

Results:

A total of 64 Gram negative uropathogens were isolated from 365 urine samples. The isolates were made up of *E.coli* (29.7%), *Salmonella arizonae* (23.1%), *Klebsiella oxytoca* (10.9%), *Enterobacter gergoviae* (9.4%), *Citrobacter freundii* (6.3%), *Serratia marscense* (6.3%), *Klebsiella pneumoniae* (4.7%), *Proteus mirabilis* (1.6%), *Enterobacter aerogenes* (1.6%), *Edwardsiella tarda* (1.6%), *Acinetobacter Iwoffii* (1.6%), *Burkholderia pseudomallei* (1.6%), and *Pseudomonas aeruginosa* (1.6%). The isolates were generally resistant to cotrimoxazole (71.9%), nalidixic acid (67.2%), ciprofloxacin (54.7%), norfloxacin (53.1%), gentamicin (50.0%), amoxicillin/clavulanate (48.4%), and nitrofurantoin (29.7%). Majoriy of the isolates were multidrug resistant (64.1%), 15.6% were extensively drug resistant (XDR), 4.7% were pandrug resistant (PDR), while 15.6% did not fall into any of the classification. Molecular analysis of the 15 MDR-ESBL-producing isolates showed that 73.3% were *bla*CTX-M, while 26.7% were *bla*OXA.

Conclusion: The prevalence of ESBL in this study was 23.4%. Molecular characterization of the selected MDR-ESBL-producing isolates showed that they harbored the *bla*CTX-M gene and *bla*OXA gene.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P156

“Ancientbiotics” from Nettles, Salt and Vinegar

Navneet Jandu, Esther Sweeney, Freya Harrison

University of Warwick, Coventry, United Kingdom

Abstract

Antibiotic resistance is currently rising at a rapid rate. The solutions to this problem could be found in ancient texts of our ancestors. The Old English *Bald's Leechbook*, an 11th century translation of the 4th century *Pseudo-Apuleius Herbarius*, the 13th Century *Lilium Medicinae* and 14th century works by the well-known physician John Arderne reveal a clear pattern of nettles (*Urtica dioica* / *U. urens*) being used to make a salve for wounds likely to be infected (“foul and rotten” wounds, dog bites, frostbite and surgical wounds). These tests suggest to use different combinations of nettles, salt and vinegar, combined with soaking or boiling, to make a paste that could cover the wound. During a Harry Smith Vacation Studentship, we tested whether combinations of these ingredients had antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. We report data showing bacteriostatic or bactericidal effects depending on how ingredients were combined.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P157

The effects of compound NX-AS-401 on Methicillin resistant *Staphylococcus aureus* (MRSA).

Phillip Butterick^{1,2}, Michael Graz², Gareth Evans², Danielle Williams², Rowena Jenkins¹

¹Swansea University, Swansea, United Kingdom. ²NEEM Biotech, Abertillery, United Kingdom

Abstract

Prevalence of antimicrobial resistant bacteria and stagnation in developing new antibiotics holds both significant challenge and significant opportunity for healthcare globally. The need for new antimicrobial agents is urgent and this project determines the effects of a new antimicrobial NX-AS-401 produced by NEEM Biotech against Methicillin Resistant *Staphylococcus aureus* (MRSA), a commonly isolated wound pathogen.

EUCAST broth microdilution methodology was used to determine minimum bactericidal/bacteriostatic concentrations (MIC/MBC) and an adapted version utilized to determine the effect of NX-AS-401 on developing and pre-established biofilms. Biofilm mass, viability and cell numbers were measured using, crystal violet, cell titre blue and total viable cell counts.

Time/Kill curves were used to deduce the effect of compound over time. Antibiotic interactions were identified via EUCAST disc diffusion methods and the effect of sub-inhibitory concentrations of NX-AS-401 on protease, lipase, gelatinase and DNase expression was determined using agar zone of inhibition assays.

NX-AS-401 had an MIC and MBC of 128mg/L and 2048mg/L respectively, with the first effects seen at 4 hours. Biofilm formation was inhibited at 128mg/L, while 256mg/L was required to disrupt preformed biofilms. Cells exposed to sub-inhibitory concentrations of NX-AS-401 and antibiotics displayed increased susceptibility to Gentamicin, Tobramycin, Ciprofloxacin and Clindamycin. Sub-inhibitory levels of the compound also caused decreased expression of lipase.

NX-AS-401 effects on growth kinetics, antibiotic interactions and virulence factor production are indicate that the compound could potentially be useful in the treatment and management of MRSA infections.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P158

Seroprevalence of Chlamydia trachomatis infection among infertile women attending Aminu Kano Teaching Hospital, Kano, Nigeria

Abdulhadi Sale Kumurya, Aminu Ibrahim, A'ishatu Kabir

Bayero University Kano, Kano, Nigeria

Abstract

Background: Infertility is the biological inability of a man or woman to contribute to conception. *Chlamydia trachomatis* is the most implicated organism in infertility. **Objective:** The objective of this study was to determine the prevalence of Chlamydial infection and some risk factors associated with the infection. **Methods:** One hundred and fifty four subjects comprising 136 infertile women as test group and 18 postnatal women as control group attending infertility and post natal clinics respectively of Aminu Kano teaching hospital were recruited. For the study, endocervical swabs were collected and screened using Chlamydia rapid test device-swab/urine (Swelab limited, China). **Results:** The overall prevalence rate of Chlamydial infection was 20.6% for the infertile groups and 5.6% for the control. The prevalence rate was higher in age groups 34-38years of age. It appeared that antigen positivity was higher in secondary infertile women (25.9%) than the primary infertile women (11.8%). Majority of the patients (93.4%) were not aware of the existence of the infection and its complications. Previous exposure to sexually transmitted disease (STD) and abortion were associated with increased risk of Chlamydial infection. **Conclusion:** Thus, this study reveals an increased prevalence for Chlamydia infection among infertile women, as one in ten is likely to harbor the infection and its occurrence may be associated with awareness about the infection and asymptomatic nature of the disease.

P159

INCIDENCE AND RISK FACTORS OF SURGICAL SITE INFECTIONS AT A TEACHING HOSPITAL IN ABUJA, NIGERIA

A. Olowo-okere¹, Y.K.E. Ibrahim², A.S. Sani³, B.O. Olayinka²

¹Usmanu Danfodiyo University, Sokoto, Nigeria. ²Ahmadu Bello University, Zaria, Nigeria. ³University of Abuja Teaching Hospital, Abuja, Nigeria

Abstract

Surgical Site Infection (SSI) is one of the adverse complications following an operation. Patient-related risk factors have been adduced as major determinants of occurrence of SSI. This study was conducted to determine the incidence and possible risk factors associated with SSI at a teaching hospital in Abuja, Nigeria.

Questionnaires were administered to collect socio-demographic and clinical data from folders of patients that willingly consented to participate in the study. Patients that undergone major surgeries in the hospital were observed prospectively over a three-month period for the occurrence of SSI based on criteria stipulated by CDC (1999). Data were analysed using IBM SPSS Statistics, version 24 (SPSS Inc., Chicago, IL, USA) and predictors of SSIs were identified using multivariable logistic regression model. P-value less than 0.05 was considered to be statistically significant.

Of the 127 surgical patients observed during the study period, 35 (27.56%) developed SSIs. A prolonged hospital stays, class of wound and cigarette smoking were found to be significantly associated with higher SSI rate. Also, alcohol consumption and sickle cell disease increased the risk of patients developing SSI by 16.8 and 3.5 times respectively when compared to their counterparts that do not consume alcohol nor had sickle cell disease.

The high incidence of SSI in the hospital highlight the need for improved surveillance of SSI and review of infection control policies of the hospital.

P160

ANTIBIOTICS RESISTANCE PATTERN OF INDOOR AIR AND FLOOR BACTERIAL ISOLATES FROM SURGICAL WARDS OF A UNIVERSITY TEACHING HOSPITAL IN ABUJA, NIGERIA

A. Olowo-okere¹, M. Babandina²

¹Usmanu Danfodiyo University, Sokoto, Nigeria. ²University of Abuja Teaching Hospital, Abuja, Nigeria

Abstract

The hospital environment is a major source of nosocomial pathogens. These pathogens could contaminate operated sites leading to surgical wound infections. This study determines the antibiotic resistance pattern of indoor air and floor bacterial isolates from surgical wards of a University Teaching Hospital in Abuja, Nigeria. A cross-sectional study was conducted over three months period at the three surgical wards of the hospital. Bacteriological quality of indoor air and floor of the wards was evaluated using passive air sampling following the 1/1/1/ scheme and swabbing methods respectively. The isolates were characterized using rapid test kits and tested for susceptibility to the commonly used antibiotics in the hospital by the modified Kirby Bauer disc diffusion method. A total of 121 bacterial isolates comprising eleven (11) species were isolated from the hospital. In the air and floor of all the surgical wards, *Staphylococcus* spp was the most prevalent Gram-positive bacteria. Among the Gram-negative bacterial isolates, *Pseudomonas aeruginosa* and *Proteus mirabilis* were respectively the most prevalent. The bacterial isolates exhibited diverse degree of susceptibility to the commonly prescribed antibiotics in the hospital. It was however observed that isolated were more susceptible to fluoroquinolones. This study documents the diverse environmental microbiota of the surgical wards of the hospital. The resistance of the isolated bacteria to the commonly prescribed antibiotics in the hospital highlights the need for improved infection control and patient safety protocol in the hospital so as to prevent the spread of nosocomial infections in the surgical wards of the hospital.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P161

INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF MEDICALLY IMPOTANT BACTERIA IN MEAT PIE: IMPLICATIONS FOR PUBLIC HEALTH

Godwin Obande¹, Ebele Umeh², Emmanuel Azua², Aleruchi Chuku¹, Peter Adikwu³

¹Department of Microbiology, Federal University Lafia, Lafia, Nigeria. ²University of Agriculture, Makurdi, Nigeria. ³Department of Biological Sciences, University of Agriculture Makurdi, Benue State, Nigeria

Abstract

Ninety samples of retailed meat pie were examined for bacterial contamination, antibiotic susceptibility *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* species and observance hygiene practices by retailers. Samples were collected from eateries, supermarkets and street hawkers, and isolates were identified by cultural and biochemical characteristics. *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus* spp, *Enterobacter* spp, *Proteus* spp, *Pseudomonas* spp, *Citrobacter* spp, *Edwardsiella* spp, *Bacillus* spp, *Klebsiella* spp and *Shigella* spp were identified. *Bacillus* spp (85.0%) occurred most. Mean viable counts of fresh meat pie samples ranged from 6.63×10^7 to 1.01×10^9 cfu/g for both fillings and crusts. Analysis of variance (ANOVA) and Chi-square tests revealed no statistically significant differences in contamination rates for samples from different sources ($p > 0.05$) but Hawked samples had the highest mean viable counts. Antibiotic susceptibility tests showed that 87.1% of *Staphylococcus aureus* isolates were resistant to Cloxacillin while 88.6% were susceptible to Ofloxacin. *Escherichia coli* was resistant to Amoxycillin (100%), Tetracycline (100%), Cloxacillin (100%) and Augmentin (100%) but susceptible to Gentamicin (80%) and Ofloxacin (80%). None of the vendors (90:100%) used hand gloves, none (90:100%) used an apron, 89 (98.9%) used no cutlery and 89 (98%) had uncovered hair while serving the product. All samples were found contaminated beyond acceptable limit (10^3 to $<10^4$) and some vital hygiene practices were neglected by vendors. Meat pie could serve as a vehicle for antibiotic resistant bacteria. Production and sales of meat pie should be appropriately regulated for the sake of public health.

P162

Emergence of New Delhi Metallo-Beta-Lactamase (NDM-1) *Klebsiella pneumoniae* in Nepal

Sushila Lekhak¹, Sunil Prasad Lekhak²

¹St.Xavier College, Kathmandu, Nepal. ²Decode Genomics and Research Center, Kathmandu, Nepal

Abstract

Abstract:

Background

New Delhi metallo β -lactamase-1 (NDM-1) producing *Klebsiella pneumoniae* isolates are potential threat to human health as they are usually resistant to all classes of antibiotics, including β -lactams, leaving physicians with limited antibiotic choices for treating infected patients. This study was conducted to detect the presence of *bla*_{NDM-1} in carbapenem resistant *K. pneumoniae* in a tertiary care center of Nepal.

Methods:

This study was conducted from July 2015 to January 2016 to isolate *K. pneumoniae* from various clinical samples. All the samples were screened for Carbapenem resistant *K. pneumoniae*. MBL production, MIC determination and NDM gene was detected using combines Disk Test (CDT), E-Test and PCR respectively,

Results:

Out of 62 *K. pneumoniae* isolates, 20 were suspected to be carbapenemase producers. The incidence rate of MBL by CDT and E-test was found to be 24.25% (15) and 32.3% (20) respectively. The Minimum Inhibitory Concentration (MIC) for imipenem and meropenem ranged between 8 to >32 μ g/ml. 16 (80%) CRKP were isolated from urine and sputum, and the rest were isolated from tracheal aspirates (15%) and catheter tips (5%). Highest number of isolates (n=7) was observed in the same age group, 41-50 years, in both male and female. NDM-1 gene was detected in three CRKP isolates. The NDM-1 producers were susceptible only to tigecycline and colistin.

Conclusion

NDM-1 is not a major mechanism mediating carbapenem resistance in *K. pneumoniae* in this center. However, continuous surveillance and screening is recommended to prevent their dissemination.

P163

STUDY OF THE EFFECT OF MOBILE PHONE RADIATION (Non Ionizing Radiation) ON ANTIBIOTIC SENSITIVITY IN MICRO ORGANISMS

Arunkumar G

College of Pharmaceutical Sciences, Trivandrum, India

Abstract

Mobile phone radiation exposure for long term is harmful to human beings and other living system. Nowadays antibiotic resistance is the common tragedy in our modern allopathic treatment especially in the case of Tuberculosis. This study was based on the effect of mobile phone radiation on the antibiotic sensitivity in Escherichia Coli. The difference in sensitivity of E.Coli that exposed to mobile phone radiation were studied. The mechanism of resistance of these pathogenic bacteria has to be found out as soon as possible for improved patient care. This study may be repeated with other type of micro organisms, both gram positive and gram negative with other antibiotics for further investigations. This study has found that, such radio frequency radiation exposed E.Coli shows decreased sensitivity than other non-radiated E.Coli towards Gentamycin. Anyway this topic helps to take preventive measures to withstand our healthy living system and it is the gateway to conclude the relationships and changes of microorganisms due to our natural environmental Electromagnetic fields. On the basis of this study, further research should be necessary about the hazardous effects of the mobile phone radiation to the pathogenic gram positive & gram negative bacteria, virus and fungus. Then only this study will achieve the success in protection of human health.

P164

ISOLATION AND CHARACTERIZATION OF NOVEL LYTIC BACTERIOPHAGE AGAINST CARBAPENEM RESISTANT *Pseudomonas aeruginosa*. "AN ALTERNATIVE APPROACH TO COMBAT MULTIDRUG RESISTANT SUPERBUG".

Guna Raj Dhungana, Rajani Malla

Central Department of Biotechnology, Tribhuvan university, Kathmandu, Nepal

Abstract

Introduction

Pseudomonas aeruginosa, Gram-negative aerobic bacterium, is ubiquitous in the environment, cause serious opportunistic infections. These pathogens are common causative agents of nosocomial infections, pneumonia, bacteremia, urinary tract, skin and soft tissue infections. Multidrug Resistant strains of *P. aeruginosa* infections have emerged as a health care crisis across the globe. They become resistant increasingly against carbapenem antibiotics. The use of bacteriophages for killing bacteria has drawn recent attention, which has potential as alternative to antibiotics.

Objective

This study aimed to isolate bacteriophage against carbapenemase- producing *Pseudomonas aeruginosa* and characterize for potential phage therapy.

Method

Lytic phages were isolated through double-agar layer method. Phages were purified by successively sub-culturing single plaque thrice & standard spot assay. The morphology of phages, biological features and multiple host range, sensitivity of phage to temperature and pH, phage life cycle was determined. Two of the potent phages were confirmed by transmission electron microscopy.

Results

Fifteen lytic phages against carbapenem resistant *Pseudomonas aeruginosa* were isolated from five different river water. The phages were stable between temperature 0-70 °C and pH 3-11. Among fifteen phages, twelve phages showed multiple host range spectrum within same genus while three were extensively specific. Electron microscopy of most potent two phages revealed that both were Caudovirales.

Conclusion

Natural predators, the phages can effectively kill multi-drug resistant *P. aeruginosa* that can possibly be used in therapeutics and as professed in scientific world, phages are not extremely host specific and have evolved to show multiple host range.

P165

Identification and Characterisation of *Staphylococcus aureus* using electrochemical impedance spectroscopy (EIS)

Aiden J Hannah, Andrew C Ward, Sara Kendrick, Patricia Connolly

Department of Biomedical Engineering, University of Strathclyde, Glasgow, United Kingdom

Abstract

Background:

Current clinical bacterial detection methods can be time consuming; EIS may provide a simple, rapid alternative. Obtaining characteristic impedance signatures for key pathogens and understanding the mechanisms underlying their features is vital to the development of a real-time infection monitoring device with applications such as monitoring wound dressings.

Methods:

In this study, carbon screen-printed electrodes were used to detect the growth of *S. aureus*, a species commonly isolated from infection. The impedance of inoculated liquid media was measured between 1 MHz and 1 Hz to create spectra. Using a novel normalisation approach, changes in these spectra can be linked to the growth and presence of *S. aureus* cells.

Results:

During growth in LB media, a significant phase trough at 1 kHz emerged 30 minutes after inoculation with a 1% overnight culture (two sample t-test, $p < 0.05$). Through further study, including that in 0.9% NaCl where no growth occurred, this change was found to be related to bacterial metabolism of the rich media. Conversely, cell settlement on the electrode surface resulted in significant concentration dependant resistance peak formation at 10 Hz from cell concentrations of 3.6×10^6 CFU/ml. Furthermore, at low frequencies additional metabolic impedance changes were present, and other direct contact features evident at high frequencies.

Conclusion:

Therefore, this study demonstrates the rapid detection of *S. aureus* using a low cost EIS-based sensor. This technology could have important applications for the real-time identification of infections in clinically relevant contexts.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P166

Virulence in *Staphylococcus aureus* small colony variants following sublethal exposure to gentamicin

Abdulrahman Bazaid¹, Sarah Forbes², Gavin Humphreys¹, Andrew McBain¹

¹The University of Manchester, Manchester, United Kingdom. ²Sheffield Hallam University, Sheffield, United Kingdom

Abstract

Aims

Small Colony Variants (SCVs) are a slow growing subpopulation of bacteria that form in response to antibiotics such as gentamicin. Gentamicin-induced SCVs have been attributed to the disruption of electron transport chains as a result of hemin mutations. Although these SCVs may reportedly exhibit reduced antibiotic susceptibility, there are conflicting reports about the comparative virulence of gentamicin induced SCV. Thus, we have evaluated relative pathogenicity and haemolysis activity in SCVs generated from a collection of *Staphylococcus aureus* isolates.

Methods

A selection of five *S. aureus* isolates (including MRSA) were passaged ten times using a previously validated gradient plating system to produce gentamicin-adapted strains (P10) and a further ten times without antimicrobial (PX10). The relative pathogenicity of P10, PX10 and parent strains (P0) were determined using the *Galleria mellonella* model. Haemolytic activity and susceptibility to gentamicin were assessed for all isolates.

Results

After repeated gentamicin exposure, 3/5 formed SCVs which exhibited gentamicin resistance. A significant ($p < 0.05$) reduction in haemolytic activity was detected in 2/3 SCVs and in 1/2 of the non-SCV forming strains. 2/3 SCVs exhibited a significant reduction in relative pathogenicity when compared to untreated isolates. When gentamicin was removed (PX10), SCVs reverted to the untreated phenotype.

Conclusions

Repeated gentamicin exposure may induce SCV with reduced gentamicin susceptibility. Importantly, we have observed attenuated relative pathogenicity and reduced haemolytic activity in SCV induced by exposure to gentamicin.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P167

Antimicrobial potential of *Erythroxylum emarginatum* Thonn.

Duncan Chalo¹, Esezah Kakudidi¹, Hannington horyem-origa¹, Abiy Yennesew², Jane Namukobe¹

¹Makerere University, Kampala, Uganda. ²University of Nairobi, Nairobi, Kenya

Abstract

Background: Antibiotic resistance to available conventional drugs is becoming dire and calls for new ones with herbal remedies being possible source. The objective of this work was to determine antimicrobial potential of roots and stem bark of *Erythroxylum emarginatum* taking into account its wide usage in traditional medicine for treatment of wounds, diarrhoea, respiratory and skin infections.

Methods: Dry grounded materials were extracted using dichloromethane: methanol (1:1), methanol and partitioned by ethyl acetate. To determine presence of phytochemicals, thin layer chromatography was done whereas antimicrobial activity was evaluated by the Kirby-Bauer test against MRSA,

Staphylococcus aureus and *Escherichia coli*. **Results:** Dichloromethane: methanol (1:1), methanol and ethyl acetate extracts of root and stem bark extracts were combined due to similarity in TLC compounds profile respectively. Roots extract of *E. emarginatum* inhibited strains of *E.coli*, MRSA and *S.aureus* in all the tested concentrations (1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml) while activity of stem extracts was observed against *S. aureus* in all tested concentrations. **Conclusion:** Findings from this study form a basis for drug development and herbal preparation for the long term management of microbial infections. This species is a treasure of potentially new medicinal compounds waiting to be discovered.

Recommendation: Determination of minimum inhibition concentration (MICs), isolation and identification of the active antimicrobial phytochemicals and their cytotoxicity activity.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P168

The Role of CwIM in *Mycobacterium tuberculosis* growth

Baleegh Kadhim

University of Leicester, Leicester, United Kingdom

Abstract

Baleegh Kadhim, Lin Hui Wen, Andrew Bottrill, Russell Wallis, Obolbek Turapov, Galina V. Mukamolova

Leicester Tuberculosis Research Group, Department of Infection, immunity and Inflammation, University of Leicester, Leicester, UK

Tuberculosis remains a major global health problem, claiming around 1.5 million lives annually, and its causative agent *Mycobacterium tuberculosis* (*Mtb*) is able to persist in humans for decades. *Mtb* has a complex cell wall which can be remodelled by multiple peptidoglycan (PG) - cleaving enzymes. CwIM is an annotated N-acetylmuramyl-L-alanine amidase that is essential for mycobacterial growth (1, 2). It can be phosphorylated by a protein kinase B (PknB), however the precise role of phosphorylation in CwIM function is not fully understood. A recombinant CwIM from *Mtb* was purified as a soluble protein and used for investigation of PG-hydrolysing activity by application of zymogram and hydrolysis of FITC-labeled PG. Both CwIM or phosphorylated CwIM showed no detectable PG-cleaving activity, however a shorter CwIM form (the predicted amidase domain) was active in both assays. D339A mutation completely abolished its activity, but this mutation was not essential for mycobacterial growth. CwIM could be easily degraded to smaller fragments, however phosphorylation improved its stability. The phosphorylated form of CwIM was previously shown to interact with MurA, a key enzyme in synthesis of PG precursors, suggesting that CwIM was critical for regulation of PG biosynthesis (2). Our current efforts are focused in investigation of other potential partners of CwIM and a possible role of phosphorylation in CwIM localization and protein-protein interactions.

P169

Nutritional Composition, Biochemical Characteristics and Antimicrobial Potentials of Plant Seed and Nut Oils against *S. aureus*

OLUWAPAMILERIN YANGOMODOU, Natalie Ferry, John Hadfield

University of Salford, Manchester, United Kingdom

Abstract

Background

Plant oils such as walnut oil, cashew oil and pumpkin oil with anti-microbial potentials are attractive agents that could help curb the menace of *S. aureus* as well as the use of conventional antibiotics. In this study we evaluated the activities of walnut oil, cashew oil and pumpkin oil on *S. aureus* ATCC 6538 biofilm.

Methods

Antibiotic susceptibility test was performed against *S. aureus* by disc diffusion method. Quantitative Real time PCR was used to identify and study the expression of adhesion genes, biofilm related genes, enterotoxins, leukotoxins and two target transcripts, RNAII and RNAlII of the accessory gene regulator (*agr-QSS* locus) at 12 h, 24 h and 48 h in *S. aureus* biofilm.

Results

Antibiotic susceptibility test result showed that *S. aureus* ATCC 6538 is susceptible to pure oil samples of walnut and pumpkin as well most conventional antibiotics tested. Using qPCR, the adhesion genes, biofilm genes, enterotoxin genes, leukotoxins ED and RNAII and RNAlII transcripts were identified in *S. aureus* biofilm at 12 h, 24 h and 48 h. At 24 h, treatment of *S. aureus* biofilm with sub-inhibitory concentrations of walnut oil and pumpkin oil significantly decreased the expression level of adhesion gene *icaC* and biofilm genes (fibronectin A, B, clumping factors A, B and elastin). However, a decrease was observed for the genes at 48 h. Rest Program 2009 was used for gene expression analysis.

Conclusion

Walnut oil and pumpkin oil could be used as natural antimicrobial agents for the treatment of *S. aureus* infections.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P170

Comparative study of virulence factors of *Klebsiellae pneumoniae* isolated from neonatal feeding tubes and neonatal sepsis cases in neonatal intensive care.

Mohamed Saad, Khaled Dahmani, Pauline Ogrodzki, Jody Winter, Stephen Forsythe

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Background;

K. pneumoniae is a major cause of nosocomial infections, responsible for 75% to 86% of *Klebsiella* spp infections. Premature babies may be exposed to bacteria with pathogenic potential through the use of feeding tubes (FT) in intensive care. This study aimed to compare between strains from neonatal FTs and sepsis cases to determine linkage and virulence potential.

Materials and Methods;

Eleven *K. pneumoniae* strains (6 strains from neonatal sepsis blood cultures and 5 strains from FT) were analyzed. Strains were characterized using standard microbiological methods, sequence typing, genome sequencing, and *in vitro* tissue culture assays.

Results;

The present study revealed that all isolates possessed adhesion genes (*fimH* and *mrkD*), were able to form biofilm on plastic, were β haemolytic on horse blood, were able to survive in pH 3.5 for up to 2 hours and could tolerate human serum and desiccation. Most of the strains were resistant to aminoglycosides, carbapenem, and 3rd generation cephalosporins. *K. pneumoniae* strains isolated from FT were significantly better at surviving within macrophage (U937) cells than strains isolated from sepsis blood cultures.

Conclusions;

These findings indicate that *K. pneumoniae* strains present in FT have the potential to cause severe disease in neonates.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P171

Csu fimbriae protein plays a role in biofilm formation and adhesion to Caco-2 epithelial cells in *A. baumannii* isolated from neonatal feeding tubes in neonatal intensive care

khaled dahmani¹, Mohamed Saad¹, Pauline Ogrodzki¹, Mohamed Elemam², Jody Winter¹, Stephen Forsythe¹

¹Nottingham Trent University, Nottingham, United Kingdom. ²Tripoli University, Tripoli, Libya

Abstract

Background: *Acinetobacter baumannii* is gaining importance as a pathogen in intensive care units (ICUs). The ability of *A. baumannii* to grow as a biofilm on abiotic surfaces plays a vital role in causing nosocomial infections. The aim of this study was to assess the virulence potential of *A. baumannii* strains isolated from neonatal feeding tubes.

Material/methods: Forty-three clinical strains recovered from neonatal feeding tubes from two ICUs in Jordan were identified by *rpoB* sequence analysis and genotyped using pulsed-field gel electrophoresis. Whole-genome sequencing was performed using the Illumina MiSeq instrument. Biofilm formation was determined using a crystal violet assay and bacterial attachment to Caco-2 cells was quantified.

Results: All strains were identified as *A. baumannii* and they clustered into five pulsetypes and two different STs (ST193 and ST113). *blaOXA-51* and *blaOXA-64* genes were present in all of the isolates whereas *blaOXA-23* was only found in strains belonging to ST113. The genome comparison study revealed that all of the sequenced ST113 isolates harboured genes of the *csu* cluster and were able to form biofilm and to adhere to the Caco-2 cell line. Non ST113 strains lacked the *csu* locus and were poor biofilm formers with low Caco-2 attachment. There was a significant correlation between epithelial cell adherence and biofilm formation ($p = 0.0376$).

Conclusions: *A. baumannii* strains from neonatal feeding tubes were highly antibiotic resistant, cell adherent and biofilm forming. These virulence factors could allow strains to persist in neonatal intensive care units.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P172

Investigating the role of the bacterial mechanosensitive channel YnaI in *Salmonella* pathogenesis

Mimi Asogwa¹, Sam Miller¹, Stefania Spano¹, Mark Stevens²

¹University of Aberdeen, Aberdeen, United Kingdom. ²University of Edinburgh, Edinburgh, United Kingdom

Abstract

Mechanosensitive channels are required for bacterial cells to survive hypoosmotic shock. YnaI is one of the mechanosensitive channels found amongst many bacterial species including *Salmonella* Typhimurium. *S. Typhimurium* is a broad host range pathogen that affects humans as well as farm animals (pigs, cattle, chicken). In these animals, *S. Typhimurium* causes diseases which threaten health and impair productivity. Previous studies have suggested that *Salmonella* YnaI may be implicated in pathogenesis as disruption of *YnaI* in *Salmonella* impaired intestinal colonization in pigs, cattle, chicken and mice.

Salmonella YnaI was cloned into an IPTG-inducible plasmid, followed by physiological characterization of the *Salmonella* YnaI constructs expressed in an *E. coli* channel-less mutant strain (MJF641).

Our data show that the *Salmonella* YnaI channel has unique characteristics when expressed in *E. coli* cells. The *Salmonella* YnaI protein conferred almost complete protection against 0.3 M NaCl hypoosmotic shock, similar to previous studies with *E. coli* YnaI but interestingly high level expression of *Salmonella* YnaI protein inhibited growth, despite high similarity between the two proteins. The nature of this inhibition is under investigation.

This is the first time the *Salmonella* YnaI channel has been characterised. The behaviour observed may provide insights into other functions of the *Salmonella* YnaI channel.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P173

Anti-microbial activity of Polyunsaturated Fatty Acids related to Chronic Wound Infections

Mohamed ESshlak

University of Salford, Manchester, United Kingdom

Abstract

Background- Wound infections present major health problems worldwide. They involve polymicrobial biofilms with Multidrug-resistant organisms. To overcome the limited therapeutic options, polyunsaturated fatty acid (PUFA) compounds including Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) offer both immunomodulatory and antimicrobial properties that could enhance wound healing. **Methods** – The Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) of PUFA compounds were evaluated against different isolates. The activity of sub-inhibitory concentrations of PUFAs (50 mg ml⁻¹ and 100 mg ml⁻¹) on growth of a clinical wound isolate of *S. aureus* (SA3). RNA was extracted in triplicate after 0, 6, 12 and 24 h exposure and cDNA was synthesized for use in RT-PCR studies. The global genes expression in virulence for *S. aureus* 3 isolate were measured. **Results** -The MIC of both EPA and DHA for *S. aureus* and *Enterococcus faecalis* were 156 mg ml⁻¹ and 39 mg ml⁻¹ for *Pseudomonas aeruginosa* isolates and *Escherichia coli*. EPA and DHA killed *E. faecalis* at slightly different concentrations (MBC 1250 and 625mg ml⁻¹ respectively), and both PUFA compounds were most efficient at killing *P. aeruginosa* 14 (MBC 625mg ml⁻¹). Both PUFAs showed the highest bactericidal effect on *S. aureus* isolates biofilm (MBEC781 mg ml⁻¹). **Conclusion** – The MIC and the MBEC for PUFA compound shows bacteriostatic activity against planktonic cells and biofilm formation of *S. aureus*, *E. faecalis* and *P. aeruginosa*. Growth at sub inhibitory concentration was considerably slower for the first 6 hours but recovered after 12 hours.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P174

A Novel *Corynebacterium* Associated with Inflammation in a Patient with Chronic Prostatitis

Rungravee Kanjanasuntree, Qi Zhang, Wonyong Kim

Chung-Ang University, Seoul, Korea, Republic of

Abstract

Chronic prostatitis is a common disease, affecting men of all ages. A cream-pigmented, slightly lipophilic, and urea-hydrolyzing coryneform bacterium, designated CAU 1463, was isolated from a urine sample obtained from a chronic prostatitis patient. Phylogenetic analysis based on 16S rRNA gene sequences exhibited that the isolate belonged to the genus *Corynebacterium* with the most closely related to *Corynebacterium lipophiloflavum* DSM 44291^T (98.3% similarity). Polyphasic taxonomic analysis revealed that the bacterium represented a new species within the genus *Corynebacterium*. This organism produced potent inflammatory mediators, NO and PGE₂ in murine macrophage RAW 264.7 cells and human prostatic epithelial cells. The results suggest that although coryneforms are generally considered as saprophytes, a novel *Corynebacterium* sp. CAU 1463 may be related to the inflammatory response in chronic prostatitis.

P175

Antigenic variation of CoA gene in *Staphylococcus* species isolated from Minas Frescal cheese

PAULA POLL, SIMONE PERECMANIS, JOSÉ RENATO BORGES, ÂNGELA PATRÍCIA SANTANA

UNB, BRASÍLIA, DF, Brazil

Abstract

Some staphylococci may express coagulase, through CoA gene, an important virulence factor and a known evasion mechanism of immune system. This is an important factor for food safety. Thus, the aim of this study was to identify the polymorphism of coagulase gene in *Staphylococcus* spp. Isolated from Minas Frescal cheeses commercialized in Federal District, Brazil. Thirteen coagulase-positive staphylococci were collected from Minas Frescal cheese during June to September, 2016, in different commercial establishments. In the present study, we use a PCR method for species identification of CoA genes of staphylococcal species. The amplicon sizes found were approximately 600bp (5/13), 700bp (3/13), 800bp (1/13), 900bp (3/13) and 1000bp (1/13). Some genotypes CoA genes are prevalent in some areas and these are presented as characteristic of increased resistance to phagocytosis and death by neutrophils. *Staphylococcus* species most frequent in this study showed the amplification products of 600bp. Considering the prevalence of amplicons between 600 and 900 bp involved in some cases of infection, may indicate they are more virulent and competitive profiles. The present study indicates a considerable heterogeneity in CoA gene of strains isolated from Minas Frescal cheeses commercialized in the Federal District, Brazil. This variation proved to be relevant to genus *Staphylococcus* and determined by environmental conditions as different sites and situations revealed distinct predominance in coagulase gene polymorphisms.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P176

Can the antimicrobial triclosan induce antibiotic tolerance in *Staphylococcus aureus*?

Dean Walsh, Birte Hollmann, Mark Perkins, Veeren Chauhan, Jon Aylott, Kim Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

The antimicrobial triclosan is used extensively in both household and hospital settings, resulting in an accumulation of the antimicrobial in a vast array of environments. Triclosan has recently been implicated in the induction of antimicrobial tolerance, but the mechanism of this effect requires further elucidation. How this response affects biofilm structure and pH gradients across the biofilm also requires exploration. Viable cell counts of ciprofloxacin treated cells, with and without triclosan pre-treatment, were determined via drop plating over 24 hours. Confocal laser scanning microscopy was used to evaluate whether triclosan pre-treatment affected biofilm formation and structure. The effect of triclosan on the pH of *S. aureus* biofilms and planktonic cultures was also determined using pH-sensitive fluorescent nanosensors. Here we show that triclosan pre-treatment can induce antimicrobial tolerance in *S. aureus* to ciprofloxacin. *S. aureus* cultures pre-treated with triclosan were found to have viable counts 10,000 fold higher after 10 hours compared to cultures without triclosan pre-treatment. Incubation with triclosan was also shown to alter the biofilm structure in a concentration dependent manner. 10 ng/ml of triclosan caused small disruptions to the biofilm structure, whilst 100 ng/ml of triclosan prompted formation of distinct, yet sparsely distributed biofilm structures. Whilst no notable pH changes were measured across treated biofilms, triclosan treatment did prevent acidification of surrounding media in planktonic cultures. These findings will help us understand how the accumulation of triclosan affects the biofilm formation and antimicrobial treatment of *S. aureus*.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P177

Developing protein antibiotics for MDRPseudomonasaeruginosa.

Madhuri Barge

University of Glasgow, Glasgow, United Kingdom

Abstract

Antibiotic resistance is a global health threat and without discovery of new antibiotics we are heading towards a pre-antibiotics era where bacterial infections will become deadly. In Dan Walker's lab we work on a group of protein antibiotics produced by gram negative bacteria that have evolved to win and survive the intra species competition. One such protein antibiotics is pyocin S5 produced by *Pseudomonas aeruginosa*. We intend to develop a pyocin S5 as a highly specific therapeutic for the treatment of *Pseudomonas aeruginosa*, the major cause of death in patients suffering from cystic fibrosis (CF). The primary objective is to generate methods for the production, formulation, manufacture and delivery of pyocin S5 and a safety profile for delivery by inhalation. To achieve this we have developed a method for pyocin S5 purification. We have tested the lyophilised protein at various time interval and temperatures to evaluate suitable formulation conditions. The long term and short-term stability tests were carried out for the formulated protein. Pyocin S5 has a great potential for development as a much needed therapeutic drug to treat antibiotic resistant *Pseudomonas aeruginosa* infection and might be a suitable drug for treating people with cystic fibrosis.

P178

**ANTIMICROBIAL RESISTANCE PROFILE FROM ISOLATES OF CANINE OTITIS CASES BETWEEN 2013-2015
IN BRASILIA, BRAZIL**

Paula Poll, diego ribeiro, bruno dallago

UNB, BRASÍLIA, DF, Brazil

Abstract

Canine otitis is characterized by inflammation of the epithelium lining the ear canal. It is a multifactorial etiology disease, in which the bacteria of animal's normal microbiota play an important role to behave as opportunistic pathogens. The aim of this work was to investigate causative agents of canine otitis cases from the Veterinary Hospital of University of Brasília (HVET/UnB) and trace resistance profiles to main antimicrobial agents used in clinic. A retrospective study was carried out during 2013 to 2015, with the canine ear diseases suspected sent to laboratory diagnosis at Medical Microbiology Laboratory of Veterinary Medicine/UnB. The samples totaled 128 cases, collected by swab of the affected area with subsequent sowing, characterization and bacterial sensitivity profile. *Staphylococcus* spp (45%), *Malassezia pachydermatis* (31,9%) and *Proteus* (4,4%) were more frequently isolated. Antimicrobial drugs with most "in vitro" effectiveness were amikacin (94,73%) and amoxicillin + clavulanic acid (89,47%). However, there was an increased resistance expressed to norfloxacin (50%) and enrofloxacin (40,25%). Canine otitis represents a significant percentage of cases in veterinary clinics in Brazil. An accurate diagnosis is fundamental, especially with aid of microbiological tests in order to carry out the most effective treatment and aiming to prevent the development of multidrug resistance.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P179

Identification and Characterisation of Novel Stress Response Chaperones in *Salmonella* Typhimurium

Claire Hews, Simone Newton-Payne, Emily Addington, Hannah Gaimster, Gary Rowley

University of East Anglia, Norwich, United Kingdom

Abstract

Salmonella is a Gram-negative, food-borne pathogen and a major cause of worldwide morbidity and mortality. *Salmonella* encounters a wide variety of stressors in its environments including changes in pH and heat, antimicrobial compounds and reactive oxygen and nitrogen species. These can result in serious damage to the bacterium, in particular the cell wall. To combat this, envelope stress response (ESR) pathways sense and respond to protein damage 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 σE and σH regulated IbpA, IbpB, AgsA and the putative chaperone STM1250 to ESRs. We have subjected single and combined deletion mutants to different stress-inducing conditions, to investigate potential functional redundancy between these proteins. Initial results demonstrate that deletion of all four chaperones renders *Salmonella* more sensitive to heat treatment at 50 °C and more sensitive to the cationic antimicrobial peptide polymyxin B. Furthermore, initial findings suggest that STM1250 is important for *Salmonella* survival inside macrophages. From here we will further investigate the role of these proteins in the tolerance of nitrosative stress using DETA NONOate, a nitric oxide donor and oxidative stress using H₂O₂. Additionally, we aim to purify STM1250 and perform chaperone activity assays to investigate potential chaperone properties of this protein. Improved knowledge of the roles of envelope stress induced chaperones may, in turn, provide avenues for future research into novel therapeutic targets against this prevalent pathogen.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P180

Manuka honey, an effective tool against multi-species biofilms associated with cystic fibrosis.

Aled Roberts¹, Rowena Jenkins²

¹Cardiff Metropolitan University, Cardiff, United Kingdom. ²Swansea University, Swansea, United Kingdom

Abstract

Background:

Patients suffering from Cystic Fibrosis (CF) are susceptible to repeated chronic lung infection with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia*, these are problematic pathogens to treat due to their antimicrobial resistance and tolerance mechanisms. The co-colonisation of bacteria within the CF lung can lead to poor patient prognosis and are potentially harder to treat. Manuka honey has been shown to have excellent antimicrobial prowess against pathogens associated with the CF lung. Here we aim to determine its effectiveness against CF-associated pathogens grown as multispecies biofilms.

Methods:

Using a Soft Tissue Infection Model (STIM) supplemented with an artificial sputum media, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* isolates associated with the CF-lung were grown as multispecies biofilms, and tested for their susceptibility to varying concentrations of manuka honey.

Results:

The STIM was capable of sustaining the growth of multispecies biofilms. *P. aeruginosa* grew much better than *S. aureus* and *B. cepacia* during co-culture, however, when *S. aureus* and *B. cepacia* were grown together, the latter was more successful. Manuka honey was capable of inducing a dose-dependent effect on both mono and co-cultures, with 32% w/v inhibiting the majority of isolates, effects similar to those seen in monocultures.

Conclusion:

Manuka honey is capable of inhibiting multispecies biofilms in a dose-dependent manner which is not affected by community composition (based on the species/strains tested). This suggests manuka honey may be an effective broad-spectrum antimicrobial for the clearing of the upper nasal cavity in patients affected with CF.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P181

The Impact of Footbath on *Dichelobacter nodosus* Colonisation of the Ovine Interdigital Skin.

Hayley Marshall¹, Katie Kelly¹, Liz Genever², Peers Davies¹, Sabine Töttemeyer¹

¹School of Veterinary Medicine and Science, University of Nottingham, Nottingham, United Kingdom.

²AHDB Beef & Lamb, Kenilworth, United Kingdom

Abstract

Ovine footrot is a highly contagious mixed bacterial infectious disease primarily caused by Gram negative bacterium *Dichelobacter nodosus*. Other bacteria such as *Treponema*, *Mycoplasma* & *Porphyromonas* have also been detected in footrot cases. Footrot is the progression of interdigital dermatitis (ID), recent studies have indicated that *D.nodosus* load is greatest on the interdigital skin of sheep with ID. The aim of this study was to investigate the impact of footbath with 2% glutaraldehyde on total bacterial numbers and *D. nodosus* colonisation of the ovine interdigital skin. Twelve sheep had the interdigital skin of each foot swabbed before, immediately afterwards and at intervals up to 29 days post footbath. Swab samples were analysed by culturing on MacConkey Agar to detect total bacterial numbers and by real-time PCR to determine prevalence and load of *D.nodosus*.

As expected, footbath treatment transiently lowered total bacteria numbers. Low levels of *D. nodosus* were detected, both before and after footbath, however both the number of feet carrying *D.nodosus* and its level increased over time post footbath. These data provide novel insight into the role footbaths may play in the prevention of the colonisation of *D.nodosus*, clearly indicating that a single footbath is not sufficient to control *D.nodosus* colonisation.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P182

AcrR and EnvR regulate *acrAB* expression in *E. coli* and *S. Typhimurium*

Abigail Colclough, Vito Ricci, Helen Smith, Doug Browning, David Grainger, Laura Piddock, Jessica Blair

University of Birmingham, Birmingham, United Kingdom

Abstract

The AcrAB and AcrEF RND efflux systems extrude many compounds including antibiotics and over-expression mediates resistance to antibiotics. RND efflux pumps are also required for virulence in many Gram negative organisms including the important human pathogen *Salmonella* Typhimurium. The regulation of the AcrAB system has been extensively studied in *E. coli* and *Salmonella* where the regulatory network is broadly similar but there are differences. In both species, expression of *acrAB* is complex and is controlled by both local and global regulators. The genes encoding the TetR family regulators AcrR and EnvR are divergently transcribed from the *acrAB* and *acrEF* operons, respectively, and act to repress expression of these efflux systems by binding to the promoter regions. Here we show that over-expression of either AcrR or EnvR in *Salmonella* leads to significantly decreased MICs to a range of antimicrobial agents of distinct classes and causes increased accumulation of the Hoechst dye indicating reduced levels of efflux. Structural models have revealed that AcrR and EnvR share a high level of structural homology. In *E. coli*, EnvR has been shown to repress *acrAB* expression (1). Here, we confirm that both AcrR and EnvR bind to the same region upstream of *acrA* and *acrE* in *S. Typhimurium*. Interestingly, our results show that EnvR, which is encoded alongside *acrEF*, binds with higher affinity upstream of both *acrA* and *acrE* than AcrR, which is encoded alongside *acrAB*.

P183

Action of Terpenes on DsbA Protein as Anti-Virulence Agents in *Shigella sonnei* Infection

Thaer Hasan, Paul Herron, Veronique Seidel

University of Strathclyde, Glasgow, United Kingdom

Abstract

Background: DsbA is a bacterial periplasmic thiol-disulphide oxidoreductase and is the key component of the disulphide bond enzyme family. Terpenes are one of many novel agents which are used as anti-virulence factors by targeting the activity of DsbA.

Methods: *In vitro* and *in vivo* experiments were established by using purified recombinant DsbA protein. An enzymatic kinetic assay was used after labelling the oxidised glutathione (GSSG) by fluorescent eosin isothiocyanate (EITC) to obtain Di-E-GSSG. Di-E-GSSG was also converted to E-GSH by DTT. *In vivo* catalysis of E-GSH by DsbA protein was used by culturing *S. sonnei* wild type, DsbA mutant and $\Delta dsbA/pDsbA$ strains. Terpenes were supplemented in experiments at 42 μ M and the RFU was monitored at excitation (525nm) and emission (545nm) wavelengths for a relevant period of time.

Results: The first labelling of Di-E-GSSG was successful and the product showed an increase in RFU after adding reducing factor (DTT). PDI and DsbA proteins showed the ability to reduce Di-E-GSSG into E-GSH within 15 min. Geraniol is one of 12 terpenes used in this study, and showed significant inhibitory activity by reducing the state of DsbA. In *In vivo* experiments, *S. sonnei* wild type and the complementary one was able to catalyse E-GSH and form Di-E-GSSG again when compared to the DsbA mutant strain. Geraniol was also able to inhibit this catalysis when it was added to media.

Conclusion: Geraniol is considered a novel agent and offers a promising anti-virulence therapeutic for *Shigella* infection by targeting the DsbA protein.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P184

Legionella pneumophila's growth and infection dynamics in Acanthamoeba polyphaga and Dictyostelium discoideum

Anaisa B Moreno, Lionel Guy

Uppsala University, Uppsala, Sweden

Abstract

Legionella pneumophila is a gram-negative intracellular pathogen, ubiquitously found in soil and water reservoirs. L. pneumophila has a broad range of hosts, most commonly protozoa, such as amoebae, but also human alveolar macrophages, where it may cause Legionnaire's disease. Although much is known about how L. pneumophila infects and proliferates inside these different hosts, the effect of prey bacteria on the host population has been less studied.

Here, the growth dynamics of L. pneumophila were studied in two different eukaryotic hosts – Acanthamoeba polyphaga and Dictyostelium discoideum. The hosts were challenged with L. pneumophila at different MOIs and temperatures for a period of 72h, and the number of infected and uninfected host cells at various time points was recorded. The pathogenicity of L. pneumophila for each host was measured, as well as its titer, and the number of secondary infections caused by the extracellular bacteria found in the media.

Amoebae serve as an important reservoir for L. pneumophila, allowing it to replicate and survive even in sub-optimal conditions and thus can be a possible source of emerging pathogens. These results can help us understand the relationships and interactions between bacteria and host.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P185

Investigating the Therapeutic Potential of Novel Antimicrobial Peptides Isolated from the Rumen Microbiome Against *Pseudomonas aeruginosa*

Adam Mulkern¹, Sharon Huws², Luis Mur¹

¹Aberystwyth University, Aberystwyth, United Kingdom. ²Queen's University, Belfast, United Kingdom

Abstract

Antimicrobial resistance poses an unprecedented threat to the future of global health. *Pseudomonas aeruginosa* is an opportunistic pathogen heavily implicated in Cystic Fibrosis (CF). Patients with the genetic disorder have severely compromised host defences; this enables drug-resistant biofilms to form and inadvertently facilitates the development of life-threatening infection. The development of biofilms greatly limits the effectiveness of common antibiotics, therefore developing alternative biofilm control agents is hugely important. Numerous AMPs that have been isolated from the rumen microbiome have proven to be effective against bacterial pathogens such as *P.aeruginosa*. This study examines the potential of these AMPs when tested against strains isolated from individuals with CF, including the Liverpool Epidemic strains (LES). Minimum inhibitory concentrations for most of the 62 *P.aeruginosa* strains remained between 8-32 µg/ml. All of the AMPs were capable of greatly reducing biofilm growth and inhibiting biofilm surface attachment. In addition, no decrease in *P.aeruginosa* susceptibility was observed after 25 days of sub-lethal exposure to the AMPs. Further characterisation and mode of action studies are currently ongoing. These promising antimicrobial peptides derived from the cow rumen may provide an alternative *P. aeruginosa* infection control and could potentially improve the quality of life for patients with CF.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P186

Displacement of antibiotic resistance plasmids from bacterial populations using a probiotic conjugative pCURE approach

Anand Prakash Maurya, Alessandro Lazdins, Christopher M. Thomas

School of Biosciences, Birmingham, United Kingdom

Abstract

Background: Since the 1940s antibiotics have enabled us to control most infections. However, the rise in antimicrobial resistance has made this increasingly difficult. Since the development of new antibiotics is slow, this study aimed to develop an alternative approach involving removal of resistance plasmids from the gastrointestinal tract using a “Plasmid Curing” approach.

Material and Methods: The present study explored IncP-1 plasmid RK2 as a broad host range vector for plasmid displacement to maximize its chance of reaching all members of a bacterial population. A previously developed cassette targeting the abundant F-like plasmids was inserted into RK2 and its derivatives. Curing experiments were carried out targeting an F’pro plasmid in *E. coli* JM109 whose loss was detected on chromogenic agar.

Results: RK2 itself was a poor vector for plasmid curing but derivatives which showed displacement from >99% of transconjugant and transformant colonies were identified and these differences were mirrored in mini RK2 derivatives constructed. An “unselected invasion assay” showed that this conjugative pCURE could spread into a target population simply by mixing donor and recipient bacteria in the absence of antibiotic selection, achieving >99.9% target plasmid loss in 4 days.

Conclusion: We have shown that derivatives of broad host range plasmid RK2 can be very effective in a laboratory “unselected invasion assay”. It now needs to be tested in an animal model to determine whether its spread in the gut could be efficient enough to clear resistance plasmids from this important reservoir of resistance and thus justify clinical trials.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P187

Saving Christmas! Combating *Histomonas meleagridis*, the causative agent of blackhead disease through administration of novel antimicrobial peptides.

James Pickup, Linda Oyama, Sharon Huws

Queens university Belfast, Belfast, United Kingdom

Abstract

Histomonas meleagridis is a protozoan parasite that causes mortality and morbidity in a wide range of gallinaceous fowl. It most notably affects turkeys, causing 80-100% mortality in a flock. There is no commercial treatment for this parasite at the moment and attempts at a vaccine have failed. In this study we investigated the potential efficacy of novel rumen microbiome-derived antimicrobial peptides (AMPs) against *H. meleagridis*. *H. meleagridis* was cultivated in growth flasks in dyer media until a concentration of $\times 10^5$ cells/mL was reached. The concentration of the parasite was determined using a Haemocytometer post-staining with 0.04% trypan blue, and the cells were counted at 50X magnification under the microscope. The challenges were carried out in 96-well plates. The starting concentration of the AMPs were 1024ug/ml down to 0.5ug/ml. The cell densities of *H. meleagridis* were checked at 24 and 48 h. The concentration of samples was checked by extracting DNA and qPCR on the samples, to determine the concentration of *H. meleagridis* cells in the sample by looking for the FeHyD gene. Microscopy was also used to check parasite densities as described above. Some of the AMPs showed decreases in the FeHyD gene and microscopical decreases suggesting that the peptides show potential therapeutic application for blackhead disease. Future work will establish how quickly the AMPs work and mechanism of action.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P188

Microbial Diversity in Uropathogenic *Escherichia coli* isolated from temporal sampling of elderly recurrent UTI patients

Charlotte More, Lauren Drage, Catherine Mobray, Hannah Bright, David Pickton, Chris Harding, Robert Pickard, Judith Hall, Phillip Aldridge

Newcastle University, Newcastle, United Kingdom

Abstract

Urinary Tract Infections (UTI's) affect 150 million people across the globe. UTI's have a significant economic impact on our healthcare systems. Uropathogen *Escherichia coli* (UPEC) is responsible for up to 85% of all UTIs. Elderly patients are highly susceptible to UPEC infections. Elderly patients present with recurrent UTIs as they carry asymptomatic bacteriuria (ABU): a diagnostically significant bacterial loads without symptoms for long periods. We have conducted a clinical study of 30 ABU patients followed for 6 months providing us with urine every two weeks. This study was designed to ask: how patients react to ABU? Our study identified UPEC in 51% of urine samples.

E. coli MLST analysis confirmed the isolates to be predominantly associated with clade B2 of *E. coli*. However, *E. coli* strains from across the its clade structure were colonising our patient cohort for significant temporal periods. We have screened this strain collection for phenotypic diversity. Our analysis has focussed on phenotypes associated with aspects of UPEC pathogenicity including: biofilm formation, motility, host-pathogen interactions and host-pathogen recognition. The data we will present exhibits a significant degree of variability impacting host-pathogen interactions during UTIs. We argue that this variation is a key conclusion from our clinical study where we argue that patients are colonised not infected by *E. coli* and potentially other uropathogenic bacterial species.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P189

Using virulence-gene mutants to understand the molecular mechanisms behind Salmonella Dublin infections in reproductive tissues.

Jemma Franklin, Paul Barrow, Sabine Töttemeyer

University of Nottingham, Nottingham, United Kingdom

Abstract

Salmonella enterica serotype Dublin is the most common cause of infectious abortion in cattle in the UK. This gram-negative bacterium has been studied extensively within the context of intestinal infections, though genes in the Salmonella Pathogenicity Island (SPI) relating to bacterial invasion are yet to be investigated specifically in reproductive tissues. The aim of this study is to determine the genes most important in bacterial invasion of reproductive tissue cells.

Bovine caruncular epithelial cells (BCECs) were used to model the bovine reproductive tract. These cells were infected for 2 or 24 hours with *S. Dublin* SPI mutants, before being treated with 100ug/ml gentamycin to kill any non-internalised bacteria. The cells were then lysed using 0.5% triton, and the lysate was plated onto nutrient agar.

SipB/SopC/SptP triple-knockout mutants were unable to invade BCECs in 2h infections ($P < 0.05\%$), whilst SipB knockouts were less able to enter the cells than their parent strains. A marginal decrease is seen in SipB/SopC/SptP triple-knockouts and SipB mutants after 24h. In contrast, *S. Dublin* lacking SopE or SopB showed no significant difference in their ability to invade or replicate in the cells compared with the parent strain.

As expected, SipB, SopC and SptP are important for *S. Dublin* to invade BCEC cells as these genes mediate bacterial adhesion and actin reformation. SopC and SptP appear to be able to compensate for the loss of SipB during invasion. This is the first characterisation of *S. Dublin* infections in bovine caruncular cells.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P190

Croton lechleri as an effective antimicrobial product against ESKAPE pathogens

Megan Mortimer, Edward Taylor, Adrian Goodman

University of Lincoln, Lincoln, United Kingdom

Abstract

Antimicrobial resistance is increasingly becoming one of the greatest dangers to human health. *Croton lechleri* has been used throughout Latin American history as medicinal plant. The sap of *C. lechleri* has been shown to be particularly effective antimicrobial product.

Using sap extracted from *C. lechleri*, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were performed on four different preparations of the sap- preparation 1 was the raw sap as it came, preparation 2 was an agitated sap sample, preparation 3 and preparation 4 were ethyl acetate extractions to concentrate the proanthocyanidins in the sap (3 was the supernatant of the extraction). Each preparation was tested against *E. coli* DC0, *E. coli* ASMB1, *P. aeruginosa* 0750, *P. aeruginosa* 01, MRSA, MSSA, *K. pneumoniae*, *A. baumannii* and VRE.

Preparation 3 (the supernatant) demonstrated the least antimicrobial activity against all bacteria, followed closely by preparation 2 (agitated), preparation 1 demonstrated a higher antimicrobial activity than the agitated sample, but not as high as preparation 4 (concentrated proanthocyanidins) which demonstrated the most antimicrobial activity. None of the preparations demonstrated activity against *K. pneumoniae*. This suggests that the proanthocyanidins responsible for the blood red pigment of the sap are also the most potent antimicrobial compounds within the sap.

Croton lechleri has demonstrated remarkable antimicrobial activity against ESKAPE pathogens, however further research is needed to concentrate the proanthocyanidins from the sap and test the toxicity of the extraction.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P191

A Fatal case of contemporaneous occurrence of Cryptococcal meningitis and Tuberculous meningitis in a Post Traumatic Psychotic Patient.

Harman Multani, Veenakumari HB, Dwarakanath Srinivas, Kruthika Perumal, Ganesh Maher, Shruthi Uppoor, Nagarathna Chandrashekar

National Institute Of Mental Health And Neuro Sciences, Bengaluru, India

Abstract

The incidences of contemporaneous infection with *Mycobacterium tuberculosis* and *Cryptococcus neoformans*, is not uncommon in immunocompromised patients. However, reports of their coexistence in immunocompetent patients is rare. To the best of our knowledge this case is second of its kind case report in India.

Case : A 23 year old man presented to neurosurgery emergency services with a past history of head injury following which he developed post traumatic psychosis and was undergoing treatment for the same with complaints of headache, fever and altered sensorium with positive meningeal signs.

CT brain showed hydrocephalus with ooze . Haematological, Liver and renal parameters were normal. CSF fluid was clear, Glucose 69mg/dl , Protein 42mg/dl, cell count was 380cells/mm³, Neutrophils-60%, Lymphocytes-40%, India ink preparation revealed round budding yeast cells , CSF Cryptococcal antigen was positive , Fungal culture grew *Cryptococcus* confirmed as *C. gattii* by biochemical methods and was sensitive to Amphotericin , Flucytosine and intermediate to Fluconazole . In addition CSF sample was processed for tubercular meningitis (ZN staining, culture by conventional method and automated method . He was started on Amphotericin and Fluconazole 400 mg.

Patient did not improve much and succumbed to infection after eight days of hospitalisation. On the 17th day MGIT flagged positive and culture grew *M. tuberculosis* complex .The final diagnosis was Tuberculous meningitis with Cryptococcal meningitis in an immunocompetent patient.

Conclusion: Objective of this case report is even if there are no signs of immunodeficiency , TB infection can be cardinal cause of paving way to other opportunistic infections like *Cryptococcus*.

P192

The role of prophage in virulence factor acquisition, regulation and expression in the Lancefield Group A *Streptococcus*

Alex Remington, Claire Turner

University of Sheffield, Sheffield, United Kingdom

Abstract

Background

The UK is experiencing a dramatic upsurge in scarlet fever, a toxin-mediated disease caused by the Lancefield Group A *Streptococcus* (GAS). Specific virulence factors are believed to be responsible for the pathogenesis of scarlet fever, notably the streptococcal superantigens. Most superantigen genes are associated with prophage elements, which may control their expression.

Methods

All available genome sequences for genotype *emm4* GAS were compared bioinformatically and the prophage regions compared. A PCR method was developed to detect integrated and excised prophage. Quantitative real time PCR was used to measure virulence gene copy number and transcription. Environmental stimuli were used to induce prophages.

Results

We identified a novel lineage within the *emm4* GAS population, a major *emm*-type associated with scarlet fever. This lineage is characterised by substantial gene loss within the three prophage regions of *emm4* GAS, yet with virulence genes intact; superantigens *speC* and *ssa*, and DNases *spd1* and *spd3*. PCR analysis detected spontaneous induction of all three prophages in *emm4* isolates with complete prophages. Conversely, induction of prophages could not be detected in isolates with prophage gene loss. Prophage induction increases virulence gene copy number which in turn can lead to increased expression. Environmental factors, such as DNA-damaging agents, antibiotics and exposure to host cells may also result in prophage induction and effect virulence gene expression.

Conclusion

This comparison of isolates with complete and incomplete prophages within the same *emm*-type will enable us to identify the impact of prophage induction on virulence gene expression and factors required for induction.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P193

Investigation of the role of *Campylobacter jejuni* Type VI secretion system during interactions with host cells

Janie Liaw, Geunhye Hong, Abderrahman Hachani, Brendan Wren, Ozan Gundogdu, Nick Dorrell

LSHTM, London, United Kingdom

Abstract

The Type VI Secretion System (T6SS) is a contact-dependent secretion machinery capable of delivering effectors through contraction of a TssBC sheath, which translocates a TssD inner tube out of the cell and across a bacterial or host membrane. The T6SS is becoming increasingly prevalent in *Campylobacter jejuni* human and chicken isolates. To further investigate the role of the *C. jejuni* T6SS, *tssB*, *tssC*, and *tssD* were mutated in *C. jejuni* human isolates 488 and 43431. Secretion of TssD from *C. jejuni* 488 and 43431 wild-type strains was detected using a TssD antibody indicating the presence of a functional T6SS. TssD was also secreted from *tssB* or *tssC* mutants. Whether this relates to the presence of TssD in *C. jejuni* outer membrane vesicles will be reported. RT-PCR and qPCR were performed to compare the effects of bile salts on the expression of T6SS genes. T6SS gene expression was up-regulated in the presence of sodium deoxycholate, a secondary bile salt. Oxidative and aerobic stress response genes were also upregulated in the T6SS-positive wild-type strain and reduced resistance to oxidative stress observed in a 488 *tssD* mutant. The role of *C. jejuni* T6SS in interactions with host cells was also investigated using *Galleria mellonella* infection assays. Reduced cytotoxicity of the 488 *tssD* mutant in *G. mellonella* infection was observed. Our results indicate that the *C. jejuni* T6SS is associated with the oxidative stress response and in interactions with host cells.

194

Investigating the biogenesis of *Campylobacter jejuni* outer membrane vesicle production

Cadi Davies¹, Aidan Taylor², Ozan Gundogdu¹, Brendan Wren¹, Abdi Elmi¹, David Kelly², Nick Dorrell¹

¹LSHTM, London, United Kingdom. ²University of Sheffield, Sheffield, United Kingdom

Abstract

Campylobacter jejuni constitutively releases outer membrane vesicles (OMVs), although the exact process is unknown. The *mla* pathway is implicated as a novel mechanism for the biogenesis of OMVs in Gram-negative bacteria. MlaA, a putative lipoprotein, and MlaC, a putative periplasmic binding protein, were found to impact vesiculation and maintain lipid asymmetry in the outer membrane. We have investigated the role of the *mla* pathway in *C. jejuni* OMV biogenesis by comparing a *mlaA* mutant against the 11168 wild-type. Protein and lipid content associated with OMVs isolated from the mutant and complement strains were compared against 11168 wild-type OMVs. Protein content was quantified using a BCA assay and lipid content was quantified using a KDO assay. There were statistically significant increases in protein and lipid associated with OMVs from the *mlaA* mutant. To determine the phenotypic effect of the *mlaA* mutation, bacterial survival was investigated following incubation with Polymyxin B and both biologically relevant and extreme concentrations of sodium taurocholate (ST). The *mlaA* mutant exhibited increased resistance to Polymyxin B, a phenotype thought to be associated with increased vesicle production. ST induced stress was not observed to impact strains during exponential growth. RT-PCR was used to determine expression of *mlaA* and *mlaC* in the absence and presence of 0.2% (w/v) ST. *mlaA* and *mlaC* are both transcribed in the 11168 wild-type and expression was affected by ST. Our results suggest MlaA and MlaC play a significant role in OMV biogenesis and maintaining the lipid asymmetry of the protective outer membrane

P195

The *Campylobacter jejuni* oxidative stress regulator RrpB is associated with a genomic hypervariable region and altered oxidative stress resistance

Ozan Gundogdu¹, Daiani Teixeira da Silva¹, Banaz Mohammad¹, Abdi Elmi¹, Brendan Wren¹, Arnoud van Vliet², Nick Dorrell¹

¹LSHTM, London, United Kingdom. ²University of Surrey, Guildford, United Kingdom

Abstract

Bacteria such as *Campylobacter jejuni* that can survive within a host or in the environment require variable responses to survive stresses after exposure to different levels of reactive oxygen species. MarR-type transcriptional regulators RrpA and RrpB play a role in controlling both the *C. jejuni* oxidative and aerobic stress responses. Analysis of 3,746 *C. jejuni* and 486 *Campylobacter coli* genome sequences showed that whilst *rrpA* is present in over 99% of *C. jejuni* strains, the presence of *rrpB* is restricted and appears to correlate with specific MLST clonal complexes (predominantly ST-21 and ST-61). However *C. coli* strains lack both *rrpA* and *rrpB*. In *C. jejuni rrpB*⁺ strains, *rrpB* is located within a variable genomic region containing the IF subtype of the type I Restriction-Modification (*hsd*) system, whilst this variable genomic region in *C. jejuni rrpB*⁻ strains contains the IAB subtype *hsd* system and not *rrpB*. *C. jejuni rrpB*⁻ strains exhibit greater resistance to peroxide and aerobic stress than *C. jejuni rrpB*⁺ strains. Inactivation of *rrpA* resulted in increased sensitivity to peroxide stress in *rrpB*⁺ strains, but not in *rrpB*⁻ strains. Gene expression changes between *C. jejuni* 11168H wild-type strain, *rrpA* and *rrpB* mutants are being investigated using RNA-Seq analysis to obtain a global picture of expression changes in response to H₂O₂ stress. Combining this with EMSA and qPCR data, we aim to present a hypothetical model for the role of RrpA and RrpB in regulating *C. jejuni* responses to both oxidative and aerobic stress.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P196

A novel *mecC* allotype, *mecC3* in a human *Staphylococcus saprophyticus* isolate

Alison C MacFadyen, Gavin K Paterson

University of Edinburgh, Edinburgh, United Kingdom

Abstract

Methicillin-resistance in staphylococci is conferred by an alternative penicillin-binding protein (PBP2a/2') with low affinity for most β -lactam antibiotics. PBP2a is encoded by *mecA* which is carried on a mobile genetic element known as *SCCmec*. A variant of *mecA*, *mecC*, was described in 2011. *mecC* has been found in *Staphylococcus aureus* from humans and a wide range of animal species as well as a small number of other staphylococcal species. Integration into the chromosome by *SCCmec* is mediated by a group of serine recombinases, CcrAB and CcrC. Recently, we have identified a novel *mecC* allotype, *mecC3*, encoded by a human carriage isolate of *Staphylococcus saprophyticus*, 82B. We carried out genome sequencing to allow for an accurate assembly and comparative genomic study of the *SCCmec* region encoding *mecC3*. Not only is *mecC3* a novel allotype, it is also encoded within a *SCCmec* element distinct from those previously associated with *mecC*, which includes a previously unseen *ccrAB* pairing (*ccrA5B3*). It is as yet unknown what role this *ccrAB* pairing may have on dissemination of the *SCCmec* of 82B.

The *SCCmec* element of *S. saprophyticus* 82B, is dissimilar to the archetypal *SCCmec* XI encoding *mecC* in *S. aureus* and to other elements encoding *mecC*, which highlights the diverse context in which *mecC* may be found. Furthermore, this is the first reported *mecC* coagulase-negative staphylococci in humans, and therefore highlights the need to be vigilant with regards to their emergence in human medicine and the diagnostic issues this presents.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P197

Drivers of host switching in *Staphylococcus aureus*.

Marta Matuszewska, Lucy Weinert

University of Cambridge, Cambridge, United Kingdom

Abstract

Some of our most serious pandemics are caused by pathogens switching and establishing in a new host species and so understanding the epidemiological factors for switching success is essential for curbing pathogen spread. *Staphylococcus aureus* is a major pathogen responsible for significant global human morbidity and mortality, and economically important infections of livestock. Using large-scale population genomic approach, aspects of the host biology, including phylogenetic distance and various immune properties of the hosts with newly developed statistical techniques we have examined factors promoting switching and establishment of *S. aureus* between hosts. We have found that the host phylogeny is a predictor of *S. aureus* host shifts. *S. aureus* is more likely to establish itself in a new host species which is closely related to the original host. There are two distinct ways in which the host phylogeny can affect host shifts. The 'distance effect' predicts that host shift will most likely occur in more closely related species resulting in the expected levels of susceptibility with distance from the natural host. The 'phylogenetic effect' will have no effect on the expected levels of susceptibility. Future laboratory experiments will help us to disentangle the different processes involved in the host shifts leading to the emergence of pathogenic clones.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P198

Molecular epidemiology of emergent pathogens associated with Canine Infectious Respiratory Diseases in the United States

GRAZIELI MABONI, Paula Bartlett, Ingrid Fernandez, Susan Sanchez

University of Georgia, ATHENS, USA

Abstract

Canine infectious respiratory disease (CIRD) is an endemic syndrome with multiple respiratory pathogens associated with disease occurrence. The clinical signs caused by different pathogens are similar, which makes differential diagnosis challenging. To attain new insights into the disease epidemiology, we conducted surveillance using molecular methods. Additionally, a novel multiplex PCR was developed to simultaneously detect and differentiate two species of *Mycoplasma*.

Nasal, oropharyngeal, tracheal swabs and lung tissues (n=562) were processed at Athens Veterinary Diagnostic Laboratory (University of Georgia, USA). Canine Adenovirus 2 (CAV-2), Canine Distemper Virus (CDV), Canine Parainfluenza Virus (CPIV), *Bordetella bronchiseptica*, Coronavirus, Influenza, *Streptococcus equi* subsp. *zooeconomicus*, *Mycoplasma canis* and *M. cynos* were detected by standard or Real-Time PCR.

Preliminary results revealed that CPIV (29%), *M. canis* (23.6%) and *M. cynos* (24.5%) were the most commonly detected pathogens followed by *Influenza* (11.2%), *B. bronchiseptica* (9%), Coronavirus (4.6%), CAV (2.5%), CDV (2%) and *S. equi* subsp. *zooeconomicus* (0.8%). Nasal-pharyngeal and oropharyngeal swabs showed the highest percentage of positive samples. Co-infections occurred in 46 specimens, which were positive for 2 to 5 different CIRD agents.

In summary, while confirming that CPIV is one of the main pathogens associated with CIRD, this study highlights the role of emerging bacteria, such as *M. canis* and *M. cynos*. Further analysis will elucidate the role of co-infections in clinically ill and healthy dogs. Our novel multiplex PCR for *M. canis* and *M. cynos* provides an efficient diagnosis alternative that will allow for accurate disease therapy and control.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P199

Exploring the interactions of *Shigella sonnei* with macrophages

Jayne Watson, Avinash Shenoy, Abigail Clements

MRC Centre for Molecular Bacteriology and Infection, London, United Kingdom

Abstract

Shigella are human-adapted pathogens causing severe dysentery and resulting in 100000 deaths annually. *Shigella flexneri* is the dominant cause of this disease and is particularly associated with poor hygiene conditions in low-income countries; as a result the majority of studies have used this species to characterise *Shigella* infection. However in middle and high-income countries *S. sonnei* replaces *S. flexneri* as the dominant cause of Shigellosis. During *S. flexneri* infection, escape from the phagocytic vacuole and induction of macrophage pyroptosis is a key aspect of infection. This allows the bacteria to escape from the macrophage and invade the colonic epithelial cells via the basolateral surface, where it can replicate and spread. *S. sonnei* is presumed to behave in a similar way however we observed that *S. sonnei* does not induce cell death to the same extent as *S. flexneri*. We have been able to attribute this to differences in the amount of cytosolic bacteria due to the combined effects of reduced phagocytosis and reduced vacuole escape by *S. sonnei*. We are currently investigating the *S. sonnei* specific factors that are involved in these processes. Our findings indicate important differences in the infection process of these two *Shigella* species.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P200

Characterisation of the UspABC carbohydrate transporter from *Mycobacterium tuberculosis*

Magdalena Karlikowska, Hadyn Parker, Elizabeth Fullam

University of Warwick, Coventry, United Kingdom

Abstract

Infection by *Mycobacterium tuberculosis* (*Mtb*) remains a major global concern with an estimated 1.7 million tuberculosis (TB) deaths in 2016. *Mtb* is an intracellular pathogen residing predominantly inside macrophages, a nutrient scarce environment. Recent studies have shown that the UspABC sugar transporter is essential during early stages of *Mtb* infection and has a key role in virulence. The objective of this study was to understand the role of UspABC transporter in carbohydrate uptake and metabolism in mycobacteria. UspC, the substrate-binding unit of the UspABC transport system, was identified to recognise amino-sugars. Specifically, it was found to interact with chiobiose, a disaccharide that is structurally similar to the peptidoglycan backbone, suggesting its role in peptidoglycan recycling in *Mtb*. The topology of UspABC transporter was investigated by expressing all of its subunits fused to β -lactamase reporter in a *Mycobacterium smegmatis* model system. We have characterised the *uspABC* operon organisation through cDNA analysis. To determine the potential carbohydrate substrates of the ABC-transport system, we knocked out the transporter from *M. smegmatis* and also overexpressed UspC and UspABC in *M. smegmatis* and *M. bovis* BCG. Biolog phenotypic microarrays were used to identify differential carbohydrate uptake profiles between wild type and knockout or overexpression strains. Quantitative uptake of potential UspC carbohydrate substrates was thoroughly examined by Reagent-Free Ion Chromatography (Dionex). In conclusion, the *Mtb* UspABC transporter has an important physiological role in nutrient acquisition which can be utilised in TB drug discovery efforts.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P201

Analysis of rumen microbial genomes in silico and in vitro cultures reveals widespread distribution of antibiotic resistance genes.

Yasmin Neves Vieira Sabino¹, Ana Julia Silva Moreira¹, Fernanda Godoy Santos¹, Linda Boniface Oyama², Mateus Ferreira Santana¹, Sharon Ann Huws², Hilario Cuquetto Mantovani¹

¹Universidade Federal de Viçosa, Viçosa, Brazil. ²Queen's University Belfast, Belfast, United Kingdom

Abstract

The misuse of antibiotics in livestock, including ruminants, has been associated with the rise of multidrug-resistant pathogens in the environment and hospital setting. Due to the diversity and ecological properties of the rumen microbial ecosystem, ruminants represent a potential reservoir for antibiotic resistance genes.

In this study, we investigated the distribution and diversity of antibiotic resistance genes in 450 genomes of ruminal bacteria. Mining of antibiotic resistance genes was performed using the Resfinder, Resfams and ARG-ANNOT platforms. The resistance phenotypes identified *in silico* were analysed *in vitro* using the E-test method and cultures representative of the bacterial genomes under study. The phylogenetic distribution of the resistance genes was represented based on a 16S tree of the bacterial genomes. Acquired resistance genes were identified in 17% of the bacterial genomes using the Resfinder platform. Resfams and ARG-ANNOT analyses confirmed most of these resistance genes and indicated even greater abundance of resistance genes in the bacterial chromosome. Some complete vancomycin resistance operons were found in bacteria of the phylum Firmicutes. Analysis of the resistance genes showed that their distribution does not follow the phylogeny of the microbial genomes. Less than 50% of the resistance identified *in silico* was in agreement with *in vitro* resistance assessments.

These results demonstrate that analyses in different platforms alongside *in vitro* assessments are important to identify antibiotic resistance genes/phenotypes. It also confirms that livestock, and rumen bacteria specifically are a potential reservoir of resistance genes, suggesting that these genes could be transmitted by horizontal transfer.

P202

The DSB system is a novel target for the treatment of ESBL and carbapenemase-producing Gram-negative bacterial pathogens

R. Christopher D. Furniss¹, Diego Gonzalez², Ciarán L. Kelly³, Laurent Dortet¹, Despoina A.I. Mavridou¹

¹Imperial College London, London, United Kingdom. ²University of Lausanne, Lausanne, Switzerland.

³University of Oxford, Oxford, United Kingdom

Abstract

The Disulfide bond (DSB) system is responsible for the introduction of disulfide bonds, covalent linkages between pairs of cysteine residues, into periplasmic and extracellular proteins. These linkages facilitate correct protein folding and impart the stability necessary for proteins to function in the harsh extra-cytoplasmic environment. A wide range of clinically important bacterial pathogens, such as *Escherichia coli* and the ESKAPE pathogens *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* spp., produce extended spectrum β -lactamases (ESBLs) and/or carbapenemases, hydrolytic enzymes that inactivate a range of β -lactam antibiotics and thus contribute to multidrug-resistance and treatment failure. Many of these enzymes contain at least one intra-molecular disulfide bond.

Using the *E. coli* MC1000 strain and an isogenic *dsbA* mutant we assessed the ability of a panel of eleven clinically important cysteine-containing β -lactamases to confer resistance to a range of β -lactam antibiotics. In the absence of *dsbA* nine of these enzymes were less able to confer resistance to at least one of the antibiotics tested, compared to the resistance conferred upon the parental *E. coli* strain. We were also able to phenocopy these results via chemical inhibition of the DSB system. Thus, inhibition of disulfide bond formation may facilitate the resensitisation of a variety of ESBL and carbapenemase-producing multidrug-resistant pathogens to currently available antibiotics. On-going work is focussed on inhibiting the DSB system in a range of clinical isolates, using both chemical and novel genetic approaches.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P203

Staphylococcus aureus targets corneodesmosin to colonise skin in atopic dermatitis

Aisling M. Towell¹, Pauline Pauline², Cécile Feuillie², Alan D. Irvine³, Yves F. Dufrêne², Joan A. Geoghegan¹

¹Trinity College, Dublin, Ireland. ²Université catholique de Louvain, Louvain-la-Neuve, Belgium. ³Our Lady's Children's Hospital Crumlin, Dublin, Ireland

Abstract

Staphylococcus aureus plays an important role in atopic dermatitis (AD), a chronic inflammatory skin disease. During an AD flare the diversity of the skin's normal microbiota is diminished and skin colonization by *S. aureus* increases the clinical severity of AD. However the molecular determinants of adhesive interactions between bacteria and skin are poorly understood. *S. aureus* adheres to dead flattened skin cells known as corneocytes in the outermost layer of the epidermis. Corneocytes in AD skin have an altered surface morphology compared to corneocytes from healthy skin. Corneodesmosin, an adhesive protein normally confined to the tight junctions between corneocytes, decorates the tips of villus-like projections on the surface of AD corneocytes. Here we identify corneodesmosin as a key ligand for *S. aureus* on AD corneocytes. We show that strains of *S. aureus* isolated from infected AD skin lesions adhere to recombinant corneodesmosin. We identify three proteins exposed on the bacterial surface as being important for adherence to corneodesmosin. All three cell proteins were expressed recombinantly and shown to bind to corneodesmosin with high affinity using surface plasmon resonance. High-resolution imaging of corneocytes from AD skin revealed that strong adhesive interactions are not uniformly distributed across the corneocyte surface but mostly concentrate on the tips of villus-like projections, consistent with corneodesmosin being a ligand. In summary this study identifies novel interactions between *S. aureus* and corneodesmosin and thus provides important new insights into the first steps in the establishment of *S. aureus* skin colonisation in AD patients.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P204

Melanised Fungal Brain Abscess: A Four Decades Review from Tertiary Neuro-Care Center India.

Kruthika Perumal, Nagarathna chandrashekar, Veena kumari HB, DWARAKANATH S

NIMHANS, BANGALORE, India

Abstract

Introduction: - Fungal brain abscess is a universal health problem with grave outcome. Fungal brain abscess (FBA) due to melanized fungi are usually cryptic and poses diagnostic challenge: majority of the times diagnosis done only after postmortem.

Materials and methods: - This is a retrospective study conducted in the department of Neuromicrobiology NIMHANS, Bangalore, India. Culture confirmed cases of melanized fungal brain abscess cases from January 1st, 1979 to December 11th, 2017 were analyzed. Clinico-mycological parameters were collected from the hospital medical record section.

Results: - Out of total 2956 brain abscess cases 36 (1.2%) grew fungi and out of these 16(0.5%) grew melanized fungi. *Cladophialophora bantiana* (87.5%) was the predominant isolate, followed by *Fonsecaea pedrosoi* (6.25%) and *Neoscytalidium dimidiatum* (6.25%) with cryptic origin. All age group affected, male: female ratio was 15:1. Head ache (75%), limb weakness (62.5%) and fever (43.7%) were the most common presentations. Craniotomy with Excision/ Decompression (68.7%) was the predominant surgical management followed by treatment with Amphotericin (68 %). Fatal outcome was seen in 50% of cases.

Conclusion: Melanized fungal brain abscess formed 44.4% of all FBA. Neurotropic *Cladophialophora bantiana* remains to be the most notorious cause for fungal brain abscess over 39 years with fatal outcome till date. Rare and non-human pathogenic fungi like *Neoscytalidium dimidiatum* can cause of brain abscess. Hence, High index of suspicion with early diagnosis and appropriate intervention alone can reduce mortality and morbidity.

Keywords: - Melanised fungal brain abscess, *Cladophialophora bantiana*, *Fonsecaea pedrosoi* and *Neoscytalidium dimidiatum*

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P205

Exploring the role of the iron-sulfur cluster regulator IscR in *Yersinia* spp.

Clair Brooks, Steve Atkinson, Paul Williams

University of Nottingham, Nottingham, United Kingdom

Abstract

Despite causing vastly different diseases *Y. pseudotuberculosis* and *Y. pestis* share an array of virulence factors and associated phenotypes, which exist as part of a complex regulatory network. Recently a novel component of this network has emerged, the iron-sulfur cluster regulator, IscR. IscR is a transcription factor best understood for its role in regulating the formation of Fe-S cluster containing proteins in *E.coli*, but recent evidence indicates it is involved in many other pathways in different species, and could be considered a global regulator of gene expression. It is now known that in *Y. pseudotuberculosis* IscR regulates type three secretion, a key virulence mechanism employed to inject effector proteins into host cells. Given the links between type three secretion and other virulence associated behaviours in *Yersinia*, including biofilm formation and motility, it is likely that IscR's full role in *Yersinia* virulence is not yet understood. Using *iscR* deletion mutants, this study aims to further characterise the role of IscR in *Y. pseudotuberculosis* and *Y. pestis*. As a potential virulence regulator IscR could be a future target for alternative antimicrobial therapies, a necessity given the threat of multidrug resistance and the classification of *Y. pestis* as a re-emerging pathogen.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P206

Fluorescent artificial cellular environments to interfere with bacterial adhesion

Oliver Creese¹, Anne-Marie Krachler², Francisco Fernandez-Trillo¹

¹University of Birmingham, birmingham, United Kingdom. ²University of Texas, Houston, USA

Abstract

Pathogenic bacteria's ability to colonise and cause disease to a host depends on the bacterium's successful attachment to host cells. Here we report the synthesis of water soluble fluorescent polymers of well-defined length with high affinity for bacterial cell walls and lectins such as fimH. These polymers are then capable of sequestering the bacteria and interfering with their adhesion while minimising evolutionary stress.

We will present the synthesis of polymeric materials displaying multiple units of sugars and /or positively charged groups which can bind to bacterial lectins and membranes via multivalent and cooperative interactions. These polymers are fluorescently labelled and we can easily modulate their chemical composition, charge and hydrophobicity to produce a library of materials with different affinities for the bacteria. The effect of these polymers on bacterial adhesion and their ability to colonise the host will be demonstrated using a combination of microbiology assays and imaging techniques using relevant human pathogen *Vibrio cholerae*. We will demonstrate how these functional polymers induce "forced communities" in this pathogen, reducing their ability to adhere to cells as well as affecting their physiology including the expression of virulence factors. These polymers are thus a promising tool to understand and control pathogenic bacteria.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P207

Characterisation of tetracycline resistant bacterial isolates from the interdigital skin of sheep collected before and after foot bathing

Katie Kelly, Adam Blanchard, Peers Davies, Sabine Totemeyer

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

Abstract

The heavy use of antibiotics throughout the world has heightened the selective pressure incurred by bacteria to develop mechanisms to resist the effects of antibiotics. In sheep farming, the antibiotic tetracycline is most commonly used to treat lameness. This study characterized tetracycline resistant putative *E. coli* isolated from ovine feet before and after footbath treatment. A broth dilution assay and antibiotic disc diffusion assay were used to further characterise tetracycline resistance as well as resistance to a range of other antibiotics.

Tetracycline resistant putative *E. coli* isolates had varying levels of resistance which affected colony phenotype. These isolates were sensitive to gentamycin and ampicillin. The latter was surprising as B-lactams are the 2nd most common antibiotic used in sheep farming.

However, 98% of the putative *E. coli* isolates were multi-drug resistant with additional resistance to Streptomycin and Sulphatriad. Sulphonamides, the class of antibiotics to which Sulphatriad belongs, account for less than 0.9% of antibiotics used in sheep, suggesting that AMR genes in bacteria transfer between food producing species easily. This study's findings warrants the implementation of careful antibiotic stewardship strategies across all sectors.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P208

Exploring gene essentiality in *Klebsiella pneumoniae*

Jessica Gray, Karl Dunne, Ian Henderson

Institute of Microbiology and Infection, Birmingham, United Kingdom

Abstract

Klebsiella pneumoniae is a gram-negative opportunistic pathogen and is associated primarily with nosocomial infections. *K. pneumoniae* infections range from urinary tract infections (UTIs) to life-threatening pneumonia and bacteraemia. Clinical isolates of *K. pneumoniae* are increasingly multidrug resistant to distinct classes of front line antibiotics and consequently the identification of novel drug targets for the treatment of these infections is essential. The elucidation of bacterial genes essential for survival is a prerequisite for identifying good potential drug targets. Essential genes are highly conserved and are favourable for broad-spectrum bacterial inhibition. Essential genes can be identified using a myriad of genetic strategies, which include transposon sequencing (Tn-seq) and transposon directed insertion-site sequencing (TraDIS). TraDIS combines transposon insertional mutagenesis with massively parallel sequencing (MPS) of the transposon insertion sites, to identify mutants in a population which lack an essential gene. We have generated a highly saturated *K. pneumoniae* TraDIS library consisting of ~1.1 million mutants. Our analysis has identified 337,262 unique insertion points (UIPs), representing an average transposon insertion every 17 base pairs within the genome. Utilising our TraDIS library, we have identified 388 genes which are essential for *K. pneumoniae* growth in laboratory conditions.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P209

Complement susceptibility in relation to genome sequence of recent *Klebsiella pneumoniae* isolates from Thai hospitals

Jessica Loraine¹, Eva Heinz², Richard Stabler³, Supayang Voravuthikunchai⁴, Potjane Srimanote⁵, Pattarachai Kiratisin⁶, Nicholas Thomson^{2,3}, Peter Taylor¹

¹UCL, London, United Kingdom. ²The Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

³London School of Hygiene and Tropical Medicine, London, United Kingdom. ⁴Prince of Songkla University, Songkla, Thailand. ⁵Thammasat University, Bangkok, Thailand. ⁶Mahidol University, Bangkok, Thailand

Abstract

Klebsiella pneumoniae has emerged in recent decades as a prime cause of systemic nosocomial and community-acquired infections in immunocompromised and healthy individuals. Survival of these heavily encapsulated opportunistic pathogens depends to a large extent on their capacity to survive in the bloodstream in the presence of potentially bactericidal complement. We determined the complement susceptibility of 170 recent *K. pneumoniae* isolates from three Thai hospitals, determined other phenotypic traits such as antibiotic susceptibility and capsule hyperviscosity and used next-generation sequencing to identify genes determining complement susceptibility. Only 19.7% of isolates were classified as multi-drug resistant although the isolates belonged to clonal lineages identified previously as representative of the global *K. pneumoniae* population as determined by phylogenetic analysis. We used the Kaptive software tool to identify K1, K2 and K51 as the major capsule types within our dataset. The proportion of complement resistant isolates varied from 55-66%, dependent on geographical site; all K1 isolates were complement resistant. In contrast to previous studies with capsule-free mutants, we found no correlations between capsule hyperviscosity, as determined by the standard string test, the physical dimensions of the capsule and complement susceptibility. A number of genes were significantly enriched in the complement resistant isolates and a proportion encoded outer membrane (OM) components not previously associated with the capacity of *K. pneumoniae* to avoid complement-mediated killing. The data supports the contention that complement resistance is determined by OM constituents that limit the capacity of complement membrane attack complexes, the lethal entity, to insert into lipid-rich OM domains.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

10

A case report of severe disseminated infection caused by an emerging strain of methicillin-sensitive *Staphylococcus aureus* (CC398) lacking typical virulence factors

P Jewell, A Kearns, A Singanayagam, E Wong, M Coleman, G Russell, J Hatcher

Imperial College Healthcare NHS Trust, London, United Kingdom

Abstract

The clonal complex 398 (CC398) strain of methicillin-resistant *Staphylococcus aureus* (MRSA) has long been known to cause livestock-associated infection. However, recently methicillin-sensitive *Staphylococcus aureus* (MSSA) of strain CC398 has been emerging as an important invasive human pathogen, with increasing reports of severe infection in humans with no prior livestock contact. Here we present a case report of severe disseminated MSSA CC398 infection in an immunocompetent man.

A 60 year old Colombian businessman, with no prior livestock contact, presented with severe sepsis on background of a week long history of muscular pains. CT and MRI scans demonstrated multiple infective foci, including a large right psoas collection; right deltoid and left subscapular pyomyositis; a retropharyngeal abscess; ventral, epidural and subdural collections predominantly in the lumbar region; and discitis and osteomyelitis of the third and fourth cervical discs and vertebrae respectively. MSSA was isolated in 5 out of 8 blood culture bottles and gene sequencing demonstrated the MLST clonal complex 398 lineage of *S. aureus*. All 14 toxin genes screened were not detected, including Panton-Valentine Leukocidin (PVL). The patient made a slow recovery over a period of months after multiple drainage procedures of various collections and a protracted course of intravenous flucloxacillin and rifampicin.

In summary, this case demonstrates the severity of infection that can be caused by CC398 MSSA, despite lacking typical virulence factors. These findings highlight the importance of this emerging highly-pathogenic strain of MSSA and further studies to elucidate its virulence are warranted.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P211

A comprehensive evaluation of the impact of organic acids on *Pseudomonas* growth, biofilm formation, and biofilm eradication.

Francesca Bushell¹, Sara Jabbari¹, Peter Tonner², Gareth Hughes^{1,3}, Amy Schmid², Peter Lund¹

¹University of Birmingham, Birmingham, United Kingdom. ²Duke University, Durham, USA. ³University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Abstract

Organic acids are growing in popularity as a alternative to antibiotic treatment for bacterial infections, particularly of wounds. However, the range of organic acids tested to date is limited and detailed studies on the combined effects of pH and concentration have not been done, despite the likely antibacterial mechanism of organic acids depending critically on their ionisation state. Here, we have compared the effects of eight different organic acids at five different pH values and three different concentrations on the growth and ability to form biofilms of two strains of *P. aeruginosa*: PA01 and a clinical isolate. Because of the large amount of data to be analysed we compared three different methods for data fitting: two using a parametric approach (with and without a lag phase) and one using Gaussian process regression. Although the general conclusions from all methods were the same, the parametric methods were less good at handling data when growth was poor or atypical and tended to either fail to produce a fit, or to produce values there were clearly inaccurate. We also evaluated the ability of a smaller range of organic acids to eradicate biofilms across a wider range of *Pseudomonas* strains. Overall, acetic and citric were the most effective at inhibiting growth and biofilm formation, and eradicating biofilms, but a significantly higher concentration was required for eradication.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P212

Structural determination of the extracellular protein CexE from Enterotoxigenic *Escherichia coli*.

Christopher Icke¹, Timothy Knowles¹, Timothy Wells², Ian R. Henderson¹

¹University of Birmingham, Birmingham, United Kingdom. ²University of Queensland, Brisbane, Australia

Abstract

Enterotoxigenic *Escherichia coli* (ETEC) and Enteroaggregative *E. coli* (EAEC) are important bacterial pathogens in both the developing and industrialised world. The prototypical EAEC strain 042 harbours the *aap* gene which encodes a product required for the correct orientation of the aggregative adherence fimbriae (AAF). In the prototypical ETEC strain H10407 an Aap homolog, CexE, is present; yet the AAF are not encoded in ETEC strains. While the role of Aap in EAEC 042 has been investigated there is no information on CexE in ETEC. From phylogenetic analysis, it was found that Aap was only present in EAEC strains and CexE was only present in ETEC strains. To understand the differences in Aap and CexE, the structure of CexE was determined by NMR. Despite the lack of sequence identity between the two proteins the structure of CexE is very similar to that of Aap, which is likely due to the shared secretion machinery of these proteins. The CexE structure is highly β -sheeted with α -helices at each pole similar to Aap. However, CexE also has an additional α -helix and a different surface charge. The slight differences between the proteins and their presence in different pathovars suggest that they may have alternate functions. These proteins appear to be required for pathogenesis and their similar structure present a promising target for vaccine development yet the role of CexE in ETEC still requires further investigation.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P213

Public Engagement in Natural Product Screening to Raise Awareness of Antimicrobial Resistance

Abdu Aldarhami^{1,2}, Florie Desriac¹, William Marsh¹, Luke Cresswell¹, Yvonne Wezel¹, Matthew Koch¹, Mathew Upton¹

¹Plymouth University Peninsula Schools of Medicine and Dentistry, Derriford Research Facility, Plymouth, United Kingdom. ²School of Medicine and Healthcare Sciences, Turabah branch, Taif University, Saudi Arabia, Taif, Saudi Arabia

Abstract

Background: Antimicrobial resistance (AMR) is one of the greatest threats to human health, but it can be difficult to educate and engage the public in this topic. This work involved members of a local University of the 3rd Age (U3A) group, aiming to increase AMR awareness. It also involved three research project students.

Material/methods:

The topic of AMR was introduced to 30 members of a Devon U3A group during an educational talk. Attendees were then given transport swabs and instructed to swab different surfaces of their choice, before swabs were returned to us for further analysis by research project students who inoculated a range of media with the swabs. Bacterial isolates were recovered and screened for natural products and identified by 16S rRNA analysis. Production in broth was optimised and antimicrobials purified from six selected strains.

Results: Approximately 100 isolates were recovered and inhibitors from six purified through three rounds of liquid chromatography. Producers were mostly *Bacillus* species. Inhibitors will be interrogated with MALDI-ToF-MS. Aside from these results, the more important outcomes were engagement of the U3A group, many of whom had limited awareness of AMR before the sessions. All data will now be made available to U3A participants through a follow up talk, which will involve the research students. This will encourage further participation in different aspects of AMR.

Conclusions: This work addressed several aspects of AMR; educating the public and encouraging engagement through participation as individuals and in collaboration with research scientists, which enhanced the project student experience.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P214

IMMUNOREGULATORY PROPERTIES OF HELICOBACTER PYLORI INFECTION IN VIVO

Ekene Ogbodo, Kazuyo Kaneko, Dona Reddiar, Karen Robinson

Centre for Biomolecular Sciences, University of Nottingham, Nottingham, NG7 2RD, Nottingham, United Kingdom

Abstract

The bacterial pathogen *H.pylori* induces a prominent CD4⁺CD25^{hi}FOXP3⁺ regulatory T-cell (Treg) response in infected humans; an anti-inflammatory cell. The *H.pylori* virulence factor vacuolating cytotoxin A (VacA) induces Treg differentiation and interleukin-10 (IL-10) production *in vitro*. The s1/i1 form of VacA is strongly associated with disease outcomes. We aimed to compare the Treg response induced *in vivo*, by *H.pylori* mutants expressing different forms of VacA.

Groups of 18 female C57BL/6 mice were infected orally with isogenic *H.pylori* mutants expressing the s1/i1 or s2/i2 form of VacA. A control group received plain Brucella broth as a placebo. Mice were killed at 3, 6 and 9 weeks post infection and their infection status confirmed. Spleen cells were isolated, stimulated with mitogens for 6 hours and stained with fluorochrome-conjugated antibodies. Treg populations were quantified by flow cytometry. Treg cells were purified and assayed for suppressive functional activity *in vitro*.

Mitogen stimulation resulted in significantly increased frequencies of IL-10+ Tregs at all time-points and in all groups (p<0.001). No statistically significant differences were found in frequencies of IL-10+ Tregs between the groups. There were also no differences in the functional suppressive activity of purified Tregs.

Despite previously finding markedly increased Treg populations in peripheral blood from infected patients, we were unable to find increased Treg numbers in the spleen of infected mice. We are now investigating frequencies of Tregs in the gastric mucosa of these mice, and studying other potential immunomodulatory molecules.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P215

Human peripheral blood cell cytokine expression in *Helicobacter pylori* infection and disease

Harry Jenkins^{1,2}, Karen Robinson^{1,2}

¹Centre for Biomolecular Sciences; School of Medicine, Faculty of Medicine and Health Sciences, University of Nottingham, Nottingham, United Kingdom. ²Nottingham Digestive Diseases Biomedical Research Centre, Queens Medical Centre, Nottingham University Hospitals, Nottingham, United Kingdom

Abstract

Helicobacter pylori is a very common bacterial pathogen, and the main cause of peptic ulcer disease and gastric cancer. Gastric cancer occurs in 1-5% of those infected, and 5-year survival rates are low (19% in the UK) due to late diagnosis. Gastric cancer is the third most common cause of cancer-associated death. Having a blood-based prognostic test for gastric cancer risk would be very beneficial. We began this project by analysing the expression of *IFNG*, *IL4*, and *IL10*, as signature cytokines of CD4⁺ T-helper (Th) subsets Th1, Th2 and Treg, in peripheral blood mononuclear cells (PBMCs).

14 *H. pylori*-positive and 25 *H. pylori*-negative patients, attending the Queen's Medical Centre for a routine upper GI endoscopy, consented to donate peripheral blood samples. PBMCs were purified and mRNA expression quantified using reverse transcriptase quantitative PCR (RT-qPCR).

RT-qPCR conditions for each transcript were optimised and produced accurate standard curves (R^2 values between 0.95-0.99). cDNA was prepared from PBMC mRNA of 12 *Hp*-negative donors for use as a comparator in RT-qPCR. Expression of *IFNG* was increased by 11-fold ($p < 0.001$); *IL4* by 5-fold ($p < 0.001$) and *IL10* by 4-fold ($p < 0.001$) in samples from infected compared to uninfected donors. We now plan to expand the number of cytokines assayed, both by PBMC RT-qPCR and serum ELISA, and to correlate responses with gastro-duodenal disease status and virulence factor genotype of the colonising strains.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P216

Biofilm Formation and Antibiotic Resistance of Helicobacter Pylori Clinical Isolates

Lolwah AlSharaf, Andreja Paulin, Jody Winter

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Background: Biofilm formation has been reported to increase antibiotic resistance in several bacterial species, and may contribute to the persistence of *H. pylori* infection. We aimed to determine whether or not *H. pylori* biofilm forming ability was associated with antibiotic resistance *in vitro* and disease *in vivo*. Methods: The biofilm-forming ability of *H. pylori* laboratory strains 60190, Tx30a and 17 clinical isolates was determined *in vitro*. Antibiotic susceptibility of biofilm and planktonic bacteria were compared for one of the most susceptible clinical strains. Results: All *H. pylori* strains tested were able to form biofilm on glass coverslips at the air-media interface but with significant variation between strains. The strain which was the best at forming biofilm, 444A, was also the most antibiotic resistant and virulent (*cagA+*, *vacA* s1i1, high Sydney scores and gastric ulcer disease in the patient). *H. pylori* strains isolated from patients with more severe gastric markers of disease generally formed better biofilm but overall no statistically significant associations between strain virulence, disease status of the patient and biofilm forming capacity were seen. An antibiotic susceptible clinical isolate was significantly more resistant to amoxicillin ($p=0.025$) and tetracycline ($p=0.0219$) in biofilm form compared with planktonic cells. Conclusion: *H. pylori* strains differ markedly in their ability to form biofilms but no significant associations between biofilm formation and virulence were detected in this study. Biofilm formation can increase the antibiotic resistance of *H. pylori*, potentially leading to increased survival.

Keywords: *Helicobacter Pylori*, Biofilm Formation, Antibiotic Resistance.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P217

Impact of an extended spectrum β -lactamase (ESBL) IncI1-harboring plasmid to *Escherichia coli* O104:H4 pathobiology

Michaela Giles¹, Shawn Cawthraw¹, Manal AbuOun¹, Chris Thomas², Diana Munera³, Matt Waldor³, Roberto La Ragione⁴, [Jennifer Ritchie](#)⁴

¹Animal Plant and Health Protection Agency, Weybridge, United Kingdom. ²University of Birmingham, Birmingham, United Kingdom. ³Brigham and Women's Hospital, Boston, USA. ⁴University of Surrey, Guildford, United Kingdom

Abstract

Evidence to support the belief that large, conjugative drug-resistant plasmids confer a fitness burden to members of the *Enterobacteriaceae* is conflicting. Despite being commonly found in the intestine of man and animals, few studies have focused on the contribution of the plasmid under these conditions. Herein we examined the impact of an extended spectrum β -lactamase (ESBL)-containing IncI1 plasmid on the colonisation and pathobiology of a Shiga toxin-negative mutant of *Escherichia coli* O104:H4 in two different mammalian hosts.

Specific-pathogen-free 3-day old New Zealand White rabbits and conventionally-reared 6-week-old weaned lambs were orally infected with wild-type *E. coli* O104:H4 or the ESBL-plasmid cured derivative, and the recovery of bacteria in intestinal homogenates and faeces monitored over time.

Carriage of the ESBL plasmid had differing impacts on *E. coli* O104:H4 colonisation of the two experimental hosts. The plasmid cured strain was recovered at significantly higher levels than wild type during late-stage colonization of rabbits, but at lower levels than wildtype in sheep. Regardless of the animal host, the ESBL plasmid was stably maintained in virtually all *in vivo* passaged bacteria that were examined. Differences in their ability to form biofilms may provide a mechanistic explanation of these results.

In conclusion, our findings suggest that carriage of ESBL plasmids may confer host-specific effects on the bacterium and that it is unlikely that generalisations about the impact of these plasmids, are possible.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P218

Antimicrobial properties of a novel drug delivery system for the eradication of *Helicobacter pylori*

Nicola Osborne, Nora Francini, Vincenzo Taresco, Martin Garnett, Karen Robinson

University of Nottingham, Nottingham, United Kingdom

Abstract

Helicobacter pylori is the leading cause of peptic ulcers and gastric cancer worldwide. It is becoming more challenging to eradicate *H. pylori* infections due to increasing antimicrobial resistance rates and poor patient compliance. We have developed a novel polymer-based nanoparticle drug delivery system for delivery of the antimicrobial fatty acid Linoleic acid (LLA) to *H. pylori*. Our nanoparticles consist of a poly(glycerol-adipate) (PGA) backbone bearing either butyryl (C4), octanoyl or stearoyl side chains.

The size of the nanoparticles was characterised using dynamic light scattering, and the addition of LLA caused an average increase in nanoparticle diameter of 48%. For example, PGA nanoparticles increased from 175.5 nm to 220.3 nm and PGA-C4 nanoparticles increased from 122.8 nm to 224.8 nm. Antimicrobial activity of both LLA loaded and unloaded nanoparticles was tested against *H. pylori in vitro*. PGA-C4 nanoparticles had bactericidal activity even without LLA. LLA-loaded PGA-C4 nanoparticles had a lower minimum bactericidal concentration compared to free LLA (0.12 mM and 0.27 mM respectively). LLA-loaded and unloaded PGA-C4 nanoparticles had minimal cytotoxicity to gastric and intestinal epithelial cell lines.

We are now formulating antibiotics and LLA into PGA-C4 nanoparticles to produce a dual mechanistic therapy.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P219

Mechanotransmission Mechanism of the MacB ABC Transporter

Nicholas Greene¹, Allister Crow^{1,2}, Elise Kaplan¹, Vassilis Koronakis¹

¹University of Cambridge, Cambridge, United Kingdom. ²University of Warwick, Coventry, United Kingdom

Abstract

Tripartite efflux pumps (TEPs) from Gram-negative bacteria span the cell envelope and mediate export of toxins, antibiotics and virulence factors. TEPs are composed of a trimeric outer-membrane exit duct, TolC, a periplasmic adaptor protein and an inner membrane transporter. Here we describe the crystal structure of MacB, an atypical ABC transporter found at the heart of a TEP responsible for secretion of enterotoxin STII and resistance to macrolide antibiotics. The structure of MacB, determined in an ATP-bound state, reveals a distinctive protein architecture with four transmembrane helices and a large periplasmic domain located between the first and second transmembrane segments. Comparison of the ATP-bound structure with nucleotide-free and ADP-bound forms determined by other groups shows that MacB undergoes profound conformational changes during its ATP-binding and hydrolysis cycle, but lacks a central cavity that would allow passage of substrates. We therefore propose that MacB uses cytoplasmic ATP binding and hydrolysis to drive long-range conformational changes in its periplasmic domain that are subsequently used to propel substrates from the periplasm to the extracellular space via TolC. We term the transmembrane coupling of periplasmic conformational changes with cytoplasmic ATP hydrolysis in MacB-like ABC transporters 'mechanotransmission'. Homologues of MacB predicted to share all the structural features that underpin mechanotransmission include essential proteins involved in cell division and lipoprotein trafficking. Mechanotransmission may therefore serve as a general model for the operation of an entire superfamily of ABC transporters related to MacB.

Forum: Microbial Infection
Zone B
Presentations: Wednesday and Thursday Evening

P220

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF SOME SELECTED BACTERIAL PATHOGENS OF LOWER RESPIRATORY TRACT TO COMMONLY USED ANTIBIOTICS AT UNIVERSITY OF ILORIN TEACHING HOSPITAL, ILORIN, NIGERIA.

ZAHARADDIN MUHAMMAD KALGO

DEPARTMENT OF MICROBIOLOGY, FEDERAL UNIVERSITY BIRNIN KEBBI, BIRNIN KEBBI, Nigeria

Abstract

Abstract: This study was aimed at investigating the prevalence and antibacterial susceptibility pattern of some selected bacterial pathogens of lower respiratory tract from patients attending University of Ilorin Teaching Hospital, Ilorin, Nigeria. The study was carried out between December, 2013 and February 2014. Sputum specimens were collected from patients and cultured on Blood agar, Chocolate agar and Mac Conkey agar media. Bacterial isolates were identified by Gram staining, series of biochemical tests and antimicrobial susceptibility test was performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Out of 103 specimens studied, 16 bacterial species were isolated, giving a prevalence rate of 15.53%. This consisted of 62.50% from male patients and 37.50% from female patients. The isolates includes: *Klebsiella pneumoniae* (81.25%), *Pseudomonas aeruginosa* (12.50%) and *Klebsiella oxytoca* (6.25%) in order of ranking. Thirteen 13 (81.25%) isolates were 92.31% susceptible to Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine and fifteen 15 (93.75%) isolates were 87.2% susceptible to Gentamycin, Ceftazidine and Piperacillin. *Klebsiella pneumoniae* was the most susceptible amongst the isolates and *Klebsiella oxytoca* displayed the highest number of resistance (83.3%) to most of the antibiotics tested except Augumentin in which, it was moderately resistant. In conclusion, *Klebsiella pneumoniae* was the most commonly recovered organism from patients with lower respiratory tract infection in this centre. Resistance to all tested antibiotics by *Klebsiella oxytoca* as recorded in this study is of clinical significance, with associated possible treatment failure. On the other hand, Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine remain useful agents in the management of LRTI in this environment if *Klebsiella oxytoca* is excluded.

Virology Workshop: Retroviruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P221

Epidemiology and risk factors in patients with AIDS (HIV +)

zahra tayebi¹, mohammad karim rahimi¹, omid sadeghi²

¹islamic azad university medical sciences, Tehran, Iran, Islamic Republic of. ²islamic azad university Kerman branch, Kerman, Iran, Islamic Republic of

Abstract

Oral candidiasis is commonly seen in HIV infection. Some risk factors, the cellular immune response causes disruption in the field of oral candidiasis. The purpose of this study was to evaluate the role of these factors in Iranian patients with HIV infection. This study is a retrospective analysis. In this study, 377 patients who had complete data regarding HIV infection in Western blot, were studied. All were positive. This clinical observation, direct smear and culture were considered choice of oral candidiasis infection. The number of CD4 cells in any of the patients receiving antiretroviral therapy at the first visit and after HAART, were measured. Of the 377 patients, 316 (84%) men and 60 (16%) were female. The mean age of the patients was 36 years. Time of diagnosis of HIV infection in about 2-9 years. most common route of transmission, use of shared needles (117 cases or 31%) 11 patients (2/9%) of antigen HBsAg, were positive. 206 (71/5%) were positive for the hepatitis C virus nucleic acid and 53 (14/1%) were infected with TB. At the first visit, 52 patients (13/9%) and the second visit, 31 cases (8/2%) had oral candidiasis infection. The average number of CD4 cells in the patient's first visit was less than other patients (193/8 vs. 349/7 cells) ($P = 0/0001$), but there was no significant difference in the second visit (276/8 vs. 333/2 cells) ($P = 0/184$). Prompt diagnosis and treatment of infection plays a significant role in prognosis and clinical course of patients with infection, reduce the rate of opportunistic infections.

Virology Workshop: Retroviruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P222

Use of a baculovirus expression system, utilising a CMV promoter, for generation of virus-like particles in mammalian cells

Layla Faqih

The University of Manchester, Manchester, United Kingdom

Abstract

Baculoviruses can be used as vehicles to efficiently deliver and express genes in mammalian cells. BacMam technology uses a recombinant baculovirus engineered to contain a mammalian expression cassette for transgene expression in mammalian cells. The mammalian gene is expressed without baculovirus replication. Virus like particles (VLPs) can be produced using this expression system. In this study, we aim to develop retrovirus-like particles to serve as a new gene therapy carrier system. Our VLP is derived from simian immunodeficiency virus (SIV) and pseudotyped with vesicular stomatitis virus (VSV) glycoprotein. The resultant VLP will be used to deliver anti-HIV IgG1 b12 antibody genes into the mammalian genome, to produce high titres of neutralizing antibody. Target genes were cloned into four altered transfer plasmids, to construct recombinant baculoviruses containing the Tat/Gag/Pol genes of SIV, plus the VSV glycoprotein. All genes are driven by a CMV promoter. Recombinant baculoviruses generated by homologous recombination between baculovirus DNA and the transfer plasmids. Mammalian cells were transduced with recombinant baculoviruses. Proteins expression was detected using western blot. We believe that the BacMam construct will deliver SIV genes into mammalian cells and produce SIV like particles pseudotyped with VSV glycoproteins. This strategy raises the possibility that IgG1 b12 antibody genes can be delivered and inserted into the genome of numerous cell types, to produce long-lasting, high titres of neutralising anti-HIV monoclonal antibody. Since baculoviruses cannot replicate in mammalian cells, this system can be used in vivo as well as in vitro.

Virology Workshop: Negative Strand RNA Viruses
Zone C
Presentations: Thursday Evening and Friday Lunchtime

P223

Mapping of functional resistance to neuraminidase inhibitors in avian influenza viruses.

Dagmara Bialy, Holly Shelton

The Pirbright Institute, Woking, United Kingdom

Abstract

Neuraminidase inhibitors (NAIs) are important antivirals used to treat or prevent influenza virus infection in humans. Mapping of functional resistance to currently licensed NAIs has been limited to human influenza viruses with only sporadic reports investigating avian influenza (AIV) NA subtypes. Since three major human pandemic influenza strains acquired NA from an avian source, it remains critical to assess whether known NAI resistance-associated changes in NA confer resistance in AIV, and identify novel motifs that could be included in surveillance programmes. We have generated by reverse genetics a panel of prevalent AIV strains representing various NA subtypes which pose a major concern to the poultry industry as well as risk to human health. We introduced previously described NAI resistance-associated mutations into the NA gene of AIV strains, including H7N9 and highly pathogenic H5N8 and H5N6. Susceptibility of AIV to NAIs was determined using the established MUNANA assay. All the AIV wild-type NAs tested are sensitive to NAI drugs and our results show the outcomes of H274Y, N294S, R292K and E119V mutations in these backgrounds. To identify novel signatures conferring NAI resistance we serially passaged NAI-sensitive viruses in increasing drug concentrations in chicken tracheal organ cultures (TOCs). Selected viruses will be subsequently tested for their NAI susceptibility and sequenced to unravel any novel motifs associated with increased resistance to antivirals.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P224

Elucidating the structure of previously inaccessible fusion intermediates of Influenza A Virus by cryo-electron microscopy

Oliver Debski-Antoniak, Alana Maerivoet, Eric Hewitt, Juan Fontana

University of Leeds, Leeds, United Kingdom

Abstract

Influenza virus of the *Orthomyxoviridae* family of viruses, 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. Influenza virus must fuse its envelope with the host's endosomal membrane in order to release its genome inside the cell and start an infection, making this process an excellent drug target. Influenza A virus (IAV) fusion is driven by the viral glycoprotein, hemagglutinin (HA), which when exposed to low pH and high K⁺ concentrations transitions from a meta-stable pre-fusion to post-fusion state.

Though pre-and post-fusion structures have been well defined, the intermediate conformational structures of HA are unknown. Elucidating these intermediates may aid the possible development of a new branch of antivirals, whilst providing an insight into how other structurally similar fusion proteins, such as human immunodeficiency virus-1 (HIV-1) gp41, mediate the fusion process.

In order to identify the intermediate structures of HA, high resolution cryo-electron microscopy will be applied to study purified HA interacting with lipid Nano discs. In parallel, complimentary experiments in terms of resolution and structural integrity will be carried out to visualise IAV fusion inside endosomes. These experiments will allow us to correlate the *in vitro* findings with *in vivo* conditions.

Overall, understanding the molecular mechanisms of Influenza virus entry will allow us to limit the detrimental impact of impending global pandemics and to decrease the social and economic consequences of seasonal flu.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P225

Role of SPLUNC1 in the Natural Transmission of Influenza A Virus

Sanaria Al Katy¹, James Stewart²

¹Institute of Infection & Global health/ University of Liverpool, Liverpool, United Kingdom. ²Institute of Infection & Global Health/ University of Liverpool, Liverpool, United Kingdom

Abstract

The respiratory system epithelium has a critical role in the initial defence against microbes and secretes a number of proteins that function in innate immune defence. SPLUNC1 also called (Short Palate, Lung, and Nasal epithelium Clone1) is continuously expressed and secreted by the respiratory system epithelium. However, its precise biological functions remain unclear. To assess whether SPLUNC1 affects the transmission of influenza A (IAV) between infected and non-infected mice, we set up two experiments. The first experiment included 20 mice (10 wild type and 10 SPLUNC1 KO). At day 0 eight mice were infected (4x WT and 4x KO) each with 5×10^4 PFU Influenza A virus X31. Six hours later, the infected mice were introduced to naïve mice 2x WT index to 3xWT contact, and 2x KO index to 3x KO contact per cage (4 cages total). All mice were euthanized at day 4 post-infection, lung and nasal tissue samples were collected for analysis of virus titre. While the second experiment was similar to the first experiment but the infected mice were introduced to naïve mice 2x WT index to 3x KO contact and vice versa per cage (4 cages total). The results showed that influenza transmitted more frequently and efficiently to SPLUNC1 KO mice, indicating a role for SPLUNC1 in the natural transmission of the virus.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P226

Ribonucleoprotein Structure in Pathogenic Orthobunyaviruses

Francis Hopkins, Neil Ranson, John Barr, Thomas Edwards

University of Leeds, Leeds, United Kingdom

Abstract

The *Orthobunyavirus* genus within the *Bunyavirales* order contains many pathogens of humans and livestock. Their genome consists of three segments of negative-sense, single-stranded RNA. These are encapsidated with polymers of nucleocapsid protein (NP) which binds RNA in a sequence-independent manner to form ribonucleoproteins (RNPs). Formation of RNPs protects the viral genome from the host immune system and has roles in transcription, genome replication and the correct packaging of segments into new virions. Thus, RNPs are an attractive target for the development of new antivirals, which is of particular importance as such therapies do not currently exist for any of the human pathogenic orthobunyaviruses.

Using a combination of structural and biophysical techniques including cryo-EM, this project aims to determine high resolution structures of orthobunyavirus RNPs to shed light on their overall architecture, assembly and mechanisms of RNA binding.

Recombinant NP from different orthobunyaviruses has been used to reconstitute RNPs for analysis by negative stain EM. This has allowed us to visualise full length RNPs and different oligomeric states of NP which are potential assembly intermediates. Infectious Bunyamwera virus has also been propagated and purified by ultracentrifugation, allowing us to extract and purify RNPs from virions which we have visualized by negative stain EM. Work is ongoing to optimise these and reconstituted RNPs for analysis by cryo-EM.

The work presented here will aid in the development of small molecules which inhibit formation of the orthobunyavirus RNP and which could be investigated further for their therapeutic potential

Virology Workshop: Negative Strand RNA Viruses
Zone C
Presentations: Thursday Evening and Friday Lunchtime

P227

Assessment of the response of swine to influenza A virus infection.

Elizabeth Coney, Alexander Byrne, Helen Everett, Sharon Brookes

Animal and Plant Health Agency (APHA Weybridge), Surrey, United Kingdom

Abstract

Since the emergence of H1N1 2009 pandemic virus (pdm09), this strain has become endemic in humans and swine. Understanding the host response to influenza A virus infection is important as host factors influence disease progression and viral mutation/adaptation, potentially leading to the emergence of new reassortant viruses and/or 'immune escape' mutants following vaccination. We studied the responses of the porcine host to swine-origin pdm09 virus infection. Bioinformatics and literature sources allowed identification of key target genes for the swine host response to influenza A virus infection. The expression of these genes was investigated in pig tracheal (NPTr) cells infected with a swine pdm09 influenza A virus (A/Swine/England/1353/2009). mRNA levels were monitored by SYBR Green RRT-qPCR.

A primer panel for detection of approximately 20 host genes has been optimised. Longitudinal RNA samples were analysed for swine pdm09 influenza A infection in NPTr cells. In comparison to non-infected cells, infected cells showed a time-dependent increase in mRNA levels corresponding to several host genes, including *Mx1* and *IDO1*. This approach is being applied to investigate the role of other host genes, such as *IFITM3* and *OASL*, recently identified as playing an important role in influenza A virus infection.

Improved understanding of host response to influenza A virus infection and the influence of prior immunity could lead to the identification of host correlates of infection or vaccine-mediated protection and thereby support the improvement and design of more effective vaccines and diagnostic assays, with the aim of mitigating future risks.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P228

Development of a lyssavirus pseudotype panel for investigations into host tropism and cellular entry

Emma R. Horncastle¹, C. Patrick McClure¹, Alessandra Vitelli², Alfredo Nicosia², Edward Wright³, Richard A. Urbanowicz¹, Jonathan K. Ball¹

¹University of Nottingham, Nottingham, United Kingdom. ²Reither SRL, Rome, Italy. ³University of Sussex, Brighton, United Kingdom

Abstract

Rabies encephalitis is a disease that kills thousands of people every year with greater than 99.9% of cases caused by the zoonotic rabies virus, however it can also be caused by viruses from one of the other lyssavirus species. These lyssaviruses are commonly found in bats, but little is known about their host tropism or if they have the potential to establish themselves in a wide variety of reservoir species similar to rabies virus. Lyssavirus cell entry mechanisms are also relatively unexplored, though there is some evidence to suggest they may use different combinations of the three proposed rabies virus receptors. The pseudotype system is modular, utilising the expression of virus glycoproteins on a replication incompetent retroviral core. Thirteen lyssaviruses, with glycoprotein sequences taken directly *ex-vivo* or from low passaged viruses, were selected to form a pseudotype panel representing all lyssavirus phylogroups. Glycoproteins were cloned into plasmid vectors via InFusion cloning. Pseudotype production was optimised by varying amounts of glycoprotein and retroviral backbone plasmids transfected into HEK293T cells. Pseudovirus data was also influenced by the choice of plasmid vector used to express glycoprotein in pseudovirus production. Through this work, we have successfully developed a panel of lyssavirus pseudotypes in order to investigate their tropism and entry pathways.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P229

Comparative analysis of Rabies Virus Glycoproteins from Pathogenic and Non-Pathogenic strains

Ibrahim Al-Masoud¹, Royle Jamie², Theo Georgious¹, Stefan Finke³, John N Barr¹, Mankouri Jamel¹

¹University of Leeds, Leeds, United Kingdom. ²CVR, Glasgow, United Kingdom. ³Friedrich-Loeffler-Institut, Greifswald, Germany

Abstract

Rabies virus (RABV) is a neurotropic virus that causes a devastating disease responsible for up to 70,000 deaths annually. The majority of RABV cases occur in economically poor developing countries and it remains endemic in some regions.

RABV is a non-segmented negative sense RNA virus classified within the family Rhabdoviridae. The RABV genome encodes five proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the RNA-dependent RNA polymerase (L). RABV-G is the only viral protein exposed on the surface of the virion and mediates RABV interactions with cellular receptors, pH-dependent virus fusion, and promotes viral entry and trans-synaptic spread within the central nervous system. However, the cellular roles of RABV-G during the RABV lifecycle post-virus entry remain to be defined.

In this work, we report a comparison of pathogenic and non-pathogenic RABV glycoproteins and observe trafficking differences between challenge virus standard (CVS) and attenuated vaccine strain SAD-B19 (SAD). To define these differences, CVS-G and SAD-G have been fused to a promiscuous biotin ligase that enables selective biotinylation of RABV-G binding partners. Using proximity-dependent biotinylation in differentiated human neuronal cells, we aim to identify differences in the cellular interactomes of CVS-G and SAD-G and assess their contribution to RABV pathogenesis.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P230

Standardising the Production of Pseudotyped Virus for Emerging Viral Outbreak Preparedness

Emma Bentley^{1,2}, Kamilla Toon¹, Mark Page¹, Giada Mattiuzzo¹, Edward Wright^{3,2}

¹National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom. ²University of Westminster, London, United Kingdom. ³University of Sussex, Falmer, Brighton, United Kingdom

Abstract

The recent outbreaks of Ebola and Zika virus have highlighted the lack of prophylactic treatments and need for effective diagnostics for emerging viruses. To support preparedness activities that will help mitigate the risks posed by future outbreaks, biological reference materials to allow evaluation of treatments and calibrated assays used in their development are needed. For treatment/vaccine efficacy trials, antibody standards will enable the comparison of results from different laboratories and between clinical trials. To evaluate this material, replication defective pseudotyped viruses (PV) are an ideal tool to avert the need for high containment levels, which are often required when handling viruses with outbreak potential. In line with the WHO priority pathogens list, we are developing PV neutralisation assays for Lassa and Nipah virus. This includes evaluating the most appropriate PV system for their production and target cell lines for use in downstream assays.

Furthermore, we have developed a pipeline to evaluate the quality of PV preparations by measuring the total number of particles, genome copies and reverse transcriptase (RT) activity, in addition to biological titre. Our results show that measures of RT activity or genome copies, in combination with biological titres, are a good method for defining assay input. We have also determined the correlation between disparate readout units of fluorescent and luminescent reporters, recorded as infectious units (IFU) and relative light units (RLU) respectively. This work enhances the assessment of the quality and input of PV preparations to be used in downstream assays.

Virology Workshop: Negative Strand RNA Viruses
Zone C
Presentations: Thursday Evening and Friday Lunchtime

P231

Live viral vaccine attenuation: study of the role of microRNAs in mumps virus host adaptation

Melanie Tanguy, Silke Schepelmann

NIBSC, Potters Bar, United Kingdom

Abstract

Mumps virus is the causative agent of the common childhood disease mumps and associated with inflammatory symptoms such as parotitis, orchitis, temporary hearing loss and rare cases of meningitis and encephalitis. The presence of mumps is attested worldwide but the availability of a vaccine led to a strong control of cases. Unlike the two other components of the MMR vaccine (measles and rubella), who are both on the verge of eradication in UK ; recent outbreaks of mumps in vaccinated populations have reinforced the need of a better understanding of attenuation of live viral vaccines.

A current focus in vaccine development and control of immunological escape is to analyse acquired mutations in vaccine strains leading to lower symptoms but preserving the ability to mount a long-lasting immunity. This approach can remain challenging, owing to fast evolution rate in RNA viruses and scarce information about gene function. It has been proposed that the use of microRNA-controlled vaccines could provide a safe and reliable design strategy for viral attenuation.

Here, we present the first profiling of microRNAs, key gene expression regulators, in cell systems commonly used in vaccine manufacturing and research. A special attention will be given to differential expression upon infection with mumps vaccine or wild-type virus. These initial results will provide a basis to understand the role of microRNAs in the onset of immune responses, the direct involvement of microRNAs in mumps virus biology and a platform for future work on mumps vaccine development and control.

Virology Workshop: Negative Strand RNA Viruses

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P232

Alternative cell-culture strategies for H3N2 influenza - avoiding selection of phenotypic variants which confound the vaccine strain selection process

Jonathan Brown¹, Wendy Barclay², Othmar Engelhardt³, Ruth Harvey³

¹Imperial College London/NIBSC, London, United Kingdom. ²Imperial College London, London, United Kingdom. ³NIBSC, Potters Bar, United Kingdom

Abstract

Every six months, the strains to be included in the seasonal influenza vaccine are selected chiefly based on data collected from serological assays. The most widely used of these is the haemagglutination inhibition (HAI) assay which relies on the ability of the virus to agglutinate red blood cells (RBC). However, in recent years many cell-passaged H3N2 isolates have demonstrated reduced titres mediated by their haemagglutinin (HA) surface protein and gained the ability to agglutinate RBC through their neuraminidase (NA). The latter phenotypic change confounds the results of HAI assays which look to determine only anti-HA immunity.

Here, next generation sequencing (NGS) of a panel of 7 MDCK/MDCK-SIAT-passaged H3N2 influenza viruses showed that all contain a mixture of variants at residues 148 and/or 151 of NA. These variants have been shown to confer NA-mediated binding. Passage in human airway epithelial (HAE) cells and sequencing revealed the selection pressures exerted upon the viruses by these cells. Subsequently, this material was amplified in MDCK-SIAT cells which have been shown to less readily select for NA-mediated binding variants. Along with NGS, this passaged material was also assessed phenotypically by haemagglutination assays in the presence and absence of a neuraminidase inhibitor, oseltamivir.

Cell culture adaptation of H3N2 influenza viruses alters their phenotype in a manner which presents issues for the vaccine strain selection process. Human airway epithelial (HAE) cell-passage may be utilised as a strategy to revert such isolates back to the phenotype of their original clinical specimen for use in serological assays.

Virology Workshop: Negative Strand RNA Viruses
Zone C
Presentations: Thursday Evening and Friday Lunchtime

P233

Preparation of Tissue culture vaccine from locally isolated highly pathogenic avian influenza virus compared with egg adapted one after exposure to reverse genetic steps.

Ahmed Maher

Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt

Abstract

Seventy three isolates of AIV were collected from 11 Egyptian governorates during the period from 2015 to 2016, from different poultry flocks, The mortality rate from examined flocks ranged from 10 - 30%.

Respiratory tract samples that collected are subjected for RT-PCR for 2 primers (M gene and H gene) then sequenced to detect cleavage site of H gene. The results revealed 43 positive samples out of 73 samples when using M gene primer while 26 positive using H gene primer out of 73 samples, whereas cleavage site sequence revealed 17 out of 73 positive with percentage 58.9%, 35.6% and 23.2%; respectively.

The phylogenetic analysis was applied and revealed that there were two major genetic groups of H5N1 AIVs were being co-circulating in the Egyptian vaccinated commercial poultry sectors during 2015- 2016. Continuous evolution of H5N1 viruses and the emergence of new antigenic variants may be partially explained by immune pressure caused by the use of vaccines, which such variants can circumnavigate vaccine-induced immunity and are called escape mutants.

AI strain (A/chicken/Egypt/M7217B/2013(H5N1) represent the most common clusters of AIV was reassorted at National research center and then adapted on MDCK cell line for preparation of Tissue culture (TC) vaccine in parallel with egg adapted one. The AIV had a titer of $10^{9.5}$ TCID₅₀/ml, while the egg adapted vaccine had titer 10^{12} .

The challenge at CLEVB results revealed that Protection % of vaccinated groups either with prepared tissue culture AI vaccine or egg adapted AI one were 50%.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P234

Microbial communication: An emerging drug target

Aijaz Ahmad

University of the Witwatersrand, Johannesburg, South Africa. National Health Laboratory Service, Johannesburg, South Africa

Abstract

Microbial communication is now highly appreciated fact, which is achieved by small chemical molecules known as quorum sensing molecules. These quorum sensing molecules are released and received by microbes that allow them to synchronize behavior on a population-wide scale. This cell-to-cell communication plays an important role in their pathogenicity and several other processes. Due to increasing drug resistance, efforts to identify new drug targets are increasing. Quorum sensing is the promising drug target, as it does not pose pressure on microbial survival, thus bypassing development of resistance. Therefore biomedical scientists are proposing to utilize this phenomenon in the development of novel antimicrobial therapies aimed at interfering with quorum sensing. Such therapies could be used to control microbial pathogenicity over killing or inhibiting growth of pathogens. With this background, we screened citral and its derivatives for inhibition of QS-regulated violacein and pyocyanin production in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, respectively. We present the results of a screening of citral and its five newly synthesized derivatives (CTS1 – CTS5) for their QS-inhibitory activities using qualitative agar diffusion and quantitative spectrophotometric assays. Three out of five derivatives along with citral showed high antimicrobial activity with MIC values ranging from 0.25 to 2 µg/ml, while as all the synthesized compounds showed potent anti-QS activity at sub-MIC values. CTS2 and CTS3 exhibited >95% violacein inhibition, suggesting their potential as QS inhibitors. The dual activities of killing and inhibiting communications of pathogens advocate in-depth study of these derivatives to develop novel antibiotics.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P235

Antagonistic Effects of Helveticin produced by *Lactobacillus helveticus* FCF20 Isolated from fermented Corn Flour

Sani Sambo Datsugwai Mohammed, Aminu Aminu Hamza

Department of Microbiology, Faculty of Science, Kaduna State University, Nigeria., Kaduna, Nigeria

Abstract

The antagonistic effects of helveticin produced by *Lactobacillus helveticus* FCF20 isolated from fermented corn flour was investigated. The *L. helveticus* FCF 20 was isolated using the pour plate technique and was characterized conventionally based on some biochemical test. This isolate was identified using standard scheme. The *L. helveticus* FCF 20 was propagated in De Man Rogosa Sharpe (MRS) broth for bacteriocin (helveticin) production. The *L. helveticus* FCF 20 was selected after vigorous screening based on its ability to grow in De Man Rogosa Sharpe broth to produce helveticin, also through spectrophotometric analysis at 580nm wave length, optimal pH and bacteriocin activity (AU/mL) (Antagonistic effect) on test isolates. The helveticin had antagonistic (inhibitory) on all test microorganisms (*Escherichia coli* N2, *Pseudomonas aeruginosa* N7, *Listeria monocytogenes* W6, *Shigella dysenteriae* N11, *Bacillus cereus* W18, *Salmonella typhimurium* N8, *Escherichia coli* W4, *Proteus vulgaris* W7, *Klebsiella aerogenes* N12, *Staphylococcus aureus* N16b, *Bacillus subtilis* N20, *Klebsiella ozaenae* W24, *Proteus mirabilis* N16a) ranging from +5mm to +6mm respectively. Bacteriocins have bactericidal or bacteriostatic effects on the cell membrane of cells of mostly closely related microorganisms. Bacteriocins, which are the antimicrobial substances of lactic acid bacteria (LAB) have gained tremendous attention as potential bio preservatives in the food and dairy industries.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P236

Aurodox: An inhibitor of the *E. coli* O157 Type Three Secretion System produced by *Streptomyces goldiniensis*.

Rebecca McHugh^{1,2}, Andrew Roe², Paul Hoskisson¹

¹University of Strathclyde, Glasgow, United Kingdom. ²University of Glasgow, Glasgow, United Kingdom

Abstract

Aurodox, a specialised metabolite of soil bacterium, *Streptomyces goldiniensis*, has recently been identified as an inhibitor of the Enteropathogenic *Escherichia coli* (EPEC) Type Three Secretion System (T3SS). In these studies, we have demonstrated the anti-virulence capacity of aurodox by fully characterising its inhibitory effect on the T3SS of EPEC, EHEC and *Citrobacter rodentium* whilst also confirming that the effect is not caused by a decrease in growth rate or cell viability. Furthermore, through the use of RNA-seq transcriptome analysis we have shown that aurodox acts a transcriptional level, by downregulating the expression of Locus of Enterocyte Effacement (LEE) genes which encode for the T3SS. We have also observed a marked reduction in expression of *ler*, the master regulator of the LEE, which we were able to show experimentally through the use of gfp-fused reporter assays. Additionally, we have demonstrated that the overexpression of *ler* allows EHEC to overcome the T3SS-knockdown phenotype, and therefore, we hypothesise that aurodox acts directly upstream of *ler*. Moreover, we have observed transcriptional effects of aurodox on other members of the *ler* regulon. We have shown at a microscopic level that aurodox inhibits EHEC (TUV93-0) attachment to epithelial cells and therefore, is a candidate compound for treatment of human EPEC and EHEC infections. In order to enhance the potential of this molecule as a drug we have sequenced the whole genome of the producing organism, *Streptomyces goldiniensis* and identified the genes encoding for aurodox in order to improve and diversify production.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P237

Dominance of *Staphylococci* sp. in bovine subclinical mastitis is negatively correlated with prevalence of *Bacillus* sp.

BALENDRA SINGH¹, Ramesh Kumar²

¹Department of Biotechnology, Bundelkhand University, Jhansi, India. ²Department of Biochemistry, Bundelkhand University, Jhansi, India

Abstract

Background: Subclinical mastitis is 30-40 times more prevalent than clinical mastitis and responsible for huge economic loss and milk reduction, worldwide. *Staphylococcus aureus*, coagulase negative *Staphylococci* become more important in recent years as causative agent of mastitis. Present study was designed to investigate co-occurrence of different bacterial pathogens in milk samples of bovine subclinical mastitis.

Methods: A total of 150 milk samples of 8 different herds, were screened +ve for SCM with CMT Test and somatic cell count, followed by microbial enrichment, isolation and characterization. Bacterial isolation and pure culture were done in accordance with Collee et. al, 2008. Mixed cultures of *Bacillus* sp. with others were compared with Pearson correlation test.

Results: Animal wise, (n=150) highest prevalence of 53.33 % was of coagulase negative *Staphylococci* sp. followed by *S.aureus*, *Streptococci* sp., *Lactobacillus*, *Corynebacterium* sp., *Klebsiella* sp., *E.coli* and others with rate of 16.66,16.66,14.00,6.67,4.67,1.33 and 3.33% respectively.80% of milk samples were found positive for more than one bacterial sp. but samples harboring *Lactobacillu/Bacillus subtilis* were observed to have decreased frequency of isolation for other sp, except *Bacillus* sp. with a correlation coefficient calculated as -0.5142, -0.5584, -0.2144, +0.6739, and -0.083 for CNS sp., *S.aureus*, *Streptococci* sp., *Corynebacterium* sp. and *Klebsiella* sp. respectively and indicate moderately –ve correlation of *S. aureus* and Coagulase negative *Staphylococci* with *Bacillus* sp. prevalence.

Conclusion: Preponderance of mastitis may be delayed by using *Lactobacillus/Bacillus subtilis* as probiotics to outcompete other associated pathogens, and its presence may indicate the quantification of infection severity.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P238

Pretty Fly for a Tsetse: Flux balance analysis of the tsetse symbiont *Sodalis glossinidius*

Rebecca Hall, Gavin Thomas, Jamie Wood, Lindsey Flanagan

University of York, York, United Kingdom

Abstract

Approximately 65 million people in sub-Saharan Africa are at risk of human African trypanosomiasis. It is a neglected tropical disease caused by the protozoan parasite *Trypanosoma brucei* and spread by the tsetse fly. There is an increasing risk of drug resistance and delivering treatment in areas of conflict and poverty can be difficult. Disease prevention via vector manipulation is an enticing alternative to an expensive vaccine or drug. The tsetse's microbiome provides a unique opportunity for this.

Sodalis glossinidius, the mutualistic secondary symbiont of the tsetse, is thought to influence trypanosome establishment within its host. Understanding aspects of *Sodalis* metabolism may enable the development of novel ways to reduce disease spread. Here, we present a new, experimentally verified flux balance analysis model of *Sodalis* metabolism. Our model provides insights into the carbon, nitrogen and vitamin requirements of *Sodalis*, supported by *in vitro* analysis. We also describe an entirely defined media proven to support *Sodalis* growth. It is hoped that this will accelerate understanding of the host-symbiont interaction with the aim of reducing trypanosome spread.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P239

Factors Affecting Resistance Plasmid Persistence in Wastewater Treatment

Helen Wilson¹, Barth Smets², David Graham³, Jan-Ulrich Kreft¹

¹University of Birmingham, Birmingham, United Kingdom. ²Technical University of Denmark, Anker Engelunds, Denmark. ³Newcastle University, Newcastle, United Kingdom

Abstract

The rise of antimicrobial resistance (AMR) is increasingly concerning, especially for those who are already at a higher risk of suffering infection, for example, the immunocompromised, elderly and children. There is an all too real possibility that we return to the days of dying from infections which we now take for granted as easily treated. A “hot bed” for AMR is found in wastewater treatment plants (WWTPs). We aim to elucidate the relative importance of the various factors that may be responsible for the persistence of resistance plasmids within WWTPs with the help of a simple mass action model. This mass action model describes the inflow of substrates, antimicrobials and Enterics from sewage and outflow into receiving waters. Within the WWTP, bacteria have a longer residence time than the solutes and the resident wastewater type bacteria consume other substrates than Enterics and may exchange plasmids with Enterics. The two bacterial populations are split into those that cannot harbour plasmids and those that can. We will show results of a comprehensive sensitivity analysis of the model to identify the relative importance of the differential growth of Enterics and indigenous wastewater bacteria (differential temperature dependence, separate growth substrates), the residence time in the WWTP, selection for resistance and transfer and loss of resistance plasmids as well as the fitness cost of the plasmid, amongst others. From this, we can identify factors that are likely important for the persistence of resistance plasmids to inform further research and efforts to develop mitigation strategies.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P240

Cooperation and conflict in solvent producing *Clostridia* populations

Jonathan Humphreys¹, Alan Cockayne¹, Stephen Diggle², Klaus Winzer¹

¹University of Nottingham, Nottingham, United Kingdom. ²Georgia Institute of Technology, Georgia, USA

Abstract

Much of the work on industrial *Clostridia* has focused on strain improvements through metabolic engineering but there has been little investigation into population dynamics. Here we examine social interactions of the solvent producing species *Clostridium beijerinckii* to investigate the evolutionary driving forces behind the phenomenon of strain degeneration. Degeneration is characterised by a reduction or complete loss in solvent and endospore formation occurring in both batch and continuous fermentations making it a serious problem for commercial applications.

To gain insights into the degeneration process we repeatedly sub-cultured *C. beijerinckii* NCIMB 8052 to promote the emergence of degenerates. Four colony morphologies were found, characterised, with the most severe morphotype FW unable to form solvents and spores.

Social interactions were studied using wild type and a naturally evolved FW-type degenerate grown in various ratios. At low initial frequencies, it displayed a higher fitness than the wild type, but its fitness rapidly decreased at higher starting frequencies. This frequency-dependent fitness is suggestive of social cheating. The mutant was however unable to sustain itself in both monoculture and mixed culture during stationary phase, suggesting defects that could not be rescued by the wild type.

We sequenced the genomes of 71 independent isolates to identify any genetic causes underlying degeneration. Four hot spots were identified which contained considerably more mutations than the rest of the genome. A combination of genetic, ecological and evolutionary approaches may be key to understanding and controlling the process of strain degeneration in industrial applications.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P241

Taxonomy is something that we do

Ale.G.A. Costa

Ospedale Policlinico San Martino, Genova, Italy

Abstract

In the last 10 years the author, a member previously active in the fields of beta-lactam mechanism of action, gram-negative anaerobes and infection control, spent much of his time in consolidate a personal taxonomic guide to microorganisms following the dictates of recognized European & international committees as well as interpretations offered by web sites (LPSN bacterio.net, Names for Life, EZ Biocloud, StrainInfo, AlgaeBase, UC Jeps, CZ Cyano, Mycobank, Index Fungorum, ICTV etc.). Today modern technologies, mainly Sequencing, Mass spectroscopy and computing, allow this field of science to grow up much faster than in the past and in many circumstances revolutionised 'time valued' grouping concepts. While fungi (including the bigger) are estimated at 2.2 to 3.8 million species and viruses touch the threshold of 4500, a wise generation of taxonomists, put at the index, published an approved list of Bacteria in 1980. A new platform, sadly out of Cyanobacteria. Something very solid on which future work can rely on: 2 divisions, 8 classes & sub, 24 orders & sub, 24 tribes, 65 families. At the end of the year 2017 I register 29 divisions, 71 classes, 170 orders, 384 families and the Archaea. Increases are not significantly disproportionated, but errors in papers and resources are found more easily than before. This must not discourage efforts in better defining microbes with which we game & play our lives. A new paperless world is coming, a changing vapour that we will not be able to fix in mind and granu salis will be crushed and crushed until They ... will be always the same

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P242

Evolutionary strategies of *Bdellovibrio bacteriovorus* predators and prey

Kim Summers, Jan-Ulrich Kreft

University of Birmingham, Birmingham, United Kingdom

Abstract

Antibiotic resistance is a serious threat to human health and new treatments for bacterial infections are urgently needed. Bacteriophages, which were first used at the beginning of the 20th century and the predatory bacterium, *Bdellovibrio bacteriovorus* (discovered in 1962) have both been proposed as potential alternatives to antibiotics.

We developed a mathematical predator prey model to predict the effects of *Bdellovibrio* and bacteriophage on prey bacterial numbers. Our system is a simple chemostat, with an abiotic resource, a single prey species, exhibiting Monod kinetics and upto two predator species with a Holling type II functional response. As *Bdellovibrio* spends considerable time in a bdelloplast stage within a dead prey cell this species is also modelled, giving a delay between prey removal and birth of predators.

We used the model to examine the effects of varying prey cell size on vulnerability to predation. We found larger cells are more easily predated, benefiting the predator. There was also a distinct difference in effectiveness between *Bdellovibrio* and bacteriophages. We also discovered an optimal attack rate for the predator.

To understand the reliance on good estimating of parameter values we performed a sensitivity analysis on the model parameters. For most settings perturbations have only modest effects, but a few parameter settings showed hypersensitivity.

Our model shows the potential effectiveness of *Bdellovibrio* and bacteriophage in combatting bacterial infections and highlights aspects of these systems which need more laboratory study.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P243

Using 'Insider Information' to Identify Novel Antibacterial Targets

Laura Nolan¹, Amy Cain², Eleni Manoli¹, Maria Sainz-Polo³, David Albesa-Jové³, Gordan Dougan², Julian Parkhill², Alain Filloux¹

¹Imperial College London, London, United Kingdom. ²Wellcome Trust Sanger Institute, Cambridge, United Kingdom. ³IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Abstract

More than 700,000 people worldwide die each year as a result of infections by bacteria resistant to current antibiotics, with this number predicted to increase to 10 million by 2050. These staggering figures must elicit drastic action if we have any chance of combating the rapid rise in antimicrobial resistance. Here we report the use of a global genomic approach to identify novel antibacterial targets.

The Type-VI secretion system (T6SS) is a weapon possessed by bacteria which delivers toxic effectors into other bacteria to kill or subvert function, allowing the producing bacteria to prevail in a niche. Within the wide range of polymicrobial settings that bacteria exist in, they have evolved T6SS-dependent toxins to most efficiently and selectively target particular bacterial components, and thus we consider the targets of these toxins to represent already validated antibacterial targets.

Transposon directed insertion sequencing-site sequencing (TraDIS) was used to identify the full repertoire of T6SS toxins in *Pseudomonas aeruginosa*, as well as a new toxin Tse8, which was found to target the GatABC transamidosome, that is required for protein synthesis in a range of bacteria but not in eukaryotes. Not only is this a completely new target for a T6SS-associated toxin but the Tse8-transamidosome interaction has directed us to a potential novel mechanism for selectively inhibiting protein synthesis in a range of bacterial pathogens. This supports the use of TraDIS to access an unexplored range of validated antibacterial targets which will be a great asset in directing development of novel antibacterial therapeutics.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P244

Probing substrate-dependent conformational dynamics of the Na⁺/succinate transporter, VcINDY, using solvent accessibility measurements.

Connor Sampson¹, Joseph Mindell², Christopher Mulligan¹

¹University of Kent, Canterbury, United Kingdom. ²NINDS, Bethesda, USA

Abstract

The Divalent Anion:Na⁺ Symporter (DASS) family transports various metabolites, including Krebs cycle intermediates and sulphate, within all domains of life, playing a key role in metabolic regulation. The Na⁺/succinate transporter, VcINDY, from *Vibrio cholerae*, is the only member of the DASS family for which we have high resolution structures, and therefore acts a structural model for this family. Furthermore, evidence suggests that seemingly unrelated bacterial transporter families involved in antimicrobial resistance (AbgT) and virulence (TRAP transporters) have the same protein fold as VcINDY and presumably a similar mechanism. Therefore, furthering our understanding of the mechanism of VcINDY could also illuminate the mechanisms of other important bacterial transporters.

In this work, we sought to gain a better understanding of the substrate-dependent conformational changes involved in VcINDY's transport mechanism. To do this, we have developed an SDS-PAGE-based band shift assay to monitor the solvent accessibility of strategically positioned single cysteine residues. Using this assay, we have probed the accessibility of several positions in the presence and absence of substrates, identifying positions that are sensitive to the presence of Na⁺/succinate and Na⁺ alone. Mapping these positions onto the protein structure will provide insight into the global and local conformational changes that are essential to the transport mechanism.

This work provides novel insights into the conformational dynamics of this model transporter with implications for the extended DASS family and other transporters with the same fold.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P245

Understanding the Role and Mechanism of the DedA Family of Integral Membrane Proteins in Antimicrobial Resistance.

Joseph Carroll, Christopher Mulligan

University of Kent, Canterbury, United Kingdom

Abstract

Membrane proteins perform critical functions such as signal transduction, ion transport, and drug efflux among many other roles. The DedA proteins are a ubiquitous family of integral membrane proteins that have been shown to be important for bacterial viability. In addition, DedA proteins contribute substantially to antimicrobial resistance in clinically relevant pathogens including *Klebsiella pneumoniae*, where a DedA family member was recently shown to be essential for resistance to colistin. Targeting these proteins for inhibition could sensitise bacteria to other antimicrobials, making them easier to kill. However, this quest is hampered by the lack of basic understanding of the structure of DedA, their mechanism of action, and their actual physiological role in the cell. To address the dearth of structural and functional information on the DedA family, we have overexpressed and purified a panel of these integral membrane proteins for biochemical and biophysical characterisation. Ultimately, this work will lead to a mechanistic understanding of DedA protein function and a better grasp of the physiological role of these enigmatic proteins that will underpin the development of inhibitors targeting these proteins.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P246

Conflict and compromise of acetate metabolism, motility and virulence in *Escherichia coli* O157:H7

Liyana Azmi, Andrew Roe

University of Glasgow, Glasgow, United Kingdom

Abstract

Background – Deletion of the bifunctional alcohol/aldehyde dehydrogenase ($\Delta adhE$) in pathogenic *Escherichia coli* O157:H7 (EHEC) unravelled two distinctive phenotypes: suppressed virulence from the reduced expression of the type three secretion system (T3SS) and an over-expression of non-functional flagella. While this presents *adhE* as an attractive anti-virulence drug target, the molecular mechanisms behind these phenotypes remain unknown.

Methods - To understand the basis of $\Delta adhE$ phenotype, we have generated $\Delta adhE$ escape mutants by cultivating $\Delta adhE$ on motility assays for an extended period of time. Whole genome sequencing (WGS) on the mutants was done to reveal mutations in the genome. To elucidate if the virulence of EHEC cross regulates with flagella expression, we compared the flagella expression of $\Delta adhE$ mutant in the non-pathogenic strain, *E. coli* K12 to EHEC via immunoblotting. Quantification of acetate in $\Delta adhE$ in EHEC was done by immunoblotting the total acetylated lysines of the total protein content.

Results – Immunoblots of acetylation profiles in $\Delta adhE$ shows that both intra and extracellular pools of acetate are increased, enhancing expression of flagella. This event, in turn cross-regulates with the expression of the T3SS, explaining the reduced virulence of EHEC. WGS revealed single nucleotide polymorphisms (SNPs) in the conserved D1 domain of *fliC* (A45V) that is thought to inhibit flagellar function. Recombineering experiments have proved that the secondary SNPs in *fliC* indeed does reinstate the motility in $\Delta adhE$.

Conclusion – Our work demonstrates the complex interplay between the T3SS, motility and acetate metabolism in EHEC in order to adapt to different environments.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P247

RsmA and AmrZ Orchestrate the Assembly of all Three Type VI Secretion Systems in *Pseudomonas aeruginosa*

Luke Allsopp, Thomas Wood, Sophie Howard, Federica Maggiorelli, Laura Nolan, Sarah Wettstädt, Alain Filloux

Imperial College London, Department of Life Sciences, MRC Centre for Molecular Microbiology and Infection, South Kensington Campus, Flowers Building, SW7 2AZ, London, United Kingdom

Abstract

The type VI secretion system (T6SS) is a weapon of bacterial warfare and host cell subversion. The Gram-negative pathogen *Pseudomonas aeruginosa* has three T6SSs involved in colonization, competition and full virulence. The H1-T6SS is a molecular gun firing seven toxins, Tse1-7, challenging survival of other bacteria and helping *P. aeruginosa* to prevail in specific niches. The H1-T6SS characterization was facilitated through studying a *P. aeruginosa* strain lacking the RetS sensor, which has a fully active H1-T6SS in contrast to the parent. Yet, study of the H2- and H3-T6SS has been neglected due to a poor understanding of the associated regulatory network. Here we performed a screen to identify H2- and H3-T6SS regulatory elements and found that the post-transcriptional regulator RsmA imposes a concerted repression on all three T6SS clusters. A higher level of complexity could be observed as we identified multiple regulators including the transcriptional regulator, AmrZ, which acts as a negative regulator of the H2-T6SS. Overall, while the level of T6SS transcripts is fine-tuned by AmrZ, all T6SS mRNAs are silenced by RsmA. We expanded this concept of global control by RsmA to VgrG spike and T6SS toxin transcripts whose genes are scattered on the chromosome. These observations triggered the characterization of a suite of H2-T6SS toxins and their implication in direct bacterial competition. Our study thus unveils a central mechanism which modulates the deployment of all T6SS weapons that may be simultaneously produced within a single cell.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P248

Peptidoglycan recognition by the lysostaphin SH3b domain

Luz Gonzalez, Stephane Mesnage, Michael P Williamson

The University of Sheffield, Sheffield, United Kingdom

Abstract

Lysostaphin is a bacteriocin produced by *Staphylococcus simulans* that specifically cleaves the pentaglycine crossbridges in *Staphylococcus aureus* peptidoglycan. Although lysostaphin has very narrow substrate specificity, how this enzyme binds to the substrate is poorly understood. The cell wall-targeting domain of lysostaphin belongs to the SH3b domain family and consists of 92 C-terminal residues sufficient for non-covalent binding to peptidoglycan. We have conducted nuclear magnetic resonance (NMR) experiments to elucidate the molecular basis for peptidoglycan recognition by the lysostaphin SH3b domain. Resonance assignment of the backbone and sidechains of the domain were made using standard triple resonance on a doubly-labelled SH3b protein. ¹⁵N-HSQC spectra of a singly-labelled SH3b and chemical shift perturbation analyses were used to measure affinities to various purified peptidoglycan fragments and to identify the residues involved in binding. In addition to these in vitro experiments, we explored single-cell binding of recombinant SH3b-GFP fusions to live bacteria using flow cytometry.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P249

To Treat or Not to Treat: Asymptomatic Bacterial Vaginosis and the Correlation to Human Papillomavirus in the United States

Brett Colbert¹, Makella Coudray², Daniel Ruiz-Perez³, Karl Krupp⁴, Giri Narasimhan³, Purnima Madhivanan², Kalai Mathee⁵

¹Department of Biology, Florida International University, Miami, USA. ²Department of Epidemiology, Florida International University, Miami, USA. ³Engineering and Computer Science, Florida International University, Miami, USA. ⁴Department of Health Promotion and Disease Prevention, Florida International University, Miami, USA. ⁵Department of Human and Molecular Genetics, Wertheim College of Medicine, Florida International University, Miami, USA

Abstract

In the United States alone, there are roughly 7 million new cases of human papillomavirus (HPV) each year; many of these are persistent and lead to an increased risk of contracting cervical and other types of cancer. It has been proposed that bacterial vaginosis (BV) may make an individual more susceptible to contracting HPV by increasing the vaginal pH and exposing the vaginal epithelial cells to the virus. This study has utilized stored vaginal swabs of eighty women with asymptomatic bacterial vaginosis collected in a randomized open-label clinical trial. Forty were treated with Metronidazole and forty were untreated, as the current standard of care is not to treat asymptomatic BV. 16S sequencing was performed on all the samples to examine the change in microbial community between groups and further examine any temporal changes. The results show that there is a clear difference between treated and untreated individuals, with the untreated having a higher diversity of microbes and complete absence of *Lactobacillus* species, which are usually associated with a healthy vaginal microbiome. The treated individuals were dominated by *Lactobacillus* species, especially *L. fornicalis*, *L. iners*, *L. jensenii*, and *L. reuteri*, suggesting that treating asymptomatic BV is indeed beneficial. The DNA from the stored swabs will be tested for 9 common oncogenic HPV types (the ones covered by the nonavalent Gardasil vaccine) and data will be available by March 2018. These data will allow a correlation between the various microbiome states that have been observed and the presence of oncogenic HPV.

The Games Microbes Play: Competition, Conflict and Cooperation In Microbiology

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P250

Multispecies biofilm formation by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* and interaction with relevant species

Priscila Vitola^{1,2,3,4}, David Cleary^{5,6,2}, Stuart Clarke^{5,6,2}, Jeremy Webb^{1,2,3,4}

¹Centre for Biological Sciences, University of Southampton, SOUTHAMPTON, United Kingdom. ²Institute for Life Sciences, University of Southampton, SOUTHAMPTON, United Kingdom. ³Southampton NIHR Respiratory Biomedical Research Unit, University Hospital Southampton NHS Foundation Trust, SOUTHAMPTON, United Kingdom. ⁴National Biofilms Innovation Centre, University of Southampton, SOUTHAMPTON, United Kingdom. ⁵Southampton NIHR Respiratory Biomedical Research Unit, University of Southampton and University Hospital Southampton NHS Foundation Trust, SOUTHAMPTON, United Kingdom. ⁶Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine, SOUTHAMPTON, United Kingdom

Abstract

Streptococcus pneumoniae (*Sp*), *Haemophilus influenzae* (*Hi*) and *Moraxella catarrhalis* (*Mc*) are amongst the most prevalent species to colonise the human nasopharynx (NP), exhibiting a ubiquitous and strong positive association. NP colonisation – and biofilm formation – is an essential step for the later development of invasive diseases, which is influenced by changes on NP microbiome's diversity.

Single- (SS) and multispecies (MS) biofilms (BF) were grown in vitro for 72 hours, and individual species were quantified at different time points by propidium monoazide real-time polymerase chain reaction or CFU enumeration. MS-BF were performed with *Sp-Mc-Hi* only, or in combination with *Staphylococcus aureus* (*Sa*), *Lactococcus lactis* (*Ll*) or *Lactobacillus casei* (*Lc*).

The results show that *Sp-Mc-Hi* form a stable MS-BF over 72 hours. In comparison to its SS-BF, *Hi* increases on MS-BF ($p \leq 0.0001$), whilst *Mc* decreases by approximately 4 log₁₀ units. Conversely, *Sp* biofilm formation is mostly unaffected by *Mc* and *Hi*. *Sp-Mc-Hi* were reduced ($p \leq 0.0001$) on MS-BF containing *Sa* or *Ll* in comparison to 3-species BF, except for an increase of *Mc* on biofilms containing *Ll*. Conversely, when in combination with a non-traditional NP coloniser, *Sp-Mc-Hi* have either increased or maintained similar numbers, even in presence of high *Lc* counts.

In conclusion, *Ll* and *Sa* – a known NP competitor – have affected but not eradicated *Sp-Mc-Hi* on MS-BF, which might indicate a shared protection or increased stability of the MS-BF. Additionally, these results might provide basis for future studies of *Ll* as a probiotic for the respiratory tract.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P251

The use of phosphate solubilizing bacterium, ammonium nitrogen (NH₄⁺-N) and nitrification inhibitor in alkaline soils

Mehmet Ogut

Selcuk University Cumra Higher Educational College, Konya, Turkey

Abstract

A series of microcosm, rhizobox and rhizosystem experiments on an alkaline soil with wheat were carried out to determine the effects of *Bacillus sp.* #189, a phosphate solubilizing bacterium on plant growth on an alkaline soil. *Bacillus sp.* #189 tend to change the microbial community structure; decrease nitrifier numbers; increase nitrogen mineralization. As ammonium:nitrate ratio increases number of nitrifiers increases. High ammonium:nitrate ratio or rock phosphate addition increases alkaline phosphatase activity. DMPP is relatively more effective on microbial community structure when there is high nitrogen dose or high ammonium:nitrate ratio. The DMPP's decreasing effect on nitrifier numbers is not dependent on nitrogen dose or ammonium:nitrate ratio. A treatment containing bacterial inoculation+rock phosphate supplying enough plant P+low level nitrogen and a treatment containing enough mineral nitrogen and phosphorus produce similar root length. Bacterium+rock phosphate+nitrogen modifies the organic acid extrusion from the plant roots especially earlier in time. Consequently, this study determined that the mechanisms by which *Bacillus sp.* #189 increases plant growth include (i) change microbial population structure; (ii) decrease nitrifier numbers; (iii) increase root length; (iv) modification in organic acid extrusion.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P252

The BLAST and the spurious - Exploring microbial communities to find novel antibiotics with the AMPLY metagenomic analysis pipeline.

Ben Thomas^{1,2}, Chris Creevey³

¹Tika Diagnostics (St. George's Hospital, Tooting), London, United Kingdom. ²Queen's University (Sharon Hughes), Belfast, United Kingdom. ³Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Bacterial antibiotic resistance is widely regarded to be one of the most pressing threats facing humanity. Finding new antibiotics is a vital research area and can now be supported by a vast reservoir of readily available 'omic data on the back of the explosion of low cost sequencing technologies.

Antimicrobial Peptides (AMPs): endogenous peptides that provide a fast and effective means of defence against pathogens as part of the innate immune response. The detection of AMPs in metagenomic data is a tantalising low-hanging fruit for computational biologists. Bioactive peptides have been isolated with sequences less than 10aas. Large reservoirs of existing sequences exist and are well annotated and understood. Post-computational wet-lab work is relatively cheap with spot synthesis of peptides cheaply available from a wide array of third party companies. A well organised screening program can screen 100s of prospects a day against model bacterial organisms to test for activity and is one of the few areas of biological science that can scale to meet the data output from computational prediction toolkits.

AMPLY, an in-house tool designed at Aberystwyth University supported by Life Science Wales and working in collaboration with Tika Diagnostics at St. George's Hospital (London) and Queen's University (Belfast) is part of a next wave of computational drug discovery platforms and is already uncovering a treasure trove of novel AMPs in diverse microbial environments. We highlight the significant benefits of forming a network between the understanding of microbial community dynamics, directed bioinformatics and confirmatory lab screening.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P253

Rapid loss of CRISPR-mediated herd immunity from bacterial populations

Sean Meaden, Loris Capria, Ellinor Opsal, Angus Buckling, Edze Westra

University of Exeter, Penryn, United Kingdom

Abstract

CRISPR-Cas are prokaryotic adaptive immune systems that insert parasite-derived DNA sequences (spacers) into genomic CRISPR loci to provide sequence-specific immunity. One feature that sets CRISPR-Cas apart from other adaptive immune systems is that immunity is heritable, resulting in trans-generational protection against fixed parasite genotypes. However, both theory and data show that levels of CRISPR-Cas immunity also depend on the diversity of spacers present within a bacterial population, with higher levels of herd immunity when the number of different spacers increases, since this constrains parasites ability to evolve to overcome CRISPR immunity. Despite the importance of spacer diversity for CRISPR herd immunity levels, the mechanisms that maintain this diversity over time are unexplored. To examine this, we performed experimental evolution using the opportunistic human pathogen *Pseudomonas aeruginosa* strain PA14, and its bacteriophage (phage) DMS3vir. We used a deep sequencing approach with a range of phenotypic assays to explore the population dynamics. Consistent with earlier work, we found that upon infection bacterial populations initially generate high population-level spacer diversity, which causes rapid phage extinction. However, levels of spacer diversity rapidly decline after phage extinction as both sensitive bacteria and partially resistant receptor mutants invade the CRISPR population. The key consequence of this process is that immunized bacterial populations that were initially highly effective in driving phage extinct, rapidly lose this ability against the same phage when it is re-introduced into the population. These data can help to explain how bacteria with CRISPR-Cas immune systems and phage can coexist in natural environments.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P254

Incorporation of *Vibrio vulnificus* into Phytoplankton-based Marine Aggregates for Oyster Uptake

Cam Hubert, Steve Michell

The University of Exeter, Exeter, United Kingdom

Abstract

Vibrio vulnificus is a halophilic bacterium found in high numbers within oysters, which sometimes causes human disease. Despite this environmental pervasiveness, there are very few clinical cases observed globally. This study's aim is to incorporate *V. vulnificus* strains into defined 'marine snow' in order to monitor bacterial ingestion by oysters. Marine snow describes the aggregation of naturally occurring phytoplankton, bacteria, debris, larvacean houses and other organic materials. In this study we have generated a defined marine snow comprised of a specific number of diatoms. The eventual objective is to monitor the uptake of a panel of *V. vulnificus* strains, varying in virulence, into oysters via defined marine aggregates. Rolling tubes containing 20 ppt artificial seawater, a predetermined number of diatoms, bacterial cultures and hyaluronic acid were rotated at 16 rpm for 24 hours to generate aggregates. Marine snow was isolated by vacuum filtration for serial dilution and enumeration. Initial results showed significantly greater aggregation of E-genotype over C-genotype *V. vulnificus* strains, supporting current findings in the literature. Addition of a known volume of diatom cells/L resulted in consistent aggregate formation with no immediate variations in size or volume. Diatom-based aggregates present a controllable and reproducible model for incorporating *V. vulnificus* into marine snow. Future studies will aim to look at the uptake rates of this marine snow into oysters and the ecological interactions between *V. vulnificus* strains of differing virulence within the oyster host.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P255

Tracking horizontal gene transfer and plasmid-host evolution in experimental soil bacterial communities

James Hall, Ellie Harrison, Michael Brockhurst

University of Sheffield, Sheffield, United Kingdom

Abstract

Horizontal gene transfer (HGT) is a fundamental and pervasive mode of bacterial evolution, with profound implications for adaptation. Mobile genetic elements (MGEs), such as plasmids, are key vectors of HGT, shuttling genes to diverse new lineages. We use experimental evolution in multi-species, spatially-structured, soil microcosm environments to investigate how ecological factors affect the spread of resistance genes. We subjected replicate species-rich soil wash microbial communities to combinations of different treatments: addition of bacteria carrying mobile mercury resistance genes, and application of mercury contamination. Here we present the effects of these treatments on community structure and resistance gene spread. First, we show that mercury can enhance the competitive ability of a plasmid-receptive species, by virtue of that species' ability to acquire resistance MGEs from neighbouring bacteria. Second, using 16S amplicon sequencing, we show how mercury application affects microbial community structure. Finally, we use an emulsion-based amplicon sequencing technique (epicPCR) to track the spread of an MGE-borne mercury resistance gene through the community, and find dissemination to diverse recipient species. Our data demonstrates the impact of ecological factors on HGT and microbial community evolution.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P256

Assessment of the long-term performance of plant microbial fuel cells using Irish Peat and *Calluna vulgaris*.

Carena Bell, Patricia Linton, Robin Sen, Craig Banks

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

The contribution of fossil fuels to current environmental challenges is widely known. Microbial fuel cell (MFC) and plant microbial fuel cell (PMFC) technologies provide a unique opportunity to address the increasing global energy demands by providing an environmentally friendly and sustainable method of generating power. MFCs generate current by using bacteria as a catalyst to oxidise organic matter. Electrons are produced by bacteria when organic matter is broken down and the electrons are transferred, firstly to the anode and secondly to the cathode thus completing a circuit.

The use of plants can make this technology self-sustaining by root exudates providing a nutrient source for electrogenic microorganisms. This study aimed to optimise PMFC technology and used a novel PMFC construction using *Calluna vulgaris* in peat using graphite felt and activated carbon electrode material in the anode and cathode chambers separated by a cation exchange membrane.

Power outputs were analysed over 90 days (measured daily) and over 24-hour cycles (measured every 10mins). In this study the novel PMFC design produced steady and reliable power output in contrast to previous studies using PMFC technology. PMFCs with *C. vulgaris* produced an average power output of 31 mW/m² and a maximum power output of 38mW/m² as compared to soil-MFCs without plants in the cathode chamber which produced an average power output of 19mW/m². The addition of plants to the MFC therefore enhances the PMFCs power generation capacity and demonstrates the potential for this design as a method of sustainable power generation.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P257

Insulative Particles Accelerated Methanogenic Propionate Degradation

Yan Huang, Hui Fan, zheng zhou, Yu Deng, [Shichun Ma](#)

Biogas Institute of Ministry of Agriculture, Chengdu, China

Abstract

Propionate degradation is an important step during the anaerobic degradation of organic matter, and it requires a syntrophic cooperation between propionate-oxidizing bacteria and methanogens. In this work, we evaluated the effect of insulative material on the syntrophic propionate oxidation and microbial community under methanogenic condition.

Batch experiments of methanogenic propionate-degrading consortium were conducted in 120 ml serum bottles containing 50 ml of BCTY medium. Bottles were incubated statically at 40 °C with 5 g insulative particles at a final concentration was 100 g /L (insulative particles group). Control group was prepared as above but in the absence of insulative particles. Gaseous products (CH₄, CO₂ and H₂) and the volatile fatty acids were measured during incubation. Bacterial and archaeal methanogenic community were analyzed by 16S rRNA genes sequencing using Illumina MiSeq or Pacbio platform.

We found that the insulative particles supplementation accelerated CH₄ production from propionate in methanogenic propionate-degrading enrichment. Compared with the control group, the time for complete degradation of propionate (3 g/L) was reduced by 24% and much more microbial aggregates were observed in insulative particles group. *Pelotomaculum* and *Methanoculleus* were predominant bacteria and archaea in both control group and insulative particles group. However, *Methanosarcina*, mainly presenting in both aggregates and the surface of particles, was identified as the second dominant methanogen in insulative particles group. Supplementation of insulative particles might triggered the growth of adept self-aggregative *Methanosarcina* and shortened the interspecies distances by forming aggregates.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P258

DISSECTING BIOMETHANE PRODUCTION IN ANAEROBIC DIGESTION PLANTS THROUGH DNA SEQUENCING

Anna Alessi¹, Bing Tao², Sonia Heaven², Charles Banks², Jane Thomas Oates¹, James Chong¹

¹University of York, York, United Kingdom. ²University of Southampton, Southampton, United Kingdom

Abstract

As natural gas consumption increases annually (EU by 7.1% since 2010), the production of methane via biologically optimised processes offers an alternative. The conversion of organic domestic waste to gaseous, energy-rich biomethane is reported across the world. This renewable energy source can be utilised in grid balancing and transportation. The relatively low methane concentration of biogas means gas produced this way requires upgrading before it can be injected to the grid. The biogas upgrading remains a cost-limiting step making the product economically unviable. Using laboratory scale reactors and DNA sequencing, we have studied the identity and physiology of the microorganisms responsible for anaerobic digestion (AD) and methane production. Reactor performance was monitored daily for one year to collect robust operational data that can be used for scaling reactor working volumes and for predicting performance at industrial scales. Biological samples were subjected to metagenomic DNA sequencing. By combining short and long read sequencing techniques, we have obtained complete or near-complete genomes for several of the most abundant microorganisms. These genome sequences have revealed metabolic pathways for sugar utilisation to produce hydrogen, CO₂ and volatile fatty acids and for subsequent conversion of these compounds to high quality biomethane. Our data provide evidence that close interactions occur between highly specialized and enriched microorganisms in AD bioreactors. Our sequencing results enable a deeper understanding of the diversity and ecology of the resident microbial community that could be manipulated to increase the efficiency and quality of the AD process.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P259

“Screening of Essential Oils and Inhibitory Bacteria as potential Control Agents of Plant Pathogens.”

David Nelson¹, Colin Fleming¹, Trevor Martin¹, Thomas Fleming¹, Richard Salvage², Juluri Rao¹

¹AFBI, Belfast, United Kingdom. ²Maxstim, Cardiff, United Kingdom

Abstract

Four plant pathogenic genera, *Fusarium*, *Phytophthora*, *Hymenoscyphus* and *Microdochium* cause significant losses to the cut flower industry, woodlands and amenity turf areas of Northern Ireland. Bacteria were isolated from infected soils by plating soil dilutions onto Potato Dextrose Agar (PDA), and any bacterial cultures showing antimicrobial activity were single colony purified, and their inhibitory properties further studied using dual-control assays on PDA. Agar plugs of pathogens were also laid onto 'lawns' incorporating dilution series of a selected range of essential oil extracts, to determine the concentration of each extract necessary to control pathogen growth. Inhibitory activity was compared with a serial dilution series of a garlic extract.

These assays isolated 4 species of bacteria (3 *Bacillus* spp. and a *Paenibacillus*) that were found to be inhibitory to *Fusarium*, *Phytophthora*, *Hymenoscyphus* and *Microdochium*. The essential oil extracts were responsible for a range of species-specific inhibition, whereas concentrations of > 3% of the garlic extract proved totally inhibitory to the growth of all 4 pathogens.

Based on these results, *Paenibacillus* and *Bacillus* isolates were chosen for further trialling as microbiocides, as suspensions for direct application to both soils and foliage, in both pot and field trials, to assess their potential primarily as prophylactics in controlling these and other plant pathogens. Considerable potential existed within the range of essential oil extracts to warrant further, more in-depth investigation.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P260

Feature Selection from Microbial Profiles via a Genetic Algorithm.

Nisha Puthiyedth¹, Kevin Stanley¹, Steven Mamet², Steven Siciliano², Tony Kusalik¹, Matthew Links^{1,3}

¹Department of Computer Science, University of Saskatchewan, Saskatoon, Canada. ²Department of Soil Science, University of Saskatchewan, Saskatoon, Canada. ³Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada

Abstract

The associations of microbes with the host they inhabit have a significant impact on host health. Identification of specific taxa from microbial profiles is a particularly challenging type of feature selection problem due to the large yet sparse datasets that represent a microbial profile. This work set out to identify microbes that were differentially associated with crop seeds using a Genetic Algorithm (GA).

Feature selection (FS) is a process of finding sub-collections of features from a high dimensional dataset. We employed a GA for FS from microbial profiles that select the best set of features according to the basic operators of GA that is fitness function, crossover and mutation probability. The fitness function assists GA to select the individuals that meets the overall problem specification. In this case, we designed the fitness function as an ANOVA and microbes with higher variance in abundance across samples were selected for crossover and mutation.

Using epiphytic seed associated microbial profiles, we applied the GA to find a set of microbes that characterize the variation between canola and wheat epiphytic microbiomes. Of the results obtained by GA, 84% were consistent with the previous reports of this microbiome. Assessment of the microbes uniquely identified by the GA is ongoing.

This work demonstrated that GAs can be used to identify collections of microbes that differentiate samples in microbiome studies. The use of GA may lead to new insights about host-microbe interactions and these techniques translate to all ecological settings where communities of microbes are being studied.

Synthetic Ecology: From Understanding Ecological Interactions To Designing Functional Microbial Communities

Zone C

Presentations: Tuesday and Wednesday Evening

P261

Novel mechanisms for overcoming phosphate limitation in rhizosphere-dwelling *Flavobacteria* spp. Implications for sustainable agriculture.

Ian Lidbury, Andrew Murphy, Gary Bending, Alex Jones, David Scanlan, Elizabeth Wellington

University of Warwick, Coventry, United Kingdom

Abstract

In soil, phosphorus (P) exists in numerous organic and inorganic forms. However, plants can only acquire inorganic orthophosphate (Pi), meaning global crop production is frequently limited by P availability. To overcome this problem, rock phosphate fertilisers are heavily applied, often with negative environmental and socio-economic consequences. Despite knowing microbes can facilitate P mobilisation, the precise mechanisms and key players facilitating this process remain unknown. Recent evidence has revealed the enrichment of *Flavobacteria* spp. in the rhizosphere of many economically important crops. To determine their potential to mobilise Pi, we isolated numerous strains from the rhizosphere of field-grown Oilseed Rape (*Brassica napus*). Unlike various other rhizobacterial groups, *Flavobacteria* strains displayed constitutive phosphatase activity. Size exclusion-chromatography on the soluble protein fraction of the model bacterium *Flavobacteria johnsoniae* UW101 revealed that multiple phosphatases were responsible for the observed phenotype, specifically the active size fractions were different when cells were harvested from either Pi-replete or Pi-deplete growth conditions. Comparative genomics and exoproteomics of numerous *Flavobacteria* strains revealed that they do not possess the traditional enzymes associated with P mobilisation and instead harbour several previously uncharacterised exoenzymes. Interestingly, our data also revealed that they lack the high affinity Pi transport system (PstSABC) and possess a novel mechanism, confirmed by mutagenesis, for the high affinity uptake of Pi under low extracellular Pi conditions. In conclusion, *Flavobacteria* strains, which are frequently enriched in various crop rhizospheres, display a distinct ability to mobilise P and thus are potential targets for bioagronomy.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P262

Innovative Assessments in Microbiology: The Bio-animation Project Experience

André Antunes¹, Lorena Fernández-Martínez¹, Paul Ashton¹, Alex Jukes²

¹Biology Department, Edge Hill University, Ormskirk, United Kingdom. ²Media Department, Edge Hill University, Ormskirk, United Kingdom

Abstract

Science and Technology are universally reported as playing an essential role in the economy, but its education is frequently understated or even unaddressed [1]. The quality of education in these fields has long been an area of concern, but efforts to improve it have been lagging behind. Promising results from several studies demonstrate the advantages of shifting from exclusive use of classic lectures and adopting and extending the use of “active learning methods” with broad and consistent benefits across different topics, teaching levels, and experimental methodologies [2-3].

The scenario in the field of Biosciences in general, and Microbiology in particular is equally bleak, and has been the focus of a few recent initiatives and studies that have identified the reliance on outdated teaching methodologies as a major shortcoming (e.g. [4]).

Here we describe the successful implementation of a Bio-animation project: an assessment for a Microbial Genetics module taught to 2nd year undergraduate students coupling them with Animation students at our University. The project promotes the use of 21st century skills, particularly critical thinking and complex communication skills, which are vital for the future employability of our students. We also provide an overview of feedback from students and peers and identify areas that need special attention.

[1]Bybee & Fuchs (2006) *J Res Sci Teach*, 43(4):349-352.

[2]Freeman et al. (2014) *Proc Natl Acad Sci U.S.A*, 111(23):8410-8415.

[3]Wieman (2014) *Proc. Natl Acad Sci U.S.A*, 111(23):8319-8320.

[4]Antunes et al. (2017) *Trends Microbiol* 24(2):77-79.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P263

Sailing towards Accreditation Paradise with Skills Passport in Hand

David Whitworth

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

For recruiting Universities the accreditation of degree schemes can substantially enhance student recruitment, as it represents a public endorsement from a respected learned society, serving as a guarantee that graduates meet acceptable levels of skills and knowledge.

Aberystwyth University is a recruiting University receiving increasing recognition for the quality of its teaching. Nevertheless it faces significant geographical challenges when recruiting students, and therefore Accreditation was recently sought from the Royal Society of Biology (RSB) for 15 degree schemes, including our two Microbiology degrees.

In preparing for accreditation we were unsure whether we met one of the accreditation criteria – namely the direct assessment of subject-specific technical skills. Our focus on skills assessment had been focussed on higher levels of cognitive function, for instance the ability to interpret and report novel data. We did not tend to directly assess students on their technical competent in generating that data.

We therefore developed a 'Skills Passport' for documenting subject-specific technical skills. The Skills Passport places the onus on the student to reflect on their skills and obtain staff signatures to confirm their technical prowess. It was also designed to minimise the impact on staff workload.

The RSB panel were overwhelming positive about our approach, to the point that we are embedding the passport within formal assessments in core modules. This poster will present the Skills Passport and our experiences of engaging staff and students with it. It will also describe our current thoughts regarding how mechanically we can embed it throughout the curriculum.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P264

Towards a core set of microbiology teaching and learning concepts for UK medical students

James Edwards

University of Plymouth, Plymouth, United Kingdom

Abstract

Medical students are acknowledged as having very specific learning needs, distinct from that of their bioscience peers. This is being increasingly recognised by various learned societies and royal colleges who have published their own suggested curricula for medical courses. To date, a dedicated set of core scientific concepts for microbiology teaching within UK medical schools has been lacking. Yet a clear list of microbiology content may offer real benefit to staff and students struggling with an already crowded curriculum.

As part of an evaluation of microbiology learning at Plymouth University Peninsula Medical School, using a combination of student interviews and questionnaires, I have explored with our undergraduate medical students microbiology content over the first 4 years of their course. Through this work, I have identified a core set of (problematic) microbiology concepts and have aligned the microbiology teaching to address these areas.

Here, I will share the results of my study along with the suggested core topics for undergraduate microbiology teaching for the UK. I will suggest that perhaps by focusing on concepts rather than generating a list of pathogens, these results may be useful regardless of how medical courses are structured. My presentation will be designed to generate discussion and explore the possibility of creating a network of microbiology teachers with a shared interest in further exploring how our microbiology expertise can be optimised within medical curricula.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P265

Medical student authored microbiology learning resources

James Edwards, Abdelrahman Elbeltagi, Isabella Parkes, Mohamed Ridha, Karen Johnstone

University of Plymouth, Plymouth, United Kingdom

Abstract

The General Medical Council require students registered at UK medical schools to attain the 'skills and attitudes of a competent teacher' as outlined in 'Outcomes for Graduates'. In order to achieve these aims, Students at Plymouth University Peninsula Medical School undertake in their third year a longitudinal module named 'Doctor as Educator' in which they develop and evaluate an educational resource of their own choosing.

Although students can choose to develop any resource they wish, microbiology resources are consistently a popular choice. From a microbiology education perspective, the development of student authored resources have revealed that gamification of microbiology learning is a particularly popular drive for resource development. This is likely to reflect student views that microbiology can be viewed as a 'dry' topic, which can make learning certain aspects of clinical microbiology such as common organism names, antibioticss and their mode of action difficult.

Here, we will present examples of student authored resources that have been created by medical students in their third year. One is a podcast designed to help students learn antibiotics and their mode of action whilst on the move. The other two resources are designed to allow students to become familiar with microorganism names and demonstrate the preference for game-based learning: a 'guess who' style game and a card game. We will present evaluation data for each of these resources.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P266

Game on! Game-enhanced learning in microbiology teaching

Alison Graham, Sara Marsham

Newcastle University, Newcastle-upon-Tyne, United Kingdom

Abstract

Game-enhanced learning is increasingly popular in university teaching as a method of increasing engagement of students with challenging concepts. This project is investigating the use of traditional games (e.g. board games, card games) and gamification (the principles of game design in a non-game context) in natural sciences teaching. This includes “escape room-style” activities and puzzles but excludes digital/electronic games. Visit our poster to see the latest results from the project, including our card game to teach accurate pipetting skills to students.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P267

'Contagious Thinking' - a Digital Educational Platform Integrated Into A Major Research Centre

Connor Bamford

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

Abstract

Connecting research scientists with students in higher education could mitigate challenges facing teaching microbiology today. However, difficulties like physical separation, time constraints and lack of training impede the realisation of this opportunity. Exploiting innovative digital communication tools (social media, blogging, podcasting and video production) to generate relevant and engaging high-quality educational resources could help address these issues, motivating researchers to engage, inspire and educate students worldwide. To this end, we have embedded a digital communications platform within a major international research centre at the MRC-University of Glasgow Centre for Virus Research. This centralised platform – called 'Contagious Thinking' - consists of integrated written, audio and video resources supported through social media and produced by a staff/student team in conjunction with the undergraduate Virology curriculum. The overall aims of Contagious Thinking are to: 1) establish a novel teaching resource; 2) use this to engage with local and international students; and 3) train and inspire researchers to become educators using digital tools and make it self-sustaining. Running for 3 years, Contagious Thinking has published ~ 30 podcasts and 50 blog posts focusing on the current science of our researchers or visitors, including local, international and even Nobel Prize winning Virologists. Having been viewed thousands of times within and outside of the university, Contagious Thinking now forms an integral educational resource and exemplary tool. We will discuss the practicalities of establishing digital platforms in a research centre and the pros- and cons- of their capability to capture, engage and aid current microbiology education techniques.

Teaching Microbiology In Higher Education
Zone C
Presentations: Tuesday and Wednesday Evening

P268

Microbiology in Primary Science

Lisa Crossman

SequenceAnalysis.co.uk, Norwich, United Kingdom. Uni, Norwich, United Kingdom

Abstract

A recent primary science report (1) surveyed a total of 1,010 teachers, 902 science leaders and 1,906 pupils aged 7-11 and was carried out by CFE Research with the University of Manchester commissioned by the Wellcome Trust. Within the report, primary school teachers are described as facing barriers in teaching science. Per week, only 1 hour 24 minutes are typically being devoted to the subject in the National Curriculum. The top barriers described were the lack of budget and resources, lack of time and curricular importance as well as other issues such as a lack of subject knowledge or confidence and concerns relating to space and resource access. Whilst 93% of pupils agreed they like to understand how things work, only 44% of pupils like science at school 'a lot'. Schools can introduce additional content into the Curriculum within the relevant Key Stage of 1 and 2 and many deliver science through dedicated science weeks.

Within this context, a Microbiology, Genomics and Bioinformatics researcher in association with a Key Stage 2 class in a Norfolk Junior School planned a joint project to take Microbiology-related science into the classroom. Here we outline the project and report on findings from both teacher and pupil's perspectives.

1) 'State of the Nation' report of UK primary science education. Baseline research for the Wellcome Trust Primary Science Campaign. S. Leonardi, H. Lamb, P. Howe & A. Choudhury. CFE Research, Pheonix Yard, Upper Brown St. Leicester LE1 5TE. September 2017.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P269

Microbiota Development Across Several Anatomical Sites in Preterm Infants at risk of Necrotising Enterocolitis

Gregory R Young¹, Darren Smith¹, Nicholas D Embleton², Janet Berrington², Christopher J van der Gast³, Clare Lanyon¹

¹Northumbria University, Newcastle, United Kingdom. ²Newcastle Neonatal Service (NuTH), Newcastle, United Kingdom. ³Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Severely premature infants have an immature immune system, increased intestinal permeability and receive significant medical intervention, contributing to a greater risk necrotising enterocolitis. Microbial dysbiosis has been linked with NEC however aetiology remains enigmatic.

We compared establishment of severely preterm gut microbiota, using 158 samples including oral and endotracheal swabs, stool and breast milk, collected longitudinally from 7 patients over the first 2 months of life. Samples were treated for non-viable DNA exclusion prior to extraction and targeted sequencing of the V4 region of the 16S rRNA gene using the Illumina MiSeq platform.

Community similarity was greatest immediately following birth and between proximal anatomical sites. Breast milk and endotracheal taxa abundances showed the strongest correlation ($R^2 = .72$, $P < 0.05$). All communities were significantly different (pairwise PERMANOVA: $P = <0.01$) and the stool microbiota showed divergence from the upstream mucosae over time (49% at WoL 1 vs 27% at WoL 8). A conserved core of four dominant taxa were observed in all samples by distribution abundance analysis, although fewer taxa constituted the core microbiota in stool than upstream mucosae. Stool microbiota diversity decreased over time, being significantly less diverse than breast milk at WoL 7 - 8 ($P < .005$) but not at WoL 1 - 2.

These findings suggest temporal development of the gut microbiota occurs, whereby speciation and divergence increase over time. This study represents the first of our knowledge to identify exclusively viable bacteria across several anatomical sites of the severely preterm infant GI tract.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P270

Characterisation of Staphylococcus aureus phage infecting MRSA clone isolates of ST22 and ST36

Elliot Whittard, James Redfern, Roobinidevi Ragupathy, Sladjana Malic, Mark Enright

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Staphylococcus aureus (including methicillin-resistant *S. aureus* - MRSA) remains a leading cause of both nosocomial and community-acquired infections globally and is a major cause of biofilm-associated infections observed in chronic wounds. The utility of antibiotics has decreased due to genomic evolution towards antibiotic resistance mechanisms, requiring alternative approaches for therapeutic and prophylactic intervention. Bacteriophage therapy exploits the natural killing ability of lytic bacteriophages (phages) as a means of controlling multidrug-resistant pathogenic bacteria. MRSA infections are caused by a small number of highly-successful clones, ST22 and ST36 are two of the most prevalent clones with global impact. Understanding the phenotypic and genotypic characteristics of these clones in relation to the phages ability to infect and disrupt established biofilms has yet to be explored.

A collection of 78 lytic *S. aureus* bacteriophages were isolated from wastewater samples across Greater Manchester, UK. The phages were screened against 120 genetically diverse *S. aureus*, including 43 ST22 and 24 ST36 strains from UK and USA.

The majority of phage (62/78) displayed a broad host-range over 70% (84/120) with four infecting 94% of study isolates. The four most effective were assessed for their anti-biofilm properties in polystyrene plates against four ST22 and four ST36 isolates. Significant ($p < 0.05$) reductions in colony forming units were observed for all phage-host combinations. Phage genome analyses confirmed each to be novel myoviruses displaying extensive genome-mosaicism. A combination of all four phages has potential to kill all study isolates and are good candidates for inclusion in future phage therapy studies.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P271

The Role of the Placental Microbiome in Chorioamnionitis

Rochelle Hockney¹, Caroline Orr¹, Gillian Taylor¹, Andrew Nelson², Stephen Cummings¹

¹Teesside University, Middlesbrough, United Kingdom. ²Northumbria University, Newcastle, United Kingdom

Abstract

Chorioamnionitis is inflammation of the foetal membranes with links to adverse maternal and neonatal outcomes including preterm birth. The detection of a genuine placental membrane microbiome remains widely debated. This research demonstrates the importance of bacterial load and specific bacterium on the foetal membrane microbiome, and the impact this may have on chorioamnionitis.

Matched paired frozen and Formalin-Fixed Paraffin-Embedded (FFPE) foetal membranes from preterm spontaneous labour with histological chorioamnionitis (n=57) were analysed alongside preterm (n=39) and term (n=17) spontaneous labour without chorioamnionitis. Genomic DNA was extracted from foetal membranes via DNA extraction kits, with DNA quality and quantity analysed via NanoDrop spectrophotometer and agarose gel electrophoresis. 16S rRNA targeted Next Generation Sequencing via Illumina MiSeq platform was used to determine microbial communities of the membranes.

A significant increase in bacterial load was observed in preterm chorioamnionitis compared to healthy preterm membranes ($p < 0.001$). Lower Shannon alpha diversity status was also detected in preterm chorioamnionitis ($p = 0.01$). *Prevotella* and *Ureaplasma* dominated the preterm chorioamnionitis patients, compared to *Escherichia coli* and *Lactobacillus* in healthy preterm and term patients. Only *Prevotella* ($p = 0.02$) and *Lactobacillus* ($p = 0.02$) were significant between groups. Non-concordance between FFPE and frozen membranes questioned the use of FFPE samples in microbiome analysis.

As bacterium were detected in all samples including healthy term membranes, the theory of a sterile placenta is rejected. An increase in bacterial load with dominance of *Prevotella* and *Ureaplasma* occurs in foetal membranes with chorioamnionitis. Overall, both bacterial load and bacterial community may influence the development of chorioamnionitis.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P272

The role of *Bifidobacterium* on antigen-specific dendritic cell responses

Shannah Lympany, Zoe Schofield, Lindsay Hall

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

The gut microbiota plays a critical role in the development and regulation of the immune system. Clinical trials have shown that *Bifidobacterium* (a dominant member of the early life microbiota), is able to enhance the humoral immune response after vaccine administration. However, the mechanisms behind these bifidobacterial-vaccine responses are currently unclear. Dendritic cells (DCs) are efficient antigen presenting cells that drive antigen-specific T cell and B cell responses, leading to immunological memory, and strains of *Bifidobacterium* have previously been shown to differentially regulate DC responses. Thus, we sought to mechanistically explore the potential role of *Bifidobacterium* in modulating DCs and vaccine responses.

DCs were isolated from the bone marrow of C57BL/6 mice and cultured in the presence of LPS (positive control), or individual novel *Bifidobacterium* strains from our in-lab culture collection. Cells were collected at specified timepoints, labelled with stimulatory and co-stimulatory molecules (MHC II, CD80, CD86) and analysed by flow cytometry, and qPCR to determine DC phenotype. Supernatants were analysed for 20-plex cytokine responses.

Analysis of DC responses revealed strain-specific maturation of DC phenotype, including antigen-presentation capacity, and corresponding anti- and pro-inflammatory cytokine responses. Strains were ranked according to their ability to promote appropriate DC responses that would be expected to play a key role during vaccine responses *in vivo*.

Future work will involve using bifidobacterial supplementation in an *in vivo* murine model to compare antigen-specific immune responses after vaccine administration. Determining how microbiota members improve antigen-specific immune responses will help deliver greater vaccine efficacy.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P273

Bifidobacteria: Shield or strain on the gut of preterm infants?

Libby Clements¹, Caroline Orr¹, Gillian Taylor¹, Stephen Cummings¹, Andrew Nelson², Nicholas Embleton³, Janet Berrington³, Christopher Stewart⁴

¹Teesside University, Middlesbrough, United Kingdom. ²Northumbria University, Newcastle, United Kingdom. ³Royal Victoria Infirmary, Newcastle, United Kingdom. ⁴Baylor College of Medicine, Houston, Texas, USA

Abstract

Necrotizing enterocolitis (NEC) is a prevalent disease within the preterm infant population, causing 21% of all deaths in infants under 32 weeks gestational age (GA). Previous examination of bacterial composition of NEC has been inconclusive, therefore the focus has shifted to understanding the metabolomic pathways involved in the interaction between gut and disease causing factors. Human milk contains an abundance and diversity of complex oligosaccharides, indigestible by preterm infants; *Bifidobacterium* utilise these human milk oligosaccharides (HMOs). Specifically, the *Bifidobacteria* strains *infantis*, *breve* and *longum* were targeted because they 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* before and after the onset of NEC by cross-referencing stool and breast milk bacterial samples. In this study breast milk and stool samples were obtained (n = 82, samples = 328) from 41 preterm infants having contracted NEC and 41 preterm infants without NEC but corresponding to the NEC samples with date of birth, day of life and gestational age. The extracted stool DNA was subjected to NGS analysis to identify bacteria, diversity and community structure. Subsequently, qPCR with species specific primers will quantify *Bifidobacteria* strains. 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.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P274

The Microbiome of the Healthy Preterm Infant: To Thrive or Not to Thrive

Cassy Ross¹, Caroline Orr¹, Gillian Taylor¹, Stephen Cummings¹, Andrew Nelson², Christopher Stewart³, Nicholas Embleton⁴, Janet Berrington⁴

¹Teesside University, Middlesbrough, United Kingdom. ²Northumbria University, Newcastle, United Kingdom. ³Baylor College of Medicine, Texas, USA. ⁴Royal Victoria Infirmary, Newcastle, United Kingdom

Abstract

Over recent years considerable attention has been paid to microbiome studies with regard to health outcomes. The aim of this study is to understand normal gut microbiome variations in preterm infants to recognise optimal healthy microbiota during early life. Previous studies investigating the preterm infant microbiome have focussed on the role of microorganisms in conditions such as Necrotising Enterocolitis (NEC), a severe gastrointestinal disorder and one of the most common illnesses occurring in 5–10% of preterm infants. Emphasis has been placed on premature intestinal defences, commensal or probiotic bacterial influences and possible genetic predisposition with little progression towards the pathogenesis of the condition. Alternatively, although they represent the great majority of infants born preterm, the development of individuals without significant disease has not been extensively researched.

A broad-range of inclusion criteria including an increase in weight and use of few antibiotics was developed to expand existing research to demonstrate that health has more measures than purely being non-diseased. The 21 preterm infants enrolled in this study had a mean gestational age of 28 weeks (ranging from 24 – 31 weeks) and a mean birth weight of 1,184 g (ranging from 705 – 1,590 g). Longitudinal stool samples (n = 155) from preterm infants cared for at the Royal Victoria Infirmary will be subjected to 16S sequencing using Illumina MiSeq system to begin to define a baseline healthy preterm microbiome. The application of these methods may potentially provide new means to prevent NEC onset and/or to improve prognosis.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P275

ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM CARP LIVER (*Cyprinus carpio*,L)

sharfina maulidayantu¹, CUT YULVIZAR²

¹AGRICULTRE UNIVERSITY, BOGOR, Indonesia. ²syiah kuala university, aceh, Indonesia

Abstract

Carp is a fish that many people are interested in both national and international market. Carp content high nutrient, but it can not be denied, there are many problems faced by carp farmer in carp cultivation. One of the problems is attacked by microorganism. The microorganism that are observed in this research is from bacteria group. The steps of the research are : sterilization (sterilization room , tools and media) , making the media (solution Oxidative / fermentative (O / F) , Motility Indole Ornithine (MIO) , Methyl Red Vogue Proskuer (MRVP) , Tryptic Soya Agar (TSA) , Triple Sugar Iron Agar (TSIA) , Lysine Iron Agar (LIA) . the process of bacteria examination consist of bacterial isolation, morphological observation, pure culture biochemical tests of bacteria (Gram- test , catalase test , oxidase test , UJIA TSIA , LIA test , test oF , test Citrate , test MRVP and test carbohydrate fermentation). The last steps of the identification of the bacteria is reading the results by using the book cowan and stell's manual of identification bacteria and Bergeys Manual of Determinative Bacteriology. These four bacteria pathogenic that infect fish , especially freshwater fish . Based on the identification performed on 11 liver carp , four species of bacteria that *A. hydrophila* , *A. caviae* , *A.* and *P. vesicularis schubertii* . Fourth These bacteria are pathogenic bacteria that infect fish , especially freshwater fish bacteria will infect the fish in a very weak state of body condition .

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P276

An unusually severe case of recalcitrant acne: Isolation and whole genome sequence analysis of a novel multi-antibiotic resistant strain of *Propionibacterium acnes*.

Joseph McLaughlin¹, Anne Eady², Alison Layton², Judit Hunyadkürti³, Balázs Bálint⁴, Istvan Nagy⁴, Andrew McDowell¹

¹Centre for Stratified Medicine, School of Biomedical Sciences, Ulster University, Derry/Londonderry BT47 6SB, United Kingdom. ²Department of Dermatology, Harrogate and District NHS Foundation Trust, Lancaster Park Road, Harrogate, HG2 7SX, United Kingdom. ³Biological Research Center of the Hungarian Academy of Sciences, 6726 Szeged, Temesvári krt 62, Hungary. ⁴SeqOmics Biotechnology Ltd, 6782 Mórahalom, Vállalkozók útja 7, Hungary

Abstract

In July 2011, an adult male with persistent recalcitrant nodular acne accompanied by extensive hypertrophic scarring was referred to the Department of Dermatology, Harrogate and District NHS Foundation Trust. His GP and dermatologist tried all conventional anti-acne medications, including oral isotretinoin, systemic antimicrobial and anti-inflammatory agents without success. Sebum output remained high. A single isolate of a novel type IA₁ *Propionibacterium acnes* strain, which was resistant to tetracycline (MIC=16mg/l), erythromycin (MIC³256 mg/l), clindamycin (MIC=8mg/l), but rifampicin sensitive (MIC=0.25 mg/l), was isolated in high numbers from the face and trunk, even after high dose isotretinoin treatment; no other propionibacteria were recovered. Addition of oral clindamycin (300 mg bd) and rifampicin (300 mg bd) to a regimen of oral isotretinoin (10 mg/daily) and methyl prednisolone (10 mg daily) brought the acne under control, eradicating the *P. acnes*. Whole genome sequencing (WGS) was conducted on this novel isolate (CP006032), designated hdn-1, using SOLiD (Life Technologies) sequencing technology. Comparative WGS alignments against completed genomes representing other type IA₁ strains did not reveal any unusual differences in content. A huge number of SNPs leading to changes at the amino acid level were, however, identified. Sequence analysis also revealed an A>G SNP at position 2058 in the 23S rRNA gene conferring high erythromycin resistance and variable resistance to other macrolides and clindamycin. This case has provided a unique opportunity to characterise a multi-antibiotic resistant strain of *P. acnes* definitely associated with severe nodular acne accompanied by extensive scarring.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P277

Effect of periodontal disease status and oral microbiome composition on pre-term birth

Erica Prosdocimi¹, Cecilia Gonzales-Marin², William Wade¹

¹Centre for Immunobiology, Blizard Institute, Queen Mary University of London, London, United Kingdom. ²Centre for Adult Oral Health, Department of Periodontology, Institute of Dentistry, Queen Mary University of London, London, United Kingdom

Abstract

Previous studies have shown periodontitis to be associated with pre-term birth. In one case, translocation of oral bacteria to the amniotic fluid has been found. The aim of this study was to determine if periodontal status and oral microbiome composition were associated with pre-term birth.

180 new mothers, half of whom gave birth pre-term, were selected from a larger cohort. Within 3 months of delivery, the Modified Gingival Index, Quigley and Hein plaque indices and a full-mouth periodontal assessment were recorded and samples of plaque and saliva collected. V1-V2 variable regions of the 16S rRNA genes were PCR-amplified from each sample and sequenced by Illumina MiSeq. After a quality filtering performed by the DADA2 R package, Mothur was used to cluster the sequences into OTUs at 98.5% similarity and classify them by comparison to the Human Oral Microbiome Database. The diversity within samples was estimated as mean inverse Simpson index over 1000 subsampling replicas of 5189 sequences each. The same subsampling approach was applied to calculate a theta-YC dissimilarity matrix between samples.

The overall most abundant genera were *Streptococcus* (saliva 29%, plaque 12%) *Prevotella* (13%, 9%), *Leptotrichia* (3%, 15%) and *Fusobacterium* (3%, 12%). There were no significant differences in richness or diversity of saliva and plaque bacterial communities (Wilcoxon test) nor in OTU composition (AMOVA) between term and pre-term groups. Moreover, no significant association was found between periodontal disease and pre-term birth.

In conclusion, this study found no relationship between pre-term birth and oral microbiome composition or gingival inflammation.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P278

CrAssphage in human faecal microbiotas: from bioinformatics to laboratory analysis

Emma Guerin¹, Andrey Shkoporov¹, Stephen Stockdale², Feargal Ryan¹, Tom Sutton¹, Paul Ross^{1,3,2}, Colin Hill^{1,4,2}

¹APC Microbiome Institute, University College Cork, Cork, Ireland. ²Department of Food Biosciences, Teagasc Food Research Centre, Moorepark, Fermoy, Ireland. ³College of Science, Engineering and Food Science, University College Cork, Cork, Ireland. ⁴School of Microbiology, University College Cork, Cork, Ireland

Abstract

CrAssphage is a highly abundant bacteriophage in the gut microbiota of humans. Recently, sequenced based classification was performed on distantly related crAss-like phages from multiple environments, generating a familial level taxonomic group. Here, we assembled the metagenomic sequencing reads from 701 human faecal virome/phageome samples and obtained 98 complete circular crAss-like phage genomes and 145 contigs ≥ 70 kb. Comparative genomics and taxonomic analysis were performed on these crAss-like phages, revealing 12 potential crAss-like phage candidate genera. Additionally, laboratory analysis was performed on the faecal sample from an individual harbouring 7 distinct crAss-like phages. In vitro propagation of crAss-like phages facilitated visualisation of Podoviridae virions by electron microscopy. Furthermore, proteomic detection of a crAss-like phage capsid protein could be linked to the metagenomic sequencing assembly, empirically confirming crAss-like phage structural annotations.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P279

Axenic culture dynamics of a bacterial endosymbiont, *Sodalis glossinidius*

Terence Markham, Poppy Stevens, Chloe James, Ian Goodhead

University of Salford, Salford, United Kingdom

Abstract

Sodalis glossinidius is the most recently-evolved secondary symbiont of tsetse flies, which transmit the neglected tropical disease Human African Trypanosomiasis. *S. glossinidius* has been shown to reduce tsetse flies' refractoriness to infection with trypanosomes, but the mechanism for this is unknown. The broad tissue tropism of *S. glossinidius* and its stochastic presence in wild tsetse populations point towards a recent evolution in comparison with the primary tsetse symbiont *Wigglesworthia glossinidia*, which has evolved closely with its host over the last 50-80 million years. *S. glossinidius* retains a large genome (~4 Mbp) compared to other symbiotic bacteria, with pseudogenes making up 50% of its genome. Axenic culture is possible but remains difficult, often restricted to undefined culture media.

We are optimising *S. glossinidius* axenic culture in custom, modifiable media, without the use of serum, and observing a number of adaptive traits, such as pH and antibiotic resistance. This is with the goal of developing a continuous culture experimental evolution system to observe *Sodalis* genome evolution. Evolving *S. glossinidius* under oxidative stress – a primary immune response of the tsetse fly to infection by trypanosomes and bacteria – will reveal novel insight into the mechanisms by which *S. glossinidius*, tsetse and trypanosomes interact. We will present Nanopore MinION whole-genome sequencing data of *S. glossinidius* strains cultured continuously under oxidative stress. These data will improve current methods for culturing *S. glossinidius* axenically, and inform future insect-vector disease control strategies using insect symbionts.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P280

Characterisation of sand fly (*Phlebotomus argentipes*) microbiota linked to *Leishmania* infection status across Bihar, India

Poppy Stevens¹, Gala Garrod², Mike Coleman², Geraldine Foster², Ian Goodhead¹

¹University of Salford, Salford, United Kingdom. ²Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Despite the importance of vector microbiota as potential targets for transmission disruption, the microbiota of *Phlebotomus argentipes* sand flies, the vector of Visceral Leishmaniasis (VL), has only been partially characterised. VL is a Neglected Tropical Disease predominantly affecting impoverished communities that causes more global fatalities than any parasitic disease except malaria. A recent paper describing the microbiota of the New World VL vector *Lutzomyia longipalpis* suggested a role for the microbiome of sand flies in restricting parasite development, and vector microbiomes have been shown to significantly impact disease transmission in other kinetoplastid systems. Eastern India is an important focus of VL; 72% of cases occur in Bihar state, where the vector species is *Phlebotomus argentipes* and the VL species is *Leishmania donovani*. We will present data on the core microbiome of *Ph. argentipes* across Bihar, and link spatial microbiota dynamics to *Leishmania* infection status.

Seven hundred female *Ph. argentipes* sand flies were collected from six sites in Bihar and assayed for VL infection status. Preliminary Illumina MiSeq 16S microbiome data for a subset of individual 240 *Leishmania*-negative flies and five *Leishmania*-positive flies shows high bacterial diversity within and between regions: common genera include Oxalobacteraceae, Moraxellaceae and Enterobacteriaceae, and uneven distribution of genera including *Spiroplasma sp.*, *Wolbachia sp.* and *Pantoea sp.* was observed. Several individuals had unusually low-diversity microbiota, potentially indicating a dysbiotic state. These data will be used to support the Indian Visceral Leishmaniasis Elimination Programme, and shed light on the mechanisms contributing to VL transmission in India.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P281

Exploring the association between the denture-associated microbiota and bacterial pneumonia

Joshua Twigg¹, Melanie Wilson¹, Matt Wise², David Williams¹

¹Cardiff University, Cardiff, United Kingdom. ²University Hospital Wales, Cardiff, United Kingdom

Abstract

Background: Pneumonia predominantly affects the frail elderly, presents diagnostic challenges, and bears a high mortality rate. Frequently, sputum or lung fluid samples yield inconclusive microbiologic results, hampering effective treatment. The oral cavity may act as a reservoir for respiratory pathogens to seed pneumonia in susceptible individuals. Denture biomaterial surfaces offer a potential niche for respiratory pathogen colonisation.

This study compared denture-associated microbiota of two cohorts: care home residents and hospital patients with bacterial pneumonia. Salivary cytokines were assessed as biomarkers for respiratory pathogen carriage.

Methods: This is an ongoing study involving analysis of microbial samples from the dorsal tongue, denture-bearing palate and denture fit-surface. Imprint cultures were transferred to selective agars targeting important respiratory pathogens. Bacterial DNA extracted from swabs will be analysed using metataxonomic sequencing of bacterial 16S rRNA genes providing microbial community profiles. Antimicrobial sensitivity profiling of cultured strains is ongoing. Salivary cytokines were analysed using a cytometric bead array and compared with cytokine profiles from oral mucosal tissue models infected with acrylic biofilms containing respiratory pathogens.

Results: To date, *C. albicans* and *S. aureus* were frequently recovered from denture surfaces. *P. aeruginosa* was isolated in 8 out of 56 individuals. Salivary cytokine analysis is ongoing.

Conclusion: Preliminary results corroborate the body of research emphasising that dentures can serve as reservoirs for pathogenic microorganisms, which could seed respiratory infections. Metataxonomic analysis of denture-associated microbiota by next generation sequencing is ongoing. The findings could aid early diagnosis and identify likely aetiologic bacteria involved in respiratory infection; guiding antimicrobial therapy.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P282

Phenotypic implications of strain diversity of the gut microbiome

Ana Zhu¹, Trevor Lawley¹, Peer Bork²

¹Sanger Institute, Cambridge, United Kingdom. ²EMBL, Heidelberg, Germany

Abstract

The gut microbiome is a complex and diverse ecosystem, an individual has on average 160 prokaryote species. I have found that an individual's strain diversity within a species is even greater, with at least 13% of their genes being different between two individuals. This large functional diversity, implies that taxonomical classification alone, such as based on 16SrRNA gene profiling is not sufficient to predict the functional potential of the gut microbiome. For example, the capacity of an individual microbiome to digest plant fibers will be determined by their strain repertoire. I have found that common properties that are considered species-specific or widespread across different phylogenetic groups, are actually strain-specific in their genetic repertoire (definition source: probiotic ISAPP expert panel). How greatly does this affect the microbiome role in human health and bacteria-bacteria interaction, would be important to investigate in the future. To elucidate this, I combined deep culturing methods to purify single isolates for whole genome sequencing and phenotypic analysis. I will present my gene content pipeline and results on strain phenotyping. Recently, the first demonstration of a commensal causal link that fulfills the Koch's postulate was found for the gut microbiome. This work will be important to shape the extent of strain phenotyping will need to be carried out in future microbiome studies.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P283

Using multi-omic approaches to compare temporal bacterial colonisation of *Lolium perenne*, *Lotus corniculatus* and *Trifolium pratense* in the rumen

Christopher Elliott^{1,2}, Joan Edwards³, Toby Wilkinson⁴, Gordon Allison², Pauline Rees Stevens², Allison Kingston-Smith¹, Sharon Huws¹

¹Queens university Belfast, Belfast, United Kingdom. ²Aberystwyth University, Aberystwyth, United Kingdom. ³Wageningen, Wageningen, Netherlands. ⁴The Roslin Institute, Edinburgh, United Kingdom

Abstract

Due to population growth, securing an adequate supply of red meat and milk is predicted to become a major challenge, requiring improvements in sustainable ruminant production. Understanding rumen plant-microbe interactions is key to developing novel methodologies allowing improvements in ruminant nutrient use efficiency. This study investigated rumen bacterial colonisation of fresh plant material and changes in plant chemistry over a period of 24h period using three different fresh forages: perennial rye grass (PRG), birds foot trefoil (BFT) and red clover (RC). We show using 16S rDNA ion torrent sequencing that post incubation, primary and secondary colonisation events occur as defined by changes in abundances of attached bacteria and changes in plant chemistry, as assessed using FT-IR. For PRG colonisation, primary colonisation was seen up to 4h and secondary colonisation from 4h onwards. The change occurred later with BFT and RC, with primary colonisation being up to 6 h and secondary colonisation post 6 h incubation. Across all 3 forages the main colonising bacteria present post-incubation were *Prevotella*, *Pseudobutyrvibrio*, *Ruminococcus*, *Olsenella*, *Butyrivibrio* and *Anaeroplasm*. Using PICRUSt analysis we predicted that lipid and amino acid metabolism were decreased in the bacteria attached during secondary colonisation, irrespective of forage type. The PICRUSt data coupled with the FT-IR data suggest that attached bacterial function is similar irrespective of forage type, with the main changes occurring between primary and secondary colonisation. These data suggest that the sward composition of pasture may have major implications for the temporal availability of nutrients for animal.

Community Interactions and the Living Host

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P491

Molecular characterization of MRSA isolates from staff members and inpatients of an Italian long-term care facility

Letizia Ceglie¹, Erika Rampazzo¹, Roberto Perin¹, Albert March², Richard Aschbacher², Giovanni Lorenzi², Elisabetta Pagani², Karin Trevisiol¹, Michela Corrà¹

¹Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy. ²Comprensorio Sanitario, Bolzano, Italy

Abstract

In 2016, a preliminary study in a long-term care facility (LTCF) of Northern Italy was conducted to investigate multi-drug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). In 2017, dogs involved in pet therapy activities in the same LTCF were also examined for MRSA. Rectal, inguinal, oropharyngeal and nasal swabs from inpatients and staff members were plated on selective media. Oral, auricular and skin swabs from dogs, at the end of a 6-month therapy session were tested. Dogs visited the facility twice a week, although they had limited contacts with the residents (i.e., feeding and stroking).

The screening showed positivity for MRSA in 14.8% (17/115) of the inpatients and 7.4% (5/67) of staff members, whereas no *Staphylococcus aureus* nor MRSA were isolated from animals. Human isolates were characterized with a microarray technology system (Alere).

The 21 isolates of human origin belonged to the clonal groups: CC22-MRSA-IV (eight isolates), CC5-MRSA-II (five isolates), CC5-MRSA-IV (four isolates), CC5-MRSA (one isolate) and CC1-MRSA-IV (three isolates). All isolates were positive for the *mecA* gene, whereas other antibiotic resistance genes (*bla_Z*, *ermA*, *ermC*, *aadD*, *aphA3*, *tetK*) were variably present. Genes for virulence factors were detected in various combinations: *lukF-PV/lukS-PV*, *sat*, *tst*, *sea*, *hla/hlab*, *sak/chp/scn*, *aur*, *icaA/B/C*.

In conclusion, MRSA isolates from LTCF residents and staff members belonged to various clonal groups, which suggests a complex epidemiology of MRSA in the screened group. In this preliminary study, an effective MRSA dissemination risk in dogs was not observed.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P285

Cooperative action of FUR and FNR on regulating iron homeostasis in *Klebsiella pneumoniae* under anaerobic condition

Ching-Ting Lin¹, Tien-Huang Lin², Cheng-Yin Tseng³, Chien-Chen Wu⁴

¹China Medical University, Taichung, Taiwan. ²Taichung Tzu Chi Hospital, Taichung, Taiwan. ³Hsinchu Mackay Memorial Hospital, Hsinchu, Taiwan. ⁴National Yang Ming University, Taipei, Taiwan

Abstract

Background:

To maintain iron homeostasis is critical for most bacterial growth and infection in response to dynamic environmental cues, especially iron availability and oxygen concentration. Ferric uptake regulator (Fur) is responsible for controlling the iron-acquisition system expression in response to iron availability. Fumarate nitrate reduction regulator (FNR) contains an [4Fe-4S] cluster in response to oxygen concentration.

Methods:

The intracellular iron levels was monitored by the iron-activated antibiotic streptonigrin, which requires iron for its bactericidal action that causes DNA degradation. The siderophore secretion was evaluated by chrome azurol S plate to observe the halo formation. The mRNA expression was measured by qRT-PCR.

Results:

The siderophore secretion in *Klebsiella pneumoniae* (Kp) was repressed under anaerobic condition. Deletion of *fnr* in Δfur abolished the halo formation of the Δfur strain in anaerobic condition, indicating that FNR has a positive role in control of siderophore secretion in Δfur strain under anaerobic condition. Based on the typical FNR box is present in P_{fepA} and P_{feoB} , the mRNA expression of *fepA* and *feoB* was determined to identify the regulatory role of FNR in siderophore production under anaerobic condition. The result was indicated that FNR could activate the *feoB* expression, but repress the *fepA* expression. Therefore, it suggested that FNR is involved in Fur regulation of ferrous iron and the enterobactin uptake to affect the iron homeostasis in Kp.

Conclusion:

Coordination of Fur and FNR in regulating the iron-acquisition system expression to maintain the iron homeostasis was demonstrated in Kp under anaerobic condition.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P286

The battle for iron: human vs bacteria

Shamim AL-Husseini, Dr.Primrose Freestone

University of Leicester, Leicester, United Kingdom

Abstract

Iron is a crucial microelement for almost all cell based lifeforms. In humans one aspect of innate immune defence involves limitation of free iron in bodily fluids and blood by the iron sequestering proteins transferrin in blood and lactoferrin in body fluids (Tf and Lf), a phenomenon called as nutritional immunity. Human bacterial pathogens have evolved many mechanisms to circumvent iron limitation, one of them is directly secure mammalian iron-binding proteins Tf and Lf on their surfaces using outer membrane-linked receptor proteins.

This study aims to investigate a novel iron acquisition pathway in *Pseudomonas aeruginosa* which involves moonlighting of outer membrane porins as human Tf and Lf capture proteins.

Methods: Proteomic techniques including western blotting, SDS PAGE and mass spectrometry were employed to explore which *P. aeruginosa* porins had the capacity to act as binding proteins for Tf/Lf. Tf and Lf affinity chromatography columns were created to confirmed Tf and Lf binding.

Results: It was found that the outer membrane porin F (OprF) of *P. aeruginosa* is a receptor for Tf and Lf, which was also confirmed for lab strains PA14 and PAO1. OprF binding of Tf was also shown for twelve *P. aeruginosa* strains isolated from cystic fibrosis patients.

Conclusion: OprF plays a new and important role in the iron biology of *P. aeruginosa*, as it is both a Tf and Lf binding protein. Therefore, to determine how important this is to the infectivity of *P. aeruginosa*, deletion of the gene for OprF is underway.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P287

A potential novel iron uptake system reserved to *Bifidobacterium longum* subsp. *longum*

Cho Zin Soe, Shabhonam Caim, Udo Wegmann, Lindsay Hall

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Iron is an essential micronutrient for almost all organisms as it is utilised in fundamental cellular processes. Iron homeostasis is tightly regulated, restricting bioavailability to microorganisms. To overcome iron starvation, bacteria have developed sophisticated and species-specific strategies to sequester extracellular iron. Whilst iron uptake systems in pathogens has been well studied, our understanding of these processes within members of the gut microbiota is limited. *Bifidobacterium* are Gram-positive resident gut bacteria, which play an important role in gut homeostasis, immune development and colonisation resistance against pathogens. In the current study, we identified iron uptake systems in *B. longum* subsp. *longum* using a comparative transcriptomic approach. Anaerobic cultures were grown under iron-deplete or iron-replete conditions, achieved via augmentation with the iron chelator 2,2'-bipyridyl +/- FeCl₃. RNA-Seq was performed on cells harvested at different growth phases and the differential gene regulation profiles compared. We identified one known (ferrous uptake), and one novel (energy-coupling factor transporter, ECF) iron uptake gene clusters upregulated under iron-deficient conditions. The ECF gene cluster contains four subunits, a cytoplasmic ATPases EcfAA', a transmembrane protein EcfT, a transmembrane substrate binding protein EcfS and a hypothetical protein. Performing comparative analysis across *Bifidobacterium* species, we determined that this system is highly conserved in *B. longum*, but not other *Bifidobacterium* spp. We also determined that these systems are important for colonisation resistance against enteric pathogens. Understanding these iron uptake pathways will allow us to tailor *Bifidobacterium* supplementation strategies in iron limited, and high pathogen burden conditions, which includes infants in developing countries.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P288

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

Dhama Alsallami

reading university, reading, United Kingdom

Abstract

C. jejuni gut colonisation depends upon its iron-acquisition ability. P19 is part of a *C. jejuni* iron-uptake system encoded by a conserved cluster of eight genes (*cj1658-cj1665*). P19 is a periplasmic homodimer that, with Ftr1 (a ferric permease), is part of a high-affinity iron transporter. The function of the last six genes is unknown; these encode a second potential transporter and two thioredoxins. The aim of this study is to explore the functions of these uncharacterised genes.

Gene context analysis showed *p19*-like genes to be organised into three main groups: I, consisting of 4 co-polar genes, apparently forming a single transcript (designated *ftrABCD*); II, consisting of ~8 co-polar genes (designated *fetMP-fetABDEF*) as in *Campylobacter*; and III, consisting of three genes – encoding cupredoxin, Ftr1 and P19. To determine if *fetABCDEF* of *C. jejuni* can enhance growth under iron restriction, a mutant *E. coli* deficient in iron transport was complemented with *fetMP* or *fetABCDEF* (or both) carried by inducible vectors. Both *fetMP* and *fetABCDEF* enhanced growth but only in minimal medium at acidic pH and under iron restriction; this effect was seen both aerobically and anaerobically. Thus, both FtrA-P19 and FetABCDEF appear to contribute to iron uptake in a surrogate host, but this effect is dependent on pH, although is not apparently influenced by oxygen. Inactivation of *ftr1*, *fetA* and *fetD* gave a significant growth reduction for *C. jejuni* in DMEM medium. The impact of iron supplementation, pH and complementation is currently being explored.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P289

Control of zinc homeostasis in *Agrobacterium tumefaciens*

Rojana Sukchawalit, Skorn Mongkolsuk

Chulabhorn Research Institute, Bangkok, Thailand

Abstract

The plant pathogenic α -proteobacterium *Agrobacterium tumefaciens* causes crown gall tumour disease. Both host and pathogen battle over access to essential metals including zinc. However, excessive amounts of intracellular zinc are toxic. Proper control of intracellular zinc levels is essentially required, that could determine the outcome of host-pathogen interactions. To study the mechanisms in the regulation of zinc homeostasis in *A. tumefaciens*, mutant strains lacking either transcriptional regulators (*zur* and *zntR*), zinc transporters (*znuA*, *znuBC*, *troCBA* and *zntA*), or chaperones (*zinT* and *yciC*) were constructed. Quantitative real-time PCR analysis was performed to determine gene expression in response to high zinc and low zinc conditions. Sensitivity to metals and EDTA (metal-limiting condition) was tested. Total cellular zinc content was measured by using an inductively coupled plasma mass spectrometer. Zur is the Fur-family transcriptional repressor of zinc acquisition genes, including *znuA*, *znuBC*, *zinT*, *troCBA* and *yciC*. Unlike many other bacteria, the ABC-type transporter ZnuABC was not the major zinc uptake system in *A. tumefaciens*. ZinT, a periplasmic protein, functioned with the high-affinity zinc importer, TroCBA. However, ZnuABC was important when TroCBA was impaired. ZinT and YciC may function as zinc chaperones that transfer Zn ions to zinc-requiring proteins in the periplasm and cytoplasm, respectively. Co-operation of zinc uptake systems and zinc chaperones is essential for *A. tumefaciens* to survive under a wide range of zinc-limiting conditions. In order to prevent excess zinc-mediated toxicity, expression of *zntA* gene encoding a P_{1B}-type ATPase exporter is turned on via activation of the MerR-family transcriptional regulator ZntR.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P290

Novel approach of antifungal high-throughput screening targeting heavy metal homeostasis.

Claudia Simm

University of Birmingham, Birmingham, United Kingdom

Abstract

Iron and zinc are essential micronutrients that play a crucial role in many cellular functions. Many invasive pathogens require these metals for proliferation and virulence within the host. Consequently, host cells are able to withhold iron and zinc from pathogens, via a process called nutritional immunity, whilst many microorganisms have in turn evolved mechanisms to scavenge micronutrients extremely effectively. *Candida albicans* uses an array of high- and low-affinity iron and zinc transporters. The expression of these transporters is regulated by metal-responsive transcription factors such as Csr1 (zinc) and Sfu1/Sef1 (iron), and their expression reflects micronutrient availability within the cell. During metal replete conditions, these transcription factors interact with metal-responsive-elements in the promoter regions of metal-regulated genes and inhibit transcription. Conversely, if metal concentrations drop gene transcription will be initiated.

Here I present an antifungal drug screen that exploits this metal regulon. The promoter regions of zinc and iron responsive genes were amplified and fused to the fluorescent proteins eGFP and dTomato, respectively. These zinc and iron sensors were integrated into the genome of *Candida albicans* and used to screen against the Prestwick library. We can identify drug hits by their induction of fluorescent protein expression as an indicator for reduced labile metal ion concentration in *Candida*. This strategy will be metal-specific and result in drug targets directly involved in metal uptake, sequestration or transcriptional regulation but no other stimuli. Drugs identified in this screen will offer an alternate therapeutic strategy for life-threatening systemic fungal infections.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P291

Role of Csp3 in the copper homeostasis of Salmonella Typhi

Emma Tarrant, Kevin Waldron

Newcastle University, Newcastle-upon-Tyne, United Kingdom

Abstract

Recently, a novel family of cytoplasmic copper storage proteins (Csp3), capable of binding numerous copper ions, was identified, but their biological role is unknown. Is it used for protection from copper toxicity, or to supply the metal to copper requiring enzymes? Bioinformatics identified these proteins encoded in numerous bacterial genomes, including pathogens such as *Salmonella*.

Salmonella Typhi is an obligate human pathogen causing the systemic infection typhoid fever and persists in host macrophages, which are believed to use copper as part of the attack on the bacteria. Therefore we are investigating copper homeostasis in *Salmonella Typhi*, which has not been well studied, and the role of Csp3 in this system.

Firstly, knock out mutants were constructed of *csp3* and all other known genes involved in copper homeostasis, and tested for any copper-dependent phenotypes, such as changes in growth and metal content. Reporter constructs for *csp3* and select other genes were utilised to investigate their expression and regulation. In addition, the role of copper supply by Csp3 were investigated using enzyme assays to determine activity of copper requiring enzymes.

Here, we will provide experimental evidence for the roles of multiple proteins of the copper homeostasis system in *Salmonella Typhi* and shed light on whether Csp3 is involved in resistance or supply of copper. With the renewed interest in copper as an antimicrobial and its use by our own immune system, it is crucial that we understand how pathogens utilise and also protect themselves from copper.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P292

Copper Storage Proteins in the Fight against Gonorrhoea

Louisa Stewart¹, Karrera Djoko², Kevin Waldron¹

¹Newcastle University, Newcastle upon Tyne, United Kingdom. ²Durham University, Durham, United Kingdom

Abstract

Background

Neisseria gonorrhoeae, a pathogenic Gram negative bacterium, causes human gonorrhoeal disease. Its genome encodes a putative periplasmic homologue of a copper storage protein (Csp1) recently characterised in methanotrophic bacteria. This project will explore whether Csp1 in *N. gonorrhoeae* has a role as: (1) a copper store, supplying copper to enzymes during low copper conditions, or (2) a metallochaperone, shuttling copper between enzymes for the switch between aerobic and anaerobic growth, or (3) a copper chelator, protecting against toxic copper excess.

Methods

Genomic mutagenesis will be used to create knockout mutants of *N. gonorrhoeae*. Phenotypic investigations, comparing wild type and mutant strains, will utilise growth analysis, whole cell metal analysis, and enzyme assays (e.g. nitrite reductase and respiratory oxidase), to explore functional interactions between Csp1 and other known copper homeostatic or copper utilising proteins in *N. gonorrhoeae*.

Results

Aerobic growth analyses of wild type and a $\Delta csp1$ knockout mutant strain of *N. gonorrhoeae* have shown an absence of a copper-dependent growth phenotype. This opposes a role for Csp1 in copper detoxification, and supports the hypothesis that Csp1 is involved with storage and/or supply of copper for copper requiring proteins and/or an involvement in the switch from aerobic to anaerobic growth.

Conclusion

In 2014 the World Health Organisation identified *Neisseria gonorrhoeae* as high priority with respect to antimicrobial resistance. An understanding of the role of Csp1 could contribute to the development of alternative antibacterial strategies that are urgently required to fight gonorrhoeal disease.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P293

Functional analysis of the iron uptake activity of the FeoABC system of *E. coli*

SALEM AL-AIDY^{1,2}, Simon Andrews¹

¹University of Reading/ School of Biological Sciences/ Biomedical sciences, Reading, United Kingdom.

²University of Wasit/College of Medicine, Wasit, Iraq

Abstract

Although iron is relatively abundant, it forms insoluble ferric complexes in the presence of oxygen leading to bioavailability problems. The more soluble ferrous iron is limited to anaerobic, reducing or acidic environments. *E. coli* K-12 encodes six ferric iron transport systems and two ferrous iron transporters, the widely distributed FeoABC and the cryptic EfeUOB. Knock-out of *feo* has been shown to reduce the virulence and/or gut colonisation capacity of several bacteria. FeoB is considered to be the ferrous permease but the functions of FeoA and FeoC have yet to be completely determined. Further understanding of Feo function may help explain why Feo operates under low-oxygen conditions.

To further characterise the Feo system, His- and FLAG-tagged variants of FeoA and FeoB were generated; the addition of these tags did not affect Feo activity but enabled their detection by Western blotting. Inducible plasmids containing various combinations of versions of WT and tagged *feoA*, *feoB* and *feoC* genes were created and transformed into *E. coli* strains that were otherwise defective for iron transport. Iron-limited bacterial growth under aerobic and anaerobic conditions was monitored as an indicator of iron-uptake activity. Results showed that both FeoA and FeoB are required for Feo activity. FeoC enhanced activity of the Feo system, but only under aerobic conditions with little impact under anaerobic conditions. These results thus suggest a role for FeoC in enhancing FeoAB iron-uptake activity under oxidising conditions and also support an absolute requirement for FeoA and FeoB for Feo-mediated iron-uptake.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P294

Manganese homeostasis in *Staphylococcus aureus* and its role in Sod activity

Ying Zhou, Kevin Waldron

Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Bacterial resistance to antibiotics has become a serious threat to human health. *Staphylococcus aureus* is one of the leading causes of bacterial infection in humans and livestock, yet our understanding of how *S. aureus* survives the host's immune response is limited.

Metals such as manganese (Mn) are essential for infectious bacteria as micronutrients. During infection, the host takes advantage of the bacterial dependency on acquiring essential metals and defends itself from invaders by reducing the availability of these essential nutrients, known as nutritional immunity. The host's defense mechanism inactivates metal-dependent enzymes such as superoxide dismutase (SOD) that are dependent on Mn, which causes bacteria to become more sensitive to the host's oxidative burst.

SodM, a superoxide dismutase that is unique to *S. aureus*, has the ability to function with either iron (Fe) or Mn. This 'cambialistic' nature of SodM allows *S. aureus* to maintain SOD activity during nutritional immunity manganese starvation induced by host. Data will be presented from our characterization of manganese homeostasis in *S. aureus*. By studying mutant strains lacking components of the Mn homeostasis system, the mechanisms by which the SODs acquire their cofactor can be learned.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P295

Developing Oxygen-sensitive Protein Expression Systems based on adaptive tools of Anaerobic Protozoa.

Ronan Egan

University of Kent, Canterbury, United Kingdom

Abstract

Inorganic Iron-sulfur clusters (ISCs) represent some of the oldest and most versatile cofactors in life. Commonly co-ordinated by cysteine and histidine-rich (C/HX_nC/H) motifs, ISCs conform to two geometric structures: rhomboid, e.g. [Fe₂S₂]²⁺ and cubane [Fe₄S₄]²⁺. These geometries together with individual protein-environments convey relatively low redox potentials (~ -700mV to +200mV vs. SHE) favouring spontaneous electron-transfer to bound substrates. Strong oxidants, e.g. molecular oxygen (O₂), however, readily react with low-potential ISCs resulting in cluster oxidation and degradation, as well as production and propagation of radical oxygen species via Fenton and Haber-Weiss reactions. This is an issue for aerobic organisms, which are dependent on many ISC-enzymes, and also limits the exploitation of ISC-enzymes for biotechnological applications such as nitrogen-fixation and cofactor-biosynthesis. Heterologous expression of a specialised protozoan ISC chaperone (BhSufCB) in *Saccharomyces cerevisiae* showed significantly increased activity, and abundance, of endogenous cytosolic ISC-enzymes (Ecm17, Leu1), as well as the heterologous ISC enzyme Hslrp1. SufCB can also restore functional Ecm17 in yeast harbouring defects in iron-utilisation and ISC-biosynthesis as well as complement growth and respiratory defects in both cytosolic and mitochondrial ISC-pathways. Ongoing work includes chemical characterisation of the SufCB protein in yeast, as well characterisation of functional partners of and exploitation of SufCB *in vivo*.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P296

Mobile genetic element-encoded hypertolerance to copper protects *Staphylococcus aureus* from killing by host phagocytes.

Marta Zapotoczna¹, Gus Peliccioli-Riboldi², Matthew T.G. Holden³, Kevin J. Waldron⁴, Joan A. Geoghegan⁵

¹Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin,, Dublin, Ireland. ²Institute for Cell & Molecular Biosciences, Faculty of Medical Sciences, Newcastle, United Kingdom. ³School of Medicine, University of St Andrews,, St. Andrews, United Kingdom. ⁴Institute for Cell & Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Framlington Place,, Newcastle, United Kingdom. ⁵Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Dublin, Ireland

Abstract

Copper toxicity is increasingly recognized as an important host defence against infecting bacteria. Some pathogenic bacteria are reliant on copper tolerance for full virulence *in vivo*, in particular to resist copper-mediated killing within the macrophage phagolysosome. The *copB-mco* operon is carried by clinically relevant strains of *S. aureus* either on a replicating plasmid or integrated into the chromosome. The *copB* gene encodes a copper efflux pump and *mco* a multicopper oxidase. In this study deletion mutants of either *copB* or *mco* were used to investigate the role of copper hypertolerance in providing resistance to phagocytic killing. Both genes contributed to copper hypertolerance and knocking out either gene reduced the growth of *S. aureus* in subinhibitory concentrations of copper. Expression of *copB* and *mco* promoted survival inside macrophages that were primed with IFN- γ and CuSO₄. The *copB-mco*-mediated advantage in resisting phagocytic killing was also shown using an *ex vivo* model of human blood infection. To study the role of the copper sensitive operon repressor CsoR in copper hypertolerance, site-directed mutagenesis was carried out on the *csoR* gene on the chromosome of *S. aureus* to introduce amino-acid substitutions C41A/H66A/C70A, generating a copper-insensitive CsoR variant. CsoR bound to *copB* promoter DNA in a copper-dependent manner and was a potent regulator of chromosomally encoded *copB-mco* but less so of the genes expressed from a plasmid. Furthermore, we demonstrate that the *copB-/mco-* are associated with two major successful clonal complexes that are responsible for invasive disease across Europe.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P297

Copper Storage Proteins in the Fight against Gonorrhoea

Safa Alsharif, Kevin Waldron

Newcastle University, Newcastle-upon-Tyne, United Kingdom

Abstract

Background

Neisseria gonorrhoeae is a pathogenic Gram negative bacterium that causes human gonorrhoeal disease. Its genome encodes a putative periplasmic homologue of a new family of copper storage proteins (Csp1) recently described in methanotrophic bacteria. Our lab is studying the form and function of these proteins in pathogens, as copper is known to play an important role in the innate immune system's ability to fight infection.

This project will explore the copper binding properties of Csp1 from *N. gonorrhoeae*, in order to understand how it may be able to aid virulence, either through sequestration of excess copper, thereby reducing copper toxicity, or by storing copper during times of abundance and subsequent release of copper during copper deficiency.

Methods

Recombinant *N. gonorrhoeae* Csp1 has been produced in *E. coli* and purified by liquid chromatography. Copper binding stoichiometry and affinity has been assessed using spectroscopic methods, including competition of Csp1 against high affinity copper chelators. The protein structure will be studied with X-ray crystallography.

Results

Data will be presented describing the copper-binding properties of the Csp1 protein.

Conclusion

In 2014 the World Health Organisation identified *Neisseria gonorrhoeae* as a priority organism with respect to antimicrobial resistance. An understanding of the role of Csp1 could contribute to the development of alternative antibacterial strategies that are urgently required to fight gonorrhoeal disease.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P298

Molecular and phylogenetic analysis of the *Candida albicans* zincophore Pra1

Mohammad Alsarraf^{1,2}, Duncan Wilson¹

¹Aberdeen Fungal Group, Medical Research Council Centre for Medical Mycology, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom. ²Public Authority of Applied Education and Training, Kuwait, Kuwait

Abstract

Candida albicans Pra1 is secreted, binds zinc, and reassociates with the fungal cell via a syntenically encoded plasma membrane transporter, Zrt1. This zincophore system contributes to *C. albicans* pathogenicity by scavenging this essential micronutrient. However, little is known about the origin, evolution and distribution of the zincophore-encoding gene in fungi.

To explore zincophore evolution, orthologues of Pra1 from *C. albicans* were identified in 87 (of 102) fungal species. These were analysed using multiple bioinformatics tools including (1) the Constraint-based Multiple Alignment Tool (COBALT) to identify the sequence similarity, (2) identification of conserved domains (CDD), (3) PSI-BLAST to search for sequence with identified conserved domain (4) Hidden Markov Model (HMM) profiling to understand the conservation of the amino acids amongst the orthologues' sequences. Moreover, to study the evolution of the Pra1 protein over different phylogenetic groups, phylogenetic tree were generated by the Maximum Likelihood methods.

BLASTp analysis indicates that Pra1 orthologues are limited to the fungal kingdom and present in ascomycetes and basidiomycetes species, but absent from members of the zygomycetes and glomeromycetes. Most (70%) *PRA1* orthologues have maintained synteny with the zincophore receptor gene, *ZRT1*. Moreover, high conservation of orthologous proteins was detected in the central HRXXH domain and the C-terminus of Pra1. Importantly, these highly conserved domains have been directly shown to bind zinc with affinity in *C. albicans* Pra1.

Therefore, it would appear that zincophore activity may be conserved throughout the fungal kingdom

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P299

Functional Characterization of a Novel *Campylobacter jejuni* Putative CDF family Zinc Exporter

Natasha Jafri, Adrian Jervis, Jen Cavet, Dennis Linton

University of Manchester, Manchester, United Kingdom

Abstract

Campylobacter jejuni is the most common bacterial cause of acute gastroenteritis in the UK and hence an important food-borne pathogen. Metal homeostasis in *C. jejuni* is poorly understood with the major focus to date on acquisition and storage of iron. We have identified and functionally characterized a *C. jejuni* gene (Cj1163c of strain NCTC 11168) involved in zinc homeostasis. This gene encodes a protein belonging to the Cobalt Zinc Cadmium resistance family predicted to act as a metal exporter across the cell inner membrane and which we termed CzcD. To test this hypothesis we constructed an insertional knockout of the Cj1163c gene by inserting an antibiotic resistance cassette. The sensitivity to a range of metals (zinc, cobalt, copper, nickel and iron) was determined for both wild type and corresponding Cj1163c insertional knock out strain. The mutant was significantly more sensitive to zinc and cobalt but not copper, nickel or iron. These phenotypes were confirmed by constructing a genetically complemented strain that restored the zinc and cobalt sensitivity to wild type levels. We further utilized this ability to genetically complement the zinc sensitive phenotype to identify specific CzcD residues required for activity. By comparing CzcD with the structurally and functionally characterised *Escherichia coli* homologue YiiP we targeted both conserved residues known to be required for activity in YiiP and more variable regions of *C. jejuni* CzcD. Our data highlight similarities and differences in the functioning of these homologues.

Microbial Metal Homeostasis: Impacts on Pathogenicity

Zone C

Presentations: Tuesday and Wednesday Evening

P300

Unraveling the zinc acquisition/homeostatic machinery of the human parasite *Leishmania*

Teresa Leão^{1,2,3}, Sandra Carvalho^{1,2}, Elyne Backx^{1,2}, Tânia Cruz^{1,2}, Helena Castro^{1,2}, Margarida Duarte^{1,2}, Ana M. Tomás^{1,2,4}

¹I3S, Porto, Portugal. ²IBMC, Porto, Portugal. ³FCUP, Porto, Portugal. ⁴ICBAS, Porto, Portugal

Abstract

Leishmania, the agents of a (often fatal) group of neglected diseases, the leishmaniasis, are intracellular parasites of macrophages. We are investigating how *Leishmania* are able to compete with their host cells for zinc without risking the toxic effects of this metal, that is, without compromising parasite zinc homeostasis.

We show that several transporters constitute the zinc acquisition machinery of *Leishmania*. The first to be identified was the zinc-regulated molecule ZIP3, a member of the ZIP family, that acts as a high-affinity zinc importer (Carvalho et al, *MolMicrobiol*, 2015,96:581-95). Using CRISPR-Cas9-mediated ablation, we now provide evidence that ZIP3 is essential under zinc-limiting situations but is dispensable in standard conditions, disclosing the existence of a second zinc importer whose identity is being pursued. The third component of the zinc acquisition machinery is CDF1, a transporter expressed in intracellular vesicles known as acidocalcisomes. The observation that only single knockout parasites (CDF^{-/+}) can be produced using CRISPR-Cas9 suggests that this protein is absolutely critical to *Leishmania*. CDF1 appears to be part of a defense mechanism against zinc toxicity. When *Leishmania* face a zinc shock, excess zinc entering the cell is channeled into acidocalcisomes, affording parasite protection at least until ZIP3 is removed from the membrane. Importantly, zinc stored in acidocalcisomes might support parasite growth during zinc shortage. How parasites are able to access stored zinc, as well as the impact of zinc and zinc transporters upon infection is being addressed through *in vivo* and *in vitro* approaches.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P301

Molecular Anatomy of an Influenza Virion

Naina Nair¹, Terry Smith², Swetha Vijayakrishnan³, Elizabeth Sloan³, David Bhella³, Edward Hutchinson³

¹Glasgow School of Art, Glasgow, United Kingdom. ²BSRC School of Biology, University of St Andrews, St Andrews, United Kingdom. ³MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Understanding the molecular biology of viral infections requires a detailed description of the virion. This is particularly challenging when, as for influenza viruses, the virions are irregular in form and incorporate substantial amounts of host-derived material. Currently no single method exists to describe such complex molecular structures. We therefore applied a multidisciplinary approach, combining proteomics, lipidomics, cryo-electron tomography and molecular modelling, to produce a detailed molecular model of the virions of influenza A/WSN/33 virus (WSN).

Using mass spectrometry, we characterised and quantified the proteins and lipids of WSN virions. The virion interior is densely packed with viral and host proteins, while the envelope is a 9:1 mixture of lipid, with a distinct composition from the bulk lipid of the host cell, and the transmembrane domains of viral and host proteins. By combining these quantitative data with electron tomography and experimental and modelled protein structures, we produced a 3D model of the virion that reconciles its overall shape with the numbers of proteins detected.

The model has enough membrane area to bind to the viral matrix and enough internal volume to accommodate the substantial amounts of host proteins detected. However, we found the standard depiction of the genome as a '7+1' array is not geometrically possible in spherical virions. Well-ordered '7+1' arrays of RNPs must become disordered when extended 'bacilliform' virions collapse to become spherical after budding. Integrating qualitative and quantitative data therefore allows us to both clarify and correct our understanding of the complex virions of influenza.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P302

Imaging polyphenolic therapeutic compounds in a eukaryotic model microbe.

Billy Ferrara, Elinor Thompson

University of Greenwich, Chatham, United Kingdom

Abstract

Flavonoids are polyphenolic metabolites that have a range of physiological and developmental functions in plants. They are the focus of much work as potential therapeutics, although investigation of specific mode of action remains a notably under-researched area. Monitoring transport and location of flavonoids in cells is difficult because, despite a role in UV-absorption in plants, they emit only low levels of fluorescence. Visualising them in plants is possible using the Naturstoff reagent (NA), reported historically to be a polyphenol-fluorescence-enhancing stain. We explored therefore whether this agent was effective during preclinical assessment of polyphenolic therapeutics in a microbial-model.

The eukaryote *Dictyostelium discoideum* has been shown to be a useful model when identifying novel drug targets for treating various diseases. For example, in the case of polycystic kidney disease, naringenin decreased *Dictyostelium* cell division whereas a polycystin-2-null *Dictyostelium* line was resistant to the flavonoid, and, subsequently, naringenin treatment proved to reduce cyst-formation in mammalian-kidney model cell lines¹. To monitor transport and site of action of the drugs investigated in such studies, we developed a method using NA-staining in this model organism. A range of polyphenolics were assayed in cells, cell-extracts and the cell-washes, and NA-enhanced imaging was evaluated in parallel with LCMS-quantification. NA-enhanced fluorescence of compounds at therapeutically relevant concentrations proved an effective and qualitative measure of transport and localisation in *Dictyostelium*, and could be used in concert with localisation dyes. Fluorescence-enhancement is limited to a subset of flavonoids, however, and not more widely applicable in our studies to date.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P303

Understanding the chemical warfare of actinomycetes across taxonomic and phylogenetic boundaries for accelerated antibiotic discovery

Laia Castano¹, Katherine Duncan¹, Paulina Rakowska²

¹SIPBS, Strathclyde University, Glasgow, United Kingdom. ²National Centre of Excellence in Mass Spectrometry Imaging, National Physical Laboratory, Teddington, United Kingdom

Abstract

The bacterial order actinomycetes, is the major producer of specialized metabolites with diverse biological activities. Approximately 45% of all antibiotics in clinical use today are produced by actinomycetes. Depending on their genome size, actinomycetes may contain over 30 biosynthetic gene clusters encoding for specialised metabolites. However, only a small fraction are transcribed under normal laboratory conditions. It has been observed that interspecies interactions may play a role in the induction of specialized metabolites. There is a vast number and taxonomic diversity of bacterial strains which might compete with actinomycetes to maintain an ecological advantage. Metabolites are likely produced as a defence mechanism. Therefore, a co-culture technique is an effective method to achieve interactions between bacteria.

To understand the chemical exchange between strains across taxonomic and phylogenetic boundaries, the impact of microbial interactions was assessed on the strains ability to produce specialised metabolites. To investigate this topic, liquid co-culture technique was used for metabolites extraction. Furthermore, the bioactivity of all metabolite extracts were tested against ESKAPE pathogens. Liquid chromatography tandem mass spectrometry (LC-MS) was performed to obtain the metabolite profiles. GNPS molecular networking has enabled the dereplication of biological extracts and comparison across the different experimental conditions. Chemically interesting interactions will be subjected to Imaging Mass Spectrometry (IMS) in collaboration with the National Physical Laboratory. The results demonstrate that microbial interactions in actinomycete strains isolated from marine environments together with the recent advances in mass spectrometry and comparative metabolomics represent an exciting strategy for prioritizing novel chemistry to combat antimicrobial resistance.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P304

Mapping the influence of the gut microbiota on small molecules in the brain through mass spectrometry imaging

Heather Hulme^{1,2}, Lynsey Meikle¹, Nicole Strittmatter³, Sheila Brown⁴, Simon Milling¹, Andrew MacDonald⁴, Richard Goodwin³, Richard Burchmore¹, Donal (Daniel) Wall¹

¹University of Glasgow, Glasgow, United Kingdom. ²Uppsala University, Uppsala, Sweden. ³AstraZeneca, Cambridge, United Kingdom. ⁴University of Manchester, Manchester, United Kingdom

Abstract

The gut microbiota is known to exert an influence over virtually all facets of human health. Recent work has highlighted a role for the gut microbiota in modulating brain health through the gut-brain axis. Microbes can influence the brain through production of neurotransmitters, the induction of host immunomodulators, and through the release, or induction, of other microbial or host molecules. Understanding the molecular basis of the gut-brain axis is an important challenge. We have employed mass spectrometry imaging (MSI) as a label-free tool to map molecular changes in the murine gut and brain caused by microbiota depletion or absence. We determined the spatial distribution and relative quantification of seven common neurotransmitters, precursors, or metabolites across whole brain and gut sections. Furthermore, using non-targeted MSI of small molecules, we detected over one thousand peaks in the resulting spectra, some 500 of which were significantly modulated by the absence or disruption of the microbiota in the gut. Only two identified metabolites, vitamin B5 and 3-hydroxy-3-methylglutarate, were significantly changed in the brains of germ-free animals whilst no significant changes were detected after one week of antibiotic treatment. This work shows that MSI could become an essential tool in understanding the gut-brain axis, allowing detailed analysis of the spatial distribution of bacterial and host metabolites within the gut and specific brain regions.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P305

Identification of Novel Surface Characteristics in *Cryptosporidium* Oocysts Using Various Microscopy Techniques

Tansy Vallintine^{1,2}, Lyne Jossé^{1,2}, Mark Price³, Ian Brown³, Wei-Feng Xue³, Anastasios Tsaousis^{1,2}

¹Laboratory of Molecular and Evolutionary Parasitology, RAPID Group, School of Biosciences, University of Kent, Canterbury, United Kingdom. ²School of Biosciences, University of Kent, Canterbury, United Kingdom. ³School of Physical Sciences, University of Kent, Canterbury, United Kingdom

Abstract

Cryptosporidium is a water borne parasite infecting the gastrointestinal tract. Infection can cause self-limiting diarrhoeal symptoms, although in infants and the immunocompromised, infection can be fatal. The infective stage of the parasite survives in a hardy 'oocyst'. Further research into oocyst composition is necessary to enable the development of effective water treatment methods and medical prophylaxis. The present research investigates the surface characteristics of two *Cryptosporidium* species, *C. parvum* and *C. ubiquitum* oocysts, using atomic force microscopy (AFM), scanning electron microscopy (SEM) and field emission SEM (FESEM).

For AFM analysis of *C. parvum* and *C. ubiquitum* samples were imaged using the Bruker Multimode 8 SPM, and analysed using NanoScope Analysis v1.40r1. For SEM analysis *C. parvum* and *C. ubiquitum* oocyst samples were imaged using the Hitachi S3400 series SEM. Here a backscatter electron sensor was used to measure density. The Hitachi S4700 (cold) field emission SEM, with Bruker "XFlash" quad-element EDX detector were used to image surface morphology and analyse chemical composition.

Preliminary data have revealed surface features not previously described in *Cryptosporidium* oocysts. These structures have a distinct chemical composition in relation to the oocyst wall. The function of these structures is unknown, but localisation to the oocyst surface indicates a possible role in environmental sensing and excystation signalling. We will present data exploring this hypothesis.

This work will enable further investigations into *Cryptosporidium* oocyst composition, host specificity and excystation control, all areas of vital importance to progress in research towards understanding the biology of this important parasite.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P306

Quantifying phenotypic variability in influenza A virus populations with fluorescence microscopy

Michael Vahey^{1,2}, Daniel Fletcher¹

¹UC Berkeley, Department of Bioengineering, Berkeley, USA. ²Washington University in St. Louis, Department of Biomedical Engineering, St. Louis, USA

Abstract

Influenza viruses inhabit a wide range of host environments using a limited repertoire of protein components. Although forming an infectious unit requires the coordinated assembly of these components into a viral particle that is well-suited to the environment at hand, the fidelity with which a virus achieves this coordination and how this subsequently influences its infectivity remains unknown. To quantify this variability at the single virus level and explore how variability influences infectivity, we developed strains of influenza virus that are amenable to fluorescence characterization through quantitative, site-specific labeling of viral proteins in locations that do not impair replication. Using these strains to measure characteristics of individual viruses within a population, we find that influenza A virus leverages variations in size and composition to modulate its adhesion to and escape from host cells, with the distribution of the receptor-destroying enzyme neuraminidase serving as a primary determinant of successful escape. Unlike genetic adaptations that require time and large populations, our experiments show that variation in the balance between receptor binding and receptor destruction is found in the progeny of an individual virus infecting an individual cell and can change rapidly and reversibly over the course of a single replication cycle. The resulting “low fidelity” of influenza A virus progeny allows the virus to sample from a large phenotypic space on short timescales and in small populations, offering a means of surviving in new or changing growth environments.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P307

Electrophotonics: Multimodal sensors for bacterial identification and phenotyping.

Stephen 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 bacterial communities and individual bacteria down to individual molecules. Multimodal sensing combines multiple transduction technologies in parallel to simultaneously probe different properties 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 measurements in a single, integrated device that has been shown to provide 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 and microorganisms. The technology can also be configured for imaging of molecular interactions with micrometre-scale resolution to provide spatial information about surface binding interactions. The use of ITO electrodes improves the electrochemical sensitivity and versatility from previous, silicon based electrophotonic technology and is compatible with voltammetry based techniques for interrogating redox behaviour, along with electrochemical impedance spectroscopy measurements. Using this innovative technology, we have shown quantitative measurements of bacterial metabolism, interrogated via the electrochemical activity of redox active molecules, in parallel with measurements of molecular binding of *Escherichia coli* to surface immobilised glycan monolayers. We believe that the multi-modal measurements with this novel technology can provide new approaches to investigate and understand microorganisms.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P308

Spatial organization of binding and release activity on the surface of influenza A virus drives directional mobility

Michael Vahey^{1,2}, Daniel Fletcher¹

¹UC Berkeley, Department of Bioengineering, Berkeley, USA. ²Washington University in St. Louis, Department of Biomedical Engineering, St. Louis, USA

Abstract

Between cycles of replication, influenza A viruses must balance the need to bind to receptors on new cells with the need to avoid non-productive attachment to decoy receptors in the host environment. To mediate these conflicting requirements, influenza A virus encodes separate proteins to bind host receptors (hemagglutinin, or HA, which binds to sialic acid) and destroy these same receptors (neuraminidase, or NA). Although recent work has focused on understanding the genetic basis for compatibility between particular HAs and NAs, we know relatively little about how the abundance and spatial organization of these proteins on the viral surface might contribute to their functional balance. Using a site-specific strategy to quantitatively and non-disruptively attach fluorescent labels to HA and NA on the surface of live filamentous viruses, we find that distributions of these proteins are spatially segregated and stable over time, with minimal diffusion of either molecule within the viral membrane. Additionally, we find that NA tends to be enriched at the genome-containing pole of the virus, establishing an asymmetric distribution of receptor binding and receptor destroying activities. When viruses bind to surfaces presenting sialic acid, this gradient in NA leads to persistent motion of the virus away from the NA-rich pole, leaving trails of cleaved sialic acid in the virus's wake that we can visualize through the binding of labeled lectins. The ability of influenza viruses to exhibit directional mobility could enable them to efficiently spread within and between hosts while maintaining the ability to bind and infect new cells.

Cool Tools for Microbial Imaging

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P309

Antimicrobial penetration and accumulation within biofilms of *Pseudomonas aeruginosa*

James Brown, Morgan Alexander, Paul Williams, Kim Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

The structure of *Pseudomonas aeruginosa* biofilms is not uniform and this influences the effectiveness of antimicrobial agents by altering their penetration and accumulation. This heterogeneity combined with mechanisms such as porin overexpression and development of persister cells contributes to the innate resistance of *Pseudomonas* in 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 to investigate the rate of cell death within the biofilm following treatment with a variety of antimicrobial agents. Using fluorescent labels we examine the effect antimicrobial agents have on the components of the biofilm. Working with partners at the National Physical Laboratory (NPL) we utilise the 3D OrbiSIMS platform to investigate the association and accumulation of antimicrobial agents within the *Pseudomonas aeruginosa* biofilm.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P310

Identification of host factors regulating nuclear size during HCMV replication using a high-throughput high-content screen.

Chen-Hsui Lee¹, Samantha Griffiths², Paul Digard¹, Nhan Pham², Manfred Auer², Juergen Haas², Finn Grey¹

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

Abstract

Regulation of nuclear size has broad impacts on cellular physiology, including gene transcription, DNA replication, and mRNA transport. Abnormal nuclear size is also linked to cancer progression. Human cytomegalovirus (HCMV) infection triggers a dramatic increase in nuclear size, providing a tractable model for investigating the mechanisms involved in nuclear size regulation. Studies have shown that the recruitment of an endoplasmic reticulum protein called BiP, SUN domain proteins and dynein are involved in remodelling the nuclear architecture during HCMV infection. However, other cellular factors and pathways are likely to be involved. Using a high-throughput RNAi screen with a high-content imaging system, we identified 116 host factors for which gene depletion led to an inhibition of nuclear enlargement by 20% or more, compared to the negative siRNA control. Failure in nuclear enlargement was highly associated with an inhibition of virus replication. Meanwhile, siRNA knockdown of 31 host factors resulted in an increase of nuclear enlargement by 20% or more. Besides the changes in nuclear size, knockdown of 85 host factors resulted in at least two-fold increase in total cellular numbers, based on the nuclear counts. This was particularly surprising as infection of HCMV induces a complete block in cell cycle progression. Studies characterising these hits are currently underway. These studies demonstrate that HCMV infection may be a useful model for investigating nuclear regulation and cellular proliferation. The results may also have important implications for cancer biology and treatment.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P311

Latency associated nuclear antigen LANA play a pivotal role in MCF disease

Ishtar Adnan Mohammed Alethari, James Stewart

Institute of Infection and global health /Liverpool university, Liverpool, United Kingdom

Abstract

Malignant catarrhal fever (MCF) is infectious, lymphoproliferative multi-systemic fatal disease of cattle and many other species of Artiodactyl, characterized by low morbidity but high mortality and it occurs in different countries in worldwide. MCF is caused by Macavirus genus (previously known as Rhadinovirus) of the family Herpesviridae, subfamily Gammaherpesvirinae. The MCF group comprises 10 known members; two of these viruses are most important to describe the MCF infection in animals, alcelaphine herpesvirus-1 (AIHV-1) and ovine herpesvirus-2 (OvHV-2). AIHV-1 is restricted to areas of Africa where wildebeest are present and to zoological collections elsewhere, and has been referred to wildebeest-associated MCF, while the OvHV-2 form occurs world-wide wherever sheep husbandry is practised and has been described as sheep-associated (SA) MCF. The mechanisms responsible for the lymphoproliferative and degenerative clinical and lesions observed in MCF are unknown. Latency associated nuclear antigen LANA is OvHV-2 protein which play a fundamental role in establishment and maintenance of the latency and also the pathogenesis of MCF. LANA protein interacts with a variety of cellular proteins, and it has multiple functional homologues to other DNA tumour virus . The aim of this project is to study the proteins that interact with oLANA using proteomic techniques by express olana as a GFP fusion protein in a retrovirus vector (pMSCV) and use this to pull down the interacting proteins in HEK293T cells. This may reveal interactions that can be disrupted using existing compounds and lead to possible interventions in MCF disease.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P312

The Effect of Mutations on Hepatitis B Surface Antigen Binding To Cell Receptors. The Effect of Mutations on Hepatitis B Surface Antigen Binding To Cell Receptors.

Chidinma Raymond, Alexander Tarr, Will Irving

The University of Nottingham, Nottingham,, United Kingdom

Abstract

Hepatitis B virus (HBV) infection is a major public health challenge with about 257 million chronically infected people worldwide and 887,000 yearly deaths, resulting from severe hepatic and non-hepatic complication. Despite the fact that HBV DNA viral load is the gold standard for diagnosis and prognosis in HBV clinical management, HBV surface antigen (HBsAg) as the initial serological marker is an important indicator of HBV infection. Serum HBsAg is useful in the identification of chronic HBV infection, prediction of infection and treatment prognosis as well as prediction of the risk of hepatocellular carcinoma [11].

The proofreading inability of the HBV polymerase, as well as the overlapping nature of the HBV genome, culminates in high mutation rates with pleiotropic effects on both the polymerase and the surface genes. Hence, mutations in the surface gene altering antigenicity and immunogenicity could potentially result in failure of HBV diagnostic assays. Our hypothesis is that mutations in the surface gene of HBV potentially influence HBsAg secretion, expression, antigenicity and immunogenicity which could be the underlying mechanism for HBsAg level fluctuations of clinical relevance.

To do this, we have identified several HBsAg mutants from clinical samples and have made HBsAg mutant constructs cloned into expression vectors. Using *in vitro* cell culture system, we will test this hypothesis by assessing the entry characteristics of HBsAg mutants, the intracellular and extracellular levels of HBsAg and HBV virion secretion as well as the binding ability of HBsAg mutants to antibodies currently used in HBV diagnostics.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P313

High-risk human Papillomaviruses downregulate the Ste20 family kinase MST1 to inhibit the Hippo pathway and promote oncogenesis

Molly Patterson, Siu Yu Lee, Christopher Wasson, Ethan Morgan, Andrew Macdonald

University of Leeds, Leeds, United Kingdom

Abstract

Cervical cancer accounts for approximately 528,000 cases of cancer a year, causing 266,000 cancer related deaths. High-risk human papillomaviruses (HPV) are the major cause of cervical cancer cases, particularly HPV 16 and HPV 18, as well as being strongly linked to a sub-set of head and neck cancers. To promote oncogenesis, HPV encoded proteins sabotage a range of host signalling pathways. One of these is the Hippo pathway, which regulates diverse physiological processes including organ size and differentiation and has been implicated in cancer progression. Whilst previous studies have shown that HPV targets the YAP transcription factor, our studies have identified that the protein kinase MST1 is down-regulated in HPV positive cervical cancer. MST1 is essential for successful activation of the Hippo pathway and are considered tumour suppressor proteins in a number of cancers. Our data shows an inverse correlation between MST1 expression and cervical cancer progression. Moreover, re-introduction of MST1 into HPV positive cervical cancer cells reverses the observed cancer phenotype. A greater understanding of the cellular targets of HPV will provide insights into their mechanisms of transformation and identify potential therapeutic targets.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P314

Identification of an Epstein Barr virus lytic cycle reactivation interactome.

Yaqi Zhou¹, Steve Sweet¹, Kate Heesom², Alison Sinclair¹

¹University of Sussex, Brighton, United Kingdom. ²University of Bristol, Bristol, United Kingdom

Abstract

Epstein-Barr virus reactivation from latency promotes lytic replication, the production of infectious virions and exposes infected cells to recognition by the immune system. The key activator of EBV lytic cycle is Zta (BZLF1), a bZIP protein that binds to the host and viral genome and acts as a transcriptional regulator and origin binding protein. Zta is a homodimer with reported interactions with several of the conserved and essential herpes virus replication proteins and with several cellular transcription factors and signal transduction molecules. We aimed to use an unbiased approach to identify the viral and cellular Zta interactome during lytic reactivation of Burkitt's lymphoma cells through the physiological approach of cross-linking surface immunoglobulins. A short spacer amino-directed cross-linker dithio-bis-(succinimidyl propionate) (DSP) was used to link Zta with its interactome. Following nuclease treatment to remove DNA, Zta and its associated proteins were isolated by immunoprecipitation with a specific antibody. The interactome was then subject to Tandem mass tags (TMT) labeling and identified by mass spectrometry. We identified 120 associated proteins (FDR<0.05). This included previously identified viral (BMRF1, BALF2 and 5) proteins. Gene ontology analysis identified several functional clusters of cellular proteins. The contribution of these clusters to EBV replication is under investigation. HSC70 is central to one cluster, we validated the interaction with Zta, demonstrated that it occurs independently of other viral proteins and showed that HSC70 contributes to EBV lytic cycle. This conclusion shows a functional conservation of HSC70 involvement in gamma herpes viruses during the viral lytic cycle.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P315

The Role of RNA4.9 in the Establishment of Human Cytomegalovirus Latency

Marianne Perera¹, Sharon Karniely², Noam Stern-Ginossar³, John Sinclair¹

¹University of Cambridge, Cambridge, United Kingdom. ²Kimron Veterinary Institute, Bet Dagan, Israel.

³Weizmann Institute of Science, Rehovot, Israel

Abstract

Human cytomegalovirus (HCMV) is a ubiquitous betaherpesvirus, infecting 50-90% of the population. In healthy people, primary infection is generally asymptomatic, and the virus goes on to establish lifelong latency in cells of the myeloid lineage. However, HCMV poses significant risk to immune-incompetent individuals, and is the leading cause of congenital birth defects. Additionally, upon reactivation from latency, HCMV causes serious disease in transplant recipients and AIDS patients.

Initial events in the establishment of latency remain incompletely understood. The latent state is believed to arise from an orchestrated effect of viral and cellular factors which result in repression of the Major Immediate Early Promoter (MIEP), thereby suppressing the lytic cascade. Recently, it has been suggested that a key mechanism to MIEP silencing involves recruitment of cellular PRC2, which results in repressive histone marks around the MIEP (H3K27 trimethylation).

Interestingly, long non-coding RNA4.9 (RNA4.9), an abundantly expressed transcript of HCMV thought also to be expressed during latency, has been shown to interact with both the MIEP and PRC2, and has thus been hypothesized to have a role in the suppression of lytic gene expression during latency.

Using the monocytic THP-1 cell line latency model, we have knocked out RNA4.9 expression using CRISPR/Cas9 and measured the effect this has on lytic gene expression during the establishment of latency. The role of RNA4.9 in latency will be discussed.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P316

Understanding temporal regulation of VACV morphogenesis proteins F13 and A26 and its effect on viral replication.

Joe Holley¹, Carlos Maluquer de Motes¹, David Ulaeto²

¹University of Surrey, Guildford, United Kingdom. ²Defence Science and Technology Laboratory, Salisbury, United Kingdom

Abstract

Vaccinia virus (VACV) and other large DNA viruses acquire variation via a number of well-defined mechanisms such as mutation rate, internal recombination, gene duplication and deletion. This variation can be confounded in vitro due to the virus' large coding capacity and functional redundancy. Here we wanted to determine how the temporal regulation of viral gene expression affects the acquisition of phenotypic variation in a non-redundant system. We explored the effect of manipulating the temporal expression of two biochemically and functionally distinct proteins critical for the VACV morphogenic cycle. The two proteins A26 and F13 are surrogate markers for the two infectious forms of VACV, the intracellular mature virus (IMV) and extracellular enveloped virus (EEV). F13 expression at 4 hpi correlates with the appearance of EEV. Conversely A26 is expressed at 6 hpi correlating with the formation of IMV, the more abundant progeny virion. This study explores this shift via swapping F13 and A26 promoter sequences. Recombinant viruses expressing FLAG-tagged F13 and A26 alleles under control of either F13 or A26 natural promoters were produced using transient dominant selection from a Δ F13 WR virus. Synchronisation of F13 expression with A26 in v(pA26)F13 results in an attenuated phenotype when compared to vF13. Moreover when A26 expression is brought forward in vF13-F/(pF13)A26 a further reduction of plaque size and viral replication is observed. This work utilises temporal regulation of key morphogenesis proteins as a tool to investigate the bifurcation of EEV and IMV production in VACV morphogenesis.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P317

Resolving the Molecular Gymnastics that Drive Herpes Simplex Virus Fusion using Cryo-Electron Microscopy Coupled with the use of Fabs

Lorelai M. Vennard¹, Doina Atanasiu², Wan Ting Saw², Roselyn J. Eisenberg², Gary H. Cohen², Juan Fontana¹

¹University of Leeds, Leeds, United Kingdom. ²University of Pennsylvania, Philadelphia, USA

Abstract

Herpes Simplex Virus (HSV) afflicts approximately 90% of the population and has no specific therapeutic regime. One of the first steps of infection is entry into the host cell, which is mediated by the HSV fusion protein, gB. Despite various structures being available for gB, a structure for its pre-fusion state is still lacking. To elucidate this structure, full length gB was previously expressed on lipid vesicles and was shown to adopt two conformations via cryo-electron microscopy techniques. These included a post-fusion form and a compact structure thought to be the elusive pre-fusion state. To increase the gB molecules in the pre-fusion conformation, we co-expressed gB and a neutralizing Fab, SS55. While this system increased the number of gB molecules in the compact pre-fusion conformation, the vesicles were produced in small quantities. The vesicle production process was further impeded by not all the gB spikes necessarily interacting with the SS55 Fab. As a result, only a low-resolution average of pre-fusion gB was obtained. To improve resolution, we will combine the 3 plasmids used; gB, and the SS55 Fab heavy and light chains via the Multibac system, which allows multiprotein expression. This will increase the transfection efficiency, producing higher yields of vesicles in which gB is trapped in the pre-fusion conformation by SS55. These vesicles will be further studied by cryo-electron microscopy to obtain a high-resolution structure of pre-fusion gB.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P318

Identification and characterization of cellular proteins that associate with viral replication centres in adenovirus-infected cells

Abeer Albalawi, Fadi Qashqari, Andrew Turnell

Institute of Cancer & Genomic Sciences, The College of Medical & Dental Sciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K., Birmingham, United Kingdom

Abstract

The recruitment of host cell proteins to viral replication centres (VRCs) can serve to either facilitate, or repress, viral replication. The proteomic composition of VRCs is however, poorly defined. The cellular RPA complex has been shown, previously, to be recruited to VRCs during adenovirus (Ad) infection, and is often used as a surrogate marker for VRCs in Ad-infected cells. As for other viruses however, the cellular proteomic composition of Ad VRCs centres has yet to be determined. In order to identify host cell proteins that are recruited to VRCs during Ad infection we utilised U2OS cells that constitutively express either GFP alone, or GFP-RPA1. Following mock infection, or infection with either Ad5 or Ad12, we performed GFP-pulldowns coupled to mass spectrometry to identify those cellular proteins that associate specifically with RPA1 in mock- and Ad-infected cells, and those cellular proteins that associate with RPA1 following Ad5 or/and Ad12 infection. These experiments identified a number of cellular proteins that are recruited to VRCs during Ad infection. The role of these proteins in Ad replication was subsequently investigated. The results of these studies will be presented and the implications of these findings discussed.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P319

Human cytomegalovirus US28 antagonises PYHIN proteins: effects on interferon and apoptosis during latency

Elizabeth Elder¹, Benjamin Krishna^{1,2}, George Sedikides¹, James Williamson¹, Paul Lehner¹, John Sinclair¹

¹University of Cambridge, Cambridge, United Kingdom. ²Cleveland Clinic, Cleveland, USA

Abstract

Human cytomegalovirus undergoes latency in cells of the early myeloid lineage, including CD14⁺ monocytes and CD34⁺ progenitor cells. In both cases, the virally encoded G protein coupled receptor US28 is essential for establishing latency. Furthermore, we and others have used a signalling mutant version of US28 (US28-R129A) to show that signalling by US28 is essential for latency.

Using proteomics technology, we identified that US28-WT, but not US28-R129A, downregulates two members of the PYHIN family of proteins, namely IFI16 and MNDA. IFI16 is a known restriction factor for HCMV and HSV-1 lytic infections, and is proposed to act as a sensor of incoming viral DNA and induce Type I interferon. In agreement with this function of IFI16, we found that US28-expressing THP-1 cells are less able to respond to DNA insults and induce interferon-beta.

MNDA has thus far had no antiviral function associated with it, but is proposed to play roles in myeloid differentiation and neutrophil apoptosis, the latter by targeting the myeloid survival protein Mcl-1 for degradation. When we overexpressed MNDA, we found that it restricted the establishment of infection in THP-1 cells. Intriguingly, we found MNDA cleavage products and intracellular localisation which are consistent with the apoptotic phenotype previously described in neutrophils. Our work identifies a novel antiviral function of MNDA, which is targeted by US28, and suggests that this process is an additional mechanism for evasion of apoptosis during HCMV latency.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P320

The Role of the cellular protein Dock5 as an egress restriction factor for Kaposi's Sarcoma-associated Herpesvirus

Ben Chadwick, James Murphy, Sophie Schumann, Martin Fuller, Juan Fontana, Adrian Whitehouse

University of Leeds, Leeds, United Kingdom

Abstract

Kaposi's sarcoma associated herpesvirus (KSHV) is the causative agent of Kaposi's Sarcoma and two lymphoproliferative diseases. KSHV replication and capsid assembly occurs in the nucleus, from where KSHV capsids migrate to the cytoplasm. Following nuclear egress, the viral capsids travel to the cellular membrane, where morphogenesis takes place. Recent analysis has demonstrated that 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. We are now employing electron microscopy to visualise this process and determine how DOCK5 is involved in KSHV 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. A better understanding of KSHV egress will identify novel antiviral targets against KSHV, and may have pan-herpesvirus activity.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P321

Investigating the relationship between adenovirus and ATR signalling pathways during infection

Reshma Nazeer, Abeer Albalawi, Fadi Qashqari, Andrew Turnell

Institute of Cancer & Genomic Sciences, The College of Medical & Dental Sciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K., Birmingham, United Kingdom

Abstract

Adenovirus (Ad) has evolved to bypass, or inactivate host cell DNA Damage Response (DDR) pathways that would otherwise inactivate cell cycle arrest or apoptotic programmes in the infected cell. Previous work from our laboratory, and others, has shown that Ad5 and Ad12 differentially regulate ATR signalling pathways during infection. Ad5 has been shown to inhibit ATR signalling through the ability of Ad5 E4orf3 to mis-localize the MRN complex during infection, whilst Ad12 inhibits the ability of ATR to phosphorylate, and activate CHK1, by promoting the Ad12 E4orf6-Cullin Ring Ligase 2-dependent degradation of the ATR activator, TOPBP1. Interestingly however, ATR retains the ability to phosphorylate RPA32 during both Ad5 and Ad12 infection, suggesting that ATR retains some of its activities during infection. Here, we investigate the relationship between Ad infection and ATR signalling pathways in more detail. We show that Ad infection promotes the ability of ATR to phosphorylate a cellular protein that is subsequently targeted by adenoviral oncoproteins for proteasomal degradation. The results of these studies will be presented and the implications of these findings discussed.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P322

Molecular evaluation of Porcine Circovirus 1 (PCV-1) from Rotavirus-vaccinated infants

Jane Mitchell¹, Leticia Botas-Perez¹, Mark Preston², Emma Summersgill¹, Machaela Palor³, Miren Iturriza-Gomara⁴, Nicola Rose¹

¹Division of Virology, NIBSC, South Mimms, United Kingdom. ²Division of Technology, Development and Infrastructure, NIBSC, South Mimms, United Kingdom. ³Institute of Immunity and Transplantation, Royal Free Hospital, London, United Kingdom. ⁴Department of Clinical Infection, University of Liverpool, Liverpool, United Kingdom

Abstract

Rotarix is a licensed live-attenuated monovalent vaccine, G1P[8]. In 2010, this vaccine was found to be contaminated with PCV-1 with viral loads estimated over 10^7 DNA copies per final dose. PCV-1 is a non-enveloped circular single-stranded DNA virus. PCV-1 infection in pigs is common and virus can be found in human stool samples however there is no disease associated with infection in either species. Regulatory authorities did not deem the vaccine unsafe for use, however, there remains the question of whether the virus could adapt to human cells.

Archive stool samples sequentially collected throughout the vaccination period from infants vaccinated with Rotarix were selected. Total viral DNA was extracted from faecal suspensions and PCV-1 load quantified by specific qPCR. Peak viral shedding was observed up to 3 days after each vaccination (2-dose regimen) with viral loads of 10^4 (first dose) and 10^3 (second dose) copies per gram of stool.

These data suggest that PCV-1 is unlikely to be replicating in vaccinees. However, this is being further assessed through the application of sensitive next generation sequencing (NGS) of full-length PCV-1 amplicons from stool with reference to the vaccine virus sequence. To date, 15 SNPs have been identified in vDNA extracted from the vaccine of which three cause non-synonymous changes. NGS of PCV-1 DNA from the vaccinees will identify whether mutations are accumulating in the virus excreted from vaccinated infants and the clinical relevance of these will be considered.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P323

Regulation of the B cell receptor pathway by Epstein-Barr virus nuclear antigens

Hildegonda Veenstra, Sarika Khasnis, Opeoluwa Ojeniyi, Michelle West

University of Sussex, Brighton, United Kingdom

Abstract

Epstein-Barr virus preferentially infects B-lymphocytes and is associated with the development of Burkitt's, Hodgkin's and post-transplant lymphoma. The EBV transcription factors, EBNA2, 3A, 3B and 3C drive immortalisation through the epigenetic reprogramming of cellular genes. Pathway analysis has shown that the B-cell receptor (BCR) signalling pathway is significantly enriched in EBNA-bound gene lists, implicating EBNA2 in its regulation. Deregulation of the BCR signalling pathway affects cell growth and survival. We have identified promoter proximal EBNA binding sites at the BCR genes *CD79A* and *CD79B* and confirmed gene repression by EBNA2 and EBNA3 proteins in cells. However, luciferase assays revealed activation by EBNA2 that was independent of the known binding partner RBPJK. Both *CD79A* and *CD79B* were activated by EBF-1 and at *CD79B*, EBNA2 inhibited EBF-1 activation. This indicates that EBNA2 may repress *CD79B* transcriptionally by interfering with EBF-1 activation. Gene expression analysis of further potential EBNA targets in the BCR signalling pathway identified repression of the downstream nuclear factor of activated T-cells genes *NFATC1* and *NFATC2* by EBNA 2 and EBNA3 proteins. Protein analysis confirmed EBNA3B and EBNA3C as negative regulators of *NFATC1* and *NFATC2* in Burkitt's lymphoma cell lines and EBNA2 as a negative regulator in LCLs. Overall, our data indicate the EBNA2 and EBNA3 proteins suppress the calcium signalling arm of the BCR pathway. This may be important to modulate the survival of EBV infected cells.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P324

Investigating the effects of human cytomegalovirus infection on adult neural progenitor cell differentiation

Hannah Beaumont, Stephen Griffin, Heiko Wurdak, Matthew Bentham

University of Leeds, Leeds, United Kingdom

Abstract

Multiple studies have detected the presence of human cytomegalovirus (HCMV) in up to 100% of glioblastoma (GBM) samples tested using an array of detection methods. However, numerous other studies have failed to detect the virus in any GBM samples and infectious particles have not been isolated. Without consensus on the mere presence of HCMV in GBM, the notion of a causal or modulatory relationship between HCMV and GBM remains highly controversial.

Based upon the brain tumour initiating cell (BTIC) hypothesis, primary adult neural progenitor (ANP) cells are a likely cell of origin for GBM in the human brain. However, whilst foetal and iPSC-derived neural progenitors have been utilised in HCMV studies, these undergo rapid cytolytic infection with HCMV and so do not recapitulate the potential scenario in the adult brain. Conversely, the behaviour of the virus in the context of human ANP is unknown. Capitalising upon unique resource, we are investigating the effects of HCMV infection on ANP differentiation. We show that HCMV efficiently infects ANP and undergoes late gene expression, yet infectious virus particles are not detectable, consistent with patient scenarios. Moreover, we will present the results of HCMV-induced effects upon ANP differentiation in culture with particular focus upon cell cycle maintenance and associated stem cell markers and proliferative pathways.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P325

Perturbation of mitochondrial number and function during human herpesvirus 6 infection

Marcus Widdess¹, Darren Wight², Benedikt Kaufer², Harriet Groom¹

¹University of Cambridge, Cambridge, United Kingdom. ²Freie Universitat Berlin, Berlin, Germany

Abstract

Perturbation of mitochondrial function occurs frequently during viral infection and pathogenesis. This is exhibited in a range of herpesviruses. Human herpesvirus 6 (HHV-6) is a betaherpesvirus and causes *exanthema subitum* (Roseola) in children, infecting virtually all children. Like other herpesviruses, it establishes life-long latency after primary infection. However, unlike other herpesviruses, it integrates into telomeres of infected cells and is thought to have germline integrations in 1 % of the general population. We hypothesise that HHV-6 infection increases mitochondrial number and/or function to meet energy demands in lytic infection.

We find by qPCR that wild-type HHV-6A upregulates mitochondrial DNA copy number by 2-fold under conditions of ongoing infection of T cell lines. These data are recapitulated in cells infected with GFP-labelled virus compared to uninfected and GFP-negative cells. Furthermore, we show by immunoblotting that certain mitochondrial oxidative phosphorylation complex proteins are upregulated during infection, but not all. This suggests that the mtDNA increase may not simply reflect an increase in mitochondrial number. These data conflict with reports suggesting that HHV-6B infection has a detrimental effect on mitochondria in T cells. These disparities are being investigated along with monitoring apoptosis and autophagy in infected cells, both of which have been linked to HHV-6 infection.

We are seeking to understand the nature and mechanism(s) of these perturbations through analysis of the control of mitochondrial gene expression during infection, and through metabolic analysis of infected cells. These data enhance our understanding of herpesvirus-host interactions in humans.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P326

African swine fever virus infection of primary macrophages results in rapid alterations of host microRNAs

Laura Dunn^{1,2}, Christopher Netherton¹, Alasdair Ivens³, Pip Bead^{1,2}

¹The Pirbright Institute, Woking, United Kingdom. ²The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom. ³Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh, United Kingdom

Abstract

African swine fever virus (ASFV) is the only member of the Asfarviridae family. However, it additionally belongs to the superfamily of Nucleocytoplasmic Large DNA Viruses (NCLDV), which also contains the *Poxviridae*. NCLDVs encode for a large number of genes (often over 200) and replicate in the cytoplasm of the cell where they undertake complex interactions with host cellular components. Previous work has shown that infection with Orthopoxviruses, such as *Vaccinia virus* (VACV), induces widespread disruption of host microRNAs (miRNAs) via a process of 3' polyadenylation and decay. This trait is also conserved in Capripoxviruses. However, the effect of ASFV infection on host miRNAs has not yet been characterised. We therefore sought to investigate this in detail by using small RNA-sequencing of RNA extracted from primary porcine macrophages infected with pathogenic ASFV taken at 0, 6, and 16 hours post infection. The results confirmed that ASFV does not share the propensity of poxviruses to induce 3' polyadenylation and decay of miRNAs. However, specific host miRNAs were differentially expressed during ASFV infection. Notably, a small number of miRNAs were substantially upregulated within the first hour of infection. This rapid miRNA upregulation was detected only in virally-infected primary macrophages and not in other cell types. Further work aims to manipulate these host miRNAs in order to uncover more information about viral pathogenesis and to modulate virus replication in porcine macrophages.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P327

Functional Proteomic Analysis of Novel Antiviral Restriction Factors in Primary Leukocytes

Lior Soday¹, Katie Nightingale¹, Ben Ravenhill¹, Luis Nobre¹, Kai-Min Lin¹, Colin Davies¹, Rich Stanton², Nick Matheson¹, Michael Weekes¹

¹University of Cambridge, Cambridge, United Kingdom. ²Cardiff University, Cardiff, United Kingdom

Abstract

The plasma membrane provides a critical interface between the cell and its environment, and is the initial portal of entry for viruses. Early interactions between a virus and cellular receptors trigger synthesis of interferons (IFN), which stimulate expression of certain antiviral restriction factors (ARFs) to inhibit viral replication and spread. An understating of these restriction factors, and how viruses interact with them, is crucial to our knowledge of infection and immunity as well as having important implications for therapy.

We have developed a functional proteomic screen to discover novel cell surface ARFs, based on two characteristic features: upregulation by IFN and downregulation by viral infection. As some ARFs are dispensable for replication in cultured cell lines yet required for efficient replication in primary cells, this screen has been applied to primary monocytes and CD4+ T cells, quantifying 647 and 441 plasma membrane proteins respectively.

Analysis of proteins that are stimulated by IFN yet degraded by diverse viruses in multiple cell types is used to delineate the most important ARFs for further characterisation. Published literature considering human cytomegalovirus (HCMV) infection of fibroblasts (Weekes et al, 2014) and HIV infection of cultured T cells (Matheson et al, 2015) was considered. Screens were validated by the identification of the known cell surface HIV restriction factor Tetherin, in addition to MHC class I, which is targeted for degradation by both HIV and HCMV. Novel data from this screen will be presented.

Virology Workshop: DNA Virus

Zone C

Presentations: Thursday Evening and Friday Lunchtime

P328

Spir-1 is an additional cellular protein that interacts with vaccinia protein K7

Amir Rashid, Alice Abreu Torres, Geoffrey Smith

Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Abstract

Vaccinia virus (VACV) encodes many proteins that aid evasion of host innate immunity. VACV K7 is a small, intracellular protein expressed early during infection that is non-essential for virus replication but affects virus virulence. K7 has a Bcl-2-like fold and binds IRAK2 and TRAF6 to inhibit NF- κ B activation. K7 also inhibits the IRF3 pathway via interaction with the DEAD-box RNA helicase 3 (DDX3). This study describes Spir-1 as an additional cellular protein targeted by K7. K7 and Spir-1 co-precipitated following ectopic expression and during VACV infection. Spir-1 belongs to a family of proteins involved in actin organisation but the interaction of K7 with Spir-1 does not require its actin binding domains, suggesting a new function of Spir-1. Based on the co-structure of K7 and an N-terminal fragment of DDX3 we used site-directed mutagenesis to create K7 mutants to investigate its interaction with binding partners. A D31A mutation abolished K7 binding to DDX3 and severely reduced binding to Spir-1. However, the D31A mutant still binds TRAF6 and hence retained other K7 functions. Immunoprecipitation assays showed a lack of association between Spir-1 and DDX3. Collectively, these data show that K7 interacts with Spir-1 and that, the N-terminal region of K7, involved in its interaction with DDX3, is also implicated in Spir-1 association. The functional consequence of K7 interacting with Spir-1 is being investigated.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P329

Analysis of the early cellular and humoral responses of *Galleria mellonella* larvae to infection by *Candida albicans*

Gerard Sheehan, Kevin Kavanagh

Maynooth University, Maynooth, Co. Kildare, Ireland

Abstract

Galleria mellonella larvae were administered an inoculum of *Candida albicans* and the response to infection over 24 hours monitored. The yeast cell density in infected larvae declined initially but replication commenced 6hrs post-infection. The hemocyte density decreased from $5.2 \times 10^6/\text{ml}$ to $2.5 \times 10^6/\text{ml}$ at 2hr but increased to $4.2 \times 10^6/\text{ml}$ at 6hr. Administration of β -glucan to larvae also caused a fluctuation in hemocyte density ($5.1 \pm 0.22 \times 10^6/\text{ml}$ (0hr) to $6.25 \pm 0.25 \times 10^6/\text{ml}$ (6hr) to $5 \pm 2.7 \times 10^6/\text{ml}$ (24hr)) and the population showed an increase in the density of small, granular cells at 24 hours ($p < 0.05$). Hemocytes from larvae inoculated with β -glucan for 6 or 24 hours showed faster killing of *C. albicans* cells ($53 \pm 4.1\%$ ($p < 0.01$), $64 \pm 3.7\%$, ($p < 0.01$), respectively) than hemocytes from control larvae ($24 \pm 11\%$) at 60min. Quantitative proteomic analysis indicated increased abundance of immune related proteins (cecropin-A (5 fold)) at 6hr post-infection but by 24hr there was elevated abundance of muscle protein (tropomyosin 2 (141 fold) and proteins indicative of cellular stress (glutathione-S-transferase-like protein (114 fold)), fungal dissemination (muscle protein 20-like protein (174 fold)) and tissue breakdown (cytochrome c (10 fold)). Proteins decreased in abundance at 24hr included β -glucan recognition protein precursor (29 fold) and prophenoloxidase subunit 2 (25 fold). Fungal dissemination was monitored microscopy. *C. albicans* infection of *G. mellonella* larvae shows strong similarities to systemic infection of mice.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P330

The importance of selecting suitable internal reference genes for quantitative real-time PCR analysis of *Staphylococcus aureus* exposed to antibiotic-induced stress conditions

Muna AL-eryan¹, Brian Jones², Curtis G Gemmell³, Sue Lang¹

¹Glasgow Caledonian University, Glasgow, United Kingdom. ²Glasgow Royal Infirmary, Glasgow, United Kingdom. ³University of Glasgow, Glasgow, United Kingdom

Abstract

Quantitative real-time PCR (qPCR) is extensively used for the evaluation of bacterial gene expression in response to external stimuli. A review of the literature indicates that 44% of *Staphylococcus aureus* studies use the 16S ribosomal RNA gene (*16SrRNA*) as an internal reference gene without prior evaluation. This practice is being questioned as it becomes clear that genes are not stably expressed under varying experimental conditions.

The aim of this study was to use the geNorm algorithm-based software to screen for *S. aureus* internal reference genes that are stably expressed under conditions of antibiotic-induced stress and determine an optimal set of reference genes for the assessment of target-gene expression.

Expression stability of eight candidate reference genes was evaluated during the mid-exponential and early stationary phases of growth, using dedicated software, after exposure to inhibitory concentrations of four antibiotics.

Using qBase plus (BioGazelle) software, algorithm geNormM demonstrated that the two gene sets *rpoB*, *rho*, *gyrB*, *ftsZ* and *ftsZ*, *rpoB*, *sodA*, *hu* were most stably expressed (*M*-value <1) with low variation in the cycle threshold (*C_t*) (0.9-1.1 cycles) in mid-exponential and early stationary phase of growth, respectively. In contrast, the *C_t* values of *16SrRNA* and *adhE* were unstable with variation in *C_t* of 8.5 and 6.6 cycles, respectively. In both growth phases geNormV indicated that 4 or 5 reference genes were required for reliable qPCR analysis.

This study demonstrates the prerequisite need to assess the constant expression of multiple reference genes for their use in the normalisation of mRNA abundance in qPCR.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P331

Transcriptomic & Proteomic map of a highly virulent, multi-drug resistant *Acinetobacter baumannii* isolate

Varsha Naidu¹, Karl Hassan^{1,2}, Karthik Kamath¹, Liam Elbourne¹, Mark Molloy¹, Ian Paulsen¹

¹Macquarie University, Sydney, Australia. ²University of Newcastle, Newcastle, Australia

Abstract

Very recently, the World health organisation published its first ever list of 12 families of bacteria for which antibiotics are needed urgently. Of these 12 families, 3 groups of Gram-negative bacteria were classified as being the highest priority “critical”, these included *Acinetobacter*, *Pseudomonas* and various Enterobacteriaceae. *Acinetobacter* species have only recently emerged as problematic pathogens in hospitals. The emergence of *Acinetobacter baumannii* as a significant hospital pathogen can be attributed to its broad resistance mechanisms.

The aim of this project was to describe the complex cellular responses of a highly virulent, drug resistant strain of *Acinetobacter baumannii* to antimicrobials of several different classes. These responses have been analysed using state-of-the-art quantitative mass spectrometry (SWATH-MS) and RNA-sequencing technologies, to define changes in expression at both the protein and transcript levels. The results have been analysed using bioinformatic tools to determine the clustering patterns of genes and proteins in response to the panel of antimicrobials, as well as to correlate mRNA expression and protein abundances across several treatments.

While each antimicrobial treatment showed a unique expression profile, overlaps in gene/protein expression demonstrated similar mechanisms of drug resistance. For example, overlaps in expression were evident in several efflux pump genes, pili genes, capsule and siderophore biosynthesis clusters, suggesting that these factors may play a common role in responding to antimicrobial treatments.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P332

Screening in research grade *Galleria mellonella* reveals that *Vibrio parahaemolyticus* *mutT* plays a role in virulence

Richard Titball¹, Olivia Champion², Sariqa Wagley¹

¹University of Exeter, Exeter, United Kingdom. ²Biosystems Technology, Exeter, United Kingdom

Abstract

Galleria mellonella (waxmoth) larvae have attracted interest over the past few years as a model for investigating mechanisms of virulence of bacterial and fungal pathogens and for measuring antibacterial and antifungal drug efficacy. Work with *G. mellonella* larvae is not subject to the same regulatory framework that governs work with higher animals. The low cost and ease of maintenance of the larvae allows experiments to be carried out flexibly and using larger cohorts, increasing the statistical power of experiments. However, the larvae used in most studies are bred as pet food or fishing bait and show variable responses to infection. In part this is because they are different ages and weights, but also because the larvae are typically bred with antibiotics and hormones added to feedstuffs. We have compared these larvae with research grade larvae and find that experiments with research grade larvae provide much more consistent data. In the case of *Vibrio parahaemolyticus*, this allowed us to show the role of *mutT*, a nudix family protein, in infection.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P333

A Rab32 trafficking pathway that prevents bacterial infections

Massimiliano Baldassarre, Virtu Solano-Collado, Domenico Mancuso, Stefania Spano

University of Aberdeen, Aberdeen, United Kingdom

Abstract

Host defence mechanisms protect complex organisms against the attack of microbes. Intracellular bacterial pathogens such as *Salmonella enterica* (*Salmonella*), evolved sophisticated, often redundant, strategies to overcome host defence and cause infection. Despite sharing many strategies to invade host cells, different *Salmonella* serovars show different pathogenic behaviors and host-specificity. We previously identified a host intracellular trafficking pathway involved in *Salmonella* Typhi host-restriction. We showed that this pathway, which depends on the host GTPase Rab32 and its guanine nucleotide exchange factor BLOC-3, prevents the human-restricted pathogen *Salmonella* Typhi from surviving in mouse macrophages and therefore infecting mice. We also showed that in contrast to *Salmonella* Typhi, the broad-host pathogen *Salmonella* Typhimurium infect mice by counteracting the Rab32 trafficking pathway through the delivery of two type-III-secretion effectors: GtgE, which is a specific protease cleaving Rab32; and SopD2, which is a Rab GTPase activating protein (GAP). These two effectors appear to act redundantly to neutralize this host defense pathway. However, the Rab32-dependent mechanisms directly involved in bacterial killing are still unknown. We now show here that the Rab32 trafficking pathway is a broad host-defense pathway. Our new results indicate that *Salmonella* is not the only pathogen susceptible to this pathway and suggest that other intracellular pathogens have to counteract this host-defence pathway to be able to survive inside the host cell and to cause infection.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P334

A Mouse Model for Non-typeable *Haemophilus influenzae* Infection and Immunization Studies

Tom Purnell, Derek Hood, Steve Brown

Medical Research Council, Oxford, United Kingdom

Abstract

Non-typeable *Haemophilus influenzae* (NTHi) is the predominant causative agent of otitis media (OM, middle ear disease) in children. The *Junbo* mouse, a mutant mouse line that develops symptoms of chronic otitis media spontaneously, has been used to study human otopathogens. These investigations have focussed upon NTHi infection and ways to improve treatment of acute OM.

A dose of 10^6 CFU of NTHi strains derived from human OM administered intranasally to 8-11 week old *Junbo* mice typically infected 70 to 90 % of middle ears and achieved high titres (average 10^4 to 10^5 CFU/ μ l) at day 7 post-inoculation. *Junbo* mouse middle ears could remain infected up to 56 days post-NTHi inoculation. We vaccinated *Junbo* mice with different NTHi bacteria using a 3-step immunization protocol then infected these mice with a single NTHi strain (162sr). There was a significant reduction in middle ear infection rate observed for mice immunized with the homologous NTHi strain whereas only a small reduction in average middle ear bacterial titre was realised in mice immunized with the heterologous NTHi strains. Blood collected from these mice allowed us to investigate the host response to NTHi immunization. These tests, including a serum bactericidal assay, indicated heterogeneity in the immune response to immunization between individual animals from the same test cohort.

We have established an efficient NTHi infection model in the *Junbo* mouse. Immunization and protection studies with NTHi demonstrate a utility for the model in translational investigations that could lead to improved treatment of otopathogen infection and OM.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P335

BactiVac, a network to support the study, development and implementation of bacterial vaccines

Adam Cunningham¹, Johanna Dean¹, Susan Pope¹, Evelina Balandyte¹, Calman MacLennan^{1,2}

¹University of Birmingham, Birmingham, United Kingdom. ²University of Oxford, Oxford, United Kingdom

Abstract

Infections account for >20% of all deaths worldwide, and are particularly problematic in low-middle income countries (LMICs). Bacterial infections contribute significantly to this burden, killing approximately 5 million people annually. The crisis of antimicrobial resistance means our options for controlling infections is narrowing. Vaccines save millions of lives yearly and are a cost-effective approach to prevent infectious disease and their devastating sequelae. There are many bacterial infections against which we lack any licensed vaccine. To address these issues, the MRC and BBSRC, through the Global Challenges Research Fund, have funded 5 vaccine-related networks. Our network, **BactiVac** (www.birmingham.ac.uk/bactivac), was launched in August 2017 following the award of £2.2m and is **led by Profs Cal MacLennan and Adam Cunningham**.

Our purpose is to establish a global bacterial vaccinology network to accelerate the development of vaccines against bacterial infections, particularly those relevant to LMICs. The **BactiVac** network will bring together academic, industrial and other partners involved in vaccine research against human and animal bacterial infections from the UK and LMICs. **BactiVac** will foster partnership and provide catalyst project and training funding to encourage cross-collaboration between academic and industrial partners. **BactiVac membership is free** – join us at <http://bit.ly/applyBactiVac>.

Benefits of membership include:

- Access to our **catalyst funding schemes**
- Invitation to our subsidised **annual network meetings**
- Access to our **Members' Directory** to develop collaborations and access distinct expertise.

We encourage you to become a member and be involved in this initiative, and to be part of its success now and in the future.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P336

Harnessing Superantigen Activity to Generate an Enhanced Vaccine Response

Coral Dominguez-Medina, Romain Paillot, Carl Robinson, Maria Lopez-Alvarez, Andrew Waller

Animal Health Trust, Kentford, Newmarket, United Kingdom

Abstract

Strangles, caused by *Streptococcus equi*, is the most common infectious disease in horses worldwide. In Europe, the only available Strangles vaccine is Equilis StrepE, a live vaccine, which provides protection to horses for only 3 months. An alternative vaccine, Strangvac, currently in development comprises 8 *S. equi* proteins and is 95% effective 2 weeks after third vaccination, but this protection only lasts for 2 months.

Superantigens activate the immune response by cross-linking MHC class II to the T cell receptor (TCR), resulting in polyclonal T cell activation. Here we show that by mutating the TCR-binding site of SzeQ, a novel superantigen, MHC class II binding properties are maintained while TCR-binding is lost. We hypothesise that fusion of the modified SzeQ to surface proteins from *Streptococcus equi* will direct vaccine components towards antigen presenting cells resulting in enhanced presentation, stronger immune responses and increased duration of protection. If successful, similar fusion proteins could be exploited to enhance the effectiveness of vaccines against other infectious diseases in different animal species, including humans.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P337

***Pseudomonas aeruginosa* as an intracellular pathogen in urinary tract infections**

John Newman, Jo Fothergill, Rachel Floyd

University of Liverpool, Liverpool, United Kingdom

Abstract

Urinary tract infections (UTIs) are the most common healthcare acquired infections and are associated with substantial economic and human cost. *Escherichia coli* is the primary causative agent of UTIs, however a small yet significant portion of UTIs are caused by *Pseudomonas aeruginosa*. *P. aeruginosa* has been identified as a pathogen of the highest concern in the global antibiotic resistance crisis. *P. aeruginosa* is frequently studied in the lung infections of cystic fibrosis patients where it has been shown capable of invading human epithelial cells. Bacterial invasion of host cells is considered to be a valuable mechanism for evading the host immune system. Several pathogens associated with UTIs have been shown to invade host cells of the urinary tract but there is no literature on the potential invasiveness of *P. aeruginosa* in the urinary tract. We attempted to elucidate whether or not several clinical isolates and lab strains of *P. aeruginosa* were capable of invading bladder urothelial cells in an *in vitro* model. Gentamicin protection and adherence assays were carried out on immortalized bladder urothelial cells. We saw variability in levels of invasiveness across several clinical isolates and lab strains of *P. aeruginosa*. Further work is needed to discover how and why the studied strains varied in their invasiveness and whether the results translate to more physiologically relevant models of the human urinary tract.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P338

Exploring the effects of Interferon α on *Cryptococcus neoformans* infection

Paula Seoane, Leanne Taylor-Smith, Robin May

University of Birmingham, Birmingham, United Kingdom

Abstract

Cryptococcus neoformans is an opportunistic human pathogen, which causes serious disease in immunocompromised hosts. Infection with this pathogen is particularly relevant in HIV⁺ patients, where it is responsible for approximately 200000 deaths pa. A key feature of cryptococcal pathogenesis is the ability of the fungi to survive and replicate within the phagosome of macrophages, as well as its ability to escape by a novel non-lytic mechanism known as vomocytosis. The impact of vomocytosis on the outcome of infection is currently unknown, although it has been proposed to be linked to dissemination.

We have been exploring the impact of inflammatory cytokine signalling on vomocytosis, focusing on the effects of the anti-viral cytokine interferon alpha (IFN α). Here we show that elevated levels of IFN α , corresponding to those produced as a result of the anti-viral immune response during HIV infection, alter cryptococcal vomocytosis rates. The same effect is observed when studying virally-infected cells. Consequently, acute viral infection may trigger the release of latent cryptococci from intracellular compartments, with significant consequences for disease progression.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P339

The antigenic structure of poliovirus type-2 determined by next generation sequencing of monoclonal antibody resistant mutants.

Bethany Charlton, Edward Mee, Helen Fox, Philip Minor, Javier Martin

National Institute for Biological Standards and Control, South Mimms, United Kingdom

Abstract

Indigenous wild poliovirus serotype 2 (PV2), one of the causative agents of paralytic poliomyelitis in humans, was declared eradicated in September 2015. However, the antigenic profile of PV2 is not yet fully established due to incomplete data on the location of independent neutralising epitopes. This study used next generation sequencing of monoclonal antibody (mAb) resistant PV2 mutants to refine the antigenic structure of PV2. The technique was repeatable in two independent populations of PV2 derived from the same vaccine seed stock with a panel of 11 PV2-specific mAbs. Nonsynonymous SNP mutations identified in the structural capsid were mapped onto a 3D model of PV2 with the aim to locate regions likely to confer mAb-resistance. Mutated residues were then grouped into antigenic sites 1, 2 and 3 based on their structural proximity and data from previous studies. These mutated epitopes grouped into regions at the 5-fold and 3-fold axes of symmetry, with some overlap identified between the neutralising epitopes of each site. Antigenic site 3 in PV2 was located at the interface between 3 pentamers at the 3-fold axis of symmetry. New vaccines with increased stability are being developed to support the post-eradication era and efficacious vaccines rely on the preservation of neutralising epitopes to ensure protection in the recipient. Characterising the full antigenic structure of PV2 is also important to better understand the impact of antigenic drift in humans and the relevance of such mutations in any vaccine-derived strains which may possibly reappear in the future.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P340

Development of an experimental system to investigate the impact of herpes simplex virus type 1 latency on human neuronal cells

Amy Jacobs^{1,2}, Martin Fritzsche³, Mark Preston³, Stacey Efstathiou¹, Michael Nicoll¹

¹Division of virology, The National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom. ²Imperial College London, London, United Kingdom. ³Bioinformatics Group, TDI, The National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom

Abstract

Herpes simplex virus 1 (HSV-1) is a highly prevalent neurotropic virus. The virus persists for the lifetime of the host with periodic reactivation. This is due to HSV-1 being able to establish neuronal latency, during which, the only abundantly transcribed HSV-1 gene is the latency-associated transcript (LAT), which can be processed into the 2.0kb major LAT intron and several microRNAs.

In order to investigate the impact of viral latency on the host cell we have established a neuronal model of HSV-1 latency using a human neuroblastoma cell-line (SH-SY5Y cells) and replication-defective HSV-1 mutants. Additionally, we have cloned the first 3.1kb of LAT or virus encoded microRNAs into lentivirus vectors. We are using these approaches to explore the impact of these non-coding RNAs on human neurons, using RNA-seq to explore any changes to the neuronal transcriptome.

We have characterised our lentiviral constructs and shown efficient transduction of SH-SY5Y cells. Preliminary RNA-seq data from neuronal cells transduced with the LAT-lentivirus construct suggests that the major LAT intron does not significantly alter the neuronal transcriptome, but when LAT splice sites are mutated – inhibiting production of the intron – host transcriptional changes are evident.

This highlights the importance of LAT splicing during latency and suggests that the LAT intron may act downstream of transcription or affect the virus directly. We are currently investigating the effect of LAT transcription on various host and viral phenotypes. Improved understanding of these interactions could help develop interventions targeting HSV-1 latency.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P341

Salmonella Typhimurium induction of tumour shrinkage in a sub-cutaneous tumour model is mediated through inflammatory monocytes.

Sile Johnson¹, Michael Ormsby¹, Stephen Tait², Karen Blyth², Donal (Daniel) Wall¹

¹University of Glasgow, Glasgow, United Kingdom. ²Cancer Research UK Beatson Institute, Glasgow, United Kingdom

Abstract

Bacteria offer unique solutions to many of the limitations of present cancer therapies. They are self-propelled and genetically amenable bearers of therapeutics that penetrate deep into tissues, stimulating intense immune responses at immune privileged sites. Long known to exert positive anti-tumour effects, recent efforts have tried to harness this ability of bacteria with varying degrees of success in animal models, but little success in clinical trials. Efforts have focused on the potential of *Salmonella* Typhimurium, amongst other bacteria, for use as a delivery vehicle and tumour-targeting agent. However the factors underlying *Salmonella* tumour-growth inhibition remain poorly understood despite the wealth of knowledge available on *Salmonella*-host interactions. Here using systemic administration of an *aroA* mutant SL7207 strain we induced tumour growth inhibition in, and increased survival of, subcutaneous tumour-bearing mice. Previous reports have identified Th1 CD4+ T cells and CD8+ T cells as being important for anti-tumour immunity. Here, however we demonstrate increased infiltration of inflammatory monocytes into SL7207 infected tumours. These cells significantly increased pro-inflammatory cytokine production whilst also increasing their phagocytic capacity. Treatment of mice with clodronate containing liposomes, which selectively remove phagocytic cells, completely inhibited *Salmonella* mediated tumour growth retardation. These findings provide important insight into another potential mechanism of bacterial mediated tumour inhibition, identifying the accumulation of monocytes and increased inflammation as necessary for *S. Typhimurium* infection mediated effects.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P342

ChIP-seq analysis to reveal the OmpR regulon implicated in the virulence and adaptive abilities of *Yersinia enterocolitica* bioserotype 1B/O:8

Marta Nieckarz, Adrianna Raczowska, Katarzyna Brzostek

Faculty of Biology University of Warsaw, Warsaw, Poland

Abstract

Background

Yersinia enterocolitica, a gram-negative human enteropathogen, exhibits a dual lifestyle. It can exist as free-living bacteria in the environment or may form an association with a host. To occupy these different ecological niches, *Y. enterocolitica* must adapt to large environmental fluctuations by altering the expression of its genes. OmpR regulates the transcription of genes by binding to sites in their promoter regions that have similar but not identical sequences.

Methods

To uncover the OmpR transcriptional regulatory network and elucidate its complex role in the virulence and adaptive abilities of *Y. enterocolitica* ChIP-seq technology was applied.

Results

The obtained results indicated that: (1) OmpR exhibits regulatory abilities that do not depend on the presence of a typical OmpR DNA-binding motif; (2) acid pH affects the DNA binding abilities of OmpR leading to the modulation of gene expression; (3) OmpR modulates the expression of genes that can promote cellular survival in acidic pH; (4) OmpR plays a role in strategies of the highly pathogenic *Y. enterocolitica* associated with survival in different niches within the host organism that vary in pH.

Conclusion

The characterization of all DNA fragments physically associated with OmpR allowed us to discern some higher functional principles that govern OmpR binding. Identification of novel OmpR-regulated genes in the clinically important *Y. enterocolitica* strain has provided valuable insights into the role of OmpR in the modulation of the virulence, thus may have implications for understanding pathophysiology of yersiniosis.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P343

Identification of cis-acting RNA sequences involved in norovirus VPg-dependent RNA synthesis

Myra Hosmillo¹, Bader Alhatlani², Edward Emmott³, Ian Goodfellow¹

¹University of Cambridge, Cambridge, United Kingdom. ²Qassim University, Qassim, Saudi Arabia.

³Northeastern University, Boston, USA

Abstract

Human noroviruses has been identified as the leading cause of viral gastroenteritis worldwide. Despite their socioeconomic impact, our understanding of the biology of human noroviruses has been limited due primarily to their inability to be efficiently cultured in the laboratory until recently. The identification of murine norovirus (MNV), which can replicate efficiently in immortalized cells and for which several robust reverse genetics systems have been developed, has transformed our understanding of the molecular details of norovirus genome translation and replication.

Using MNV as a model system, we have previously shown that while norovirus negative sense RNA is likely primed via a *de novo* mechanism, positive sense RNA is VPg primed and that the RNA is linked to a highly conserved tyrosine residue at position 26. However, the mechanism by which this linkage occurs and the template for norovirus VPg nucleotidylation has yet to be fully characterized.

We have now interrogated the role of evolutionarily conserved RNA structures at the 3' end of the viral genome in the viral life cycle. Using mutagenesis and reverse genetics we identified a series of mutations within the ORF3 coding region that were deleterious for viral replication. Further studies revealed that these mutations function by reducing the ability of the viral negative sense RNA to function as a template for VPg nucleotidylation. Biochemical analysis indicates that this complex required NS5 (VPg), NS6 (3C-like protease) and NS7 (the viral RNA-dependent RNA polymerase), but that precursors forms may also function in the nucleotidylation reaction.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P344

Effect of faecal matter on the bioactivity of anti-*Clostridium difficile* antibiotics

Beccy Sinclair¹, Isobel Twose¹, Mark Wilcox^{1,2,3}, Caroline Chilton¹

¹University of Leeds, Leeds, United Kingdom. ²Leeds Teaching Hospitals, Leeds, United Kingdom. ³Public Health England, England, United Kingdom

Abstract

Background: *C. difficile* is the leading cause of antibiotic-associated diarrhoea. Treatment success is variable between patients and recurrence rates are high. For anti-*C. difficile* antibiotics to be effective, inhibitory intracolonic levels need to be achieved. We investigated the effects of faeces, including inter-subject variability, on antibiotic bioactivity. Methods: Faeces were collected from 6 healthy volunteers. The anti-*C. difficile* antibiotics, metronidazole, vancomycin and fidaxomicin (200mg/L), were incubated anaerobically in each faecal sample (20% w/v in pre-reduced PBS) and in a PBS control. After 24 hours, samples were centrifuged and active antimicrobial concentrations determined by large plate bioassay. Results: Bioactivity was lower in faeces compared with the PBS control for all 3 antibiotics. The bioactive concentrations of metronidazole were below the level of detection following incubation in 5/6 raw faecal samples; the remaining sample showed a 30-fold decrease in metronidazole activity compared with the control. Active concentrations of fidaxomicin in the 6 faecal samples decreased by 3.5-10-fold compared with control; a 1.9-3.7-fold decrease in activity was observed for vancomycin. Conclusion: To treat *C. difficile* infection effectively, antibiotics must achieve sufficient intra-colonic concentrations. We have demonstrated that faeces have a considerable inhibitory effect on the active concentrations of metronidazole and fidaxomicin, and to a lesser degree on vancomycin. The degree of inhibition varied between individuals, which could contribute to the variability of treatment success. Further exploration of the gut microbiota is warranted to determine any correlations and investigate mechanisms of inhibition.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P345

Molecular insights on the SaPI inducing mechanism

Mohammed AlQasmi¹, Julio Bacarizo¹, Alberto Marina², Jose Penades¹

¹Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom. ²Instituto de Biomedicina de Valencia (IBV-CSIC) and CIBER de Enfermedades Raras, Valencia, Spain

Abstract

S. aureus pathogenicity islands (SaPIs) are parasitic mobile genetic elements that exploit phages for induction and transfer. SaPIs integrate to the host chromosome and are repressed by StI, the SaPI master regulator. SaPI induction is occurred when the SaPI StI/DNA complex is disrupted via a specific protein encoded by helper phage. The inducer for SaPI1 is a phage protein called Sri. Interestingly, and in addition to SaPI1 de-repression, Sri blocks bacterial DNA replication by binding to the helicase loader protein (DnaI). The fact that a small protein like Sri (52aa) interacts with two unrelated proteins raises several interesting questions; are DnaI and StI sharing similar structural conformations being recognized by the same interacting residues on Sri, or by contrast, Sri has two interacting faces, one to interact with DnaI and the other to interact with StI? Unraveling those questions would define SaPIs nature. If the DnaI and StI share a conserved domain or similar fold, SaPIs would be then considered as phage parasites. By contrast, if Sri has two different interacting regions, this would imply in somehow that SaPIs can provide unrecognized advantages for the phage. To solve these questions, we have used molecular, biochemical and protein crystallography techniques. Our results provide insights on the mechanism that is used by SaPI1 to interact with Sri protein, highlighting these elements as one of the most fascinating mobiles genetic elements in nature.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P346

Antagonistic Activity of Lactobacilli on Skin Infections' Pathogens: In Vitro and In Vivo Studies.

Duaa Al-Dulaimy, Julian Marchesi, Eshwar mahenthiralingame

School of Biosciences-Cardiff University, Cardiff, United Kingdom

Abstract

Infectious diseases are considered a public health problem in most countries. The occurrence of antibiotic resistant pathogens is increasing worldwide. As antibiotics are presently losing their effectiveness, there is an urgent need to develop safe alternatives for treating bacterial infections especially those of the skin. One of these alternatives is "Probiotics". Health- enhancing properties of probiotics should be performed by both *in vitro* and *in vivo* techniques. The greater wax moth *Galleria mellonella* is used as an *in vivo* model for host-pathogen interactions. A group of *Lactobacillus* type strains was obtained from culture collections, while the other group was isolated from fermented food products: yogurt and olives. Pathogens were isolated from skin infections' patients. All the bacterial species were identified by 16S rRNA gene sequencing. *In vitro* antagonistic activity of lactobacilli was investigated on the major causes of skin infections (*Staphylococcus aureus* and *Streptococcus pyogenes*) by performing both overlay and well diffusion assays during different incubation times and conditions. *In vivo* susceptibility of the wax moth larvae to both lactobacilli and pathogenic species was assessed by injection with serial dilutions of three preparations of each *Lactobacillus* food isolates: bacterial suspension, the supernatant and washed cells. Serial dilutions of pathogenic bacterial suspensions were injected inside the larvae as well. All the tested pathogens were sensitive to the antibacterial effect of lactobacilli. The maximum antagonism was achieved after 72h under anaerobic incubation. Injection of the larvae with both of lactobacilli and pathogens displayed differences in the survival percentages of larvae.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P347

Survival of staphylococcus in human gut

Amjed Alsultan

University of reading, readin, United Kingdom

Abstract

Resistant to the bactericidal effects of bile is crucial for the survival of *Staphylococcus aureus* in the human gut. This study is being conducted to identify and characterise components of the bacteria, which allow it to resist bile acids. comparative proteomics were used to investigate the natural protein diversity within the *Staphylococcus* with relation to bile tolerance. Imaging of one dimension gel electrophoresis show there is unique protein band in sample prepared from bile treated *s.aureus* and disappear in non-treated bacteria.. Mass spectrometry and database analysis show that 3-hydroxyacyl-CoA dehydrogenase FadB homolog is suspect protein. a fadB has a role in lipid metabolism. Several studies documented that genes of lipid metabolism induced as a response to bile salts exposure in enteric bacteria. To confirm the role of fadB in bile salt resistance it was cloned under the control of the arabinose-inducible P_{BAD} promoter of plasmid pBAD/HisA, which enabled arabinose-dose-dependent expression of fadB in $\hat{\imath}$ "fadB *E. coli* JW113. Expression of fadB recombinant *E. coli* showed significantly increment (P<0.01) in MICs to CA, GCA, when compared to $\hat{\imath}$ "fadB *E. coli* JW113. We hypothesized that fadB was responsible for the observed bile salt resistance phenotype, to test this, $\hat{\imath}$ "fadB strain was created in *S. aureus* SH1000. The mutant phenotype showed significant decrease (P<0.01) in viability upon exposure to crude bile as compared with wild type. the study concluded that 3-hydroxyacyl-CoA dehydrogenase enzyme plays a role in resistance of staphylococcus to bile.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P348

Impact of human TRIM21 gene polymorphisms on its antiviral function

Jingwei Zeng, Leo James

MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Abstract

Tripartite motif containing protein 21 (TRIM21) is a ubiquitously expressed cytosolic antibody receptor that binds to the Fc region of IgG with high affinity. Previous studies using a mouse infection model have demonstrated that TRIM21 is a critical component of antibody mediated immunity *in vivo*. Mechanistically, TRIM21 has been shown to trigger simultaneous viral degradation and pro-inflammatory pathway activation upon binding to antibody coated virus.

However, there is no data to indicate the importance of TRIM21 mediated anti-viral immunity in humans. Understanding its significance may aid the future development of antibody-based therapeutics.

We studied the functional impact of naturally occurring missense mutations in the human *TRIM21* gene reported in phase III of the 1000 Genomes Project. Experiments using Lymphoblastoid Cell lines derived from project volunteers revealed that a heterozygous loss of function mutation does not prevent TRIM21 carrying out antibody dependent intracellular neutralization of Adenovirus. A combination of CRISPR/Cas9 gene editing and lentiviral reconstitution techniques were used to introduce naturally occurring point mutants of TRIM21 into HEK-293T cells. Using this system, we investigated the ability of naturally occurring TRIM21 variants to mediate viral neutralization and NF- κ B pathway activation in response to infection by antibody-coated Adenovirus. The results revealed that the antiviral function of TRIM21 is well conserved in the human population and provides evidence that TRIM21 immune function is important in humans.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P349

Identification of genes involved in survival and proliferation of *Clostridium difficile* in vivo using Transposon Directed Insertion Site Sequencing (TraDIS)

Nadia Fernandes, Roy Chaudhuri, Robert Fagan

University of Sheffield, Sheffield, United Kingdom

Abstract

Clostridium difficile is an anaerobic, spore-forming, gram-positive bacterium and the leading cause of antibiotic-associated diarrhoea in hospitals across the globe. Little is known about the mechanisms by which the bacterium colonises and persists within the gut environment; or the signals required by the bacterium for the expression of its toxin genes.

Transposon directed insertion-site sequencing (TraDIS) combines high-density transposon mutagenesis with high-throughput sequencing to allow the identification of essential genes. The technique was applied to *C. difficile* to generate over 70,000 unique mutants and helped identify genes essential for growth and sporulation under *in vitro* conditions. We have further exploited this method to assess gene fitness of a pool of *C. difficile* mutants in a mouse gut, and to gain insights into the basic biology of the organism.

Our TraDIS studies have allowed us to identify genes essential for colonisation, survival and pathogenesis of *C. difficile* and may even aid in identifying novel antimicrobial targets. A total of 62 *in vivo* datasets and 16 *in vitro* dataset have been analysed, one of the largest studies of its kind, and production of new libraries is underway.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P350

Engineering Microbial Responses with Synthetic Polymers

Paco Fernandez-Trillo

University of Birmingham, Birmingham, United Kingdom

Abstract

Here, our efforts towards the understanding of how synthetic polymers interfere with bacteria microbiology, adhesion and virulence will be presented. We will describe polymers that can either sequester signalling molecules, to interfere with microbial communication; or that sequester the bacteria into clusters, thus stopping adhesion to host. Using a combination of computational methods, *in vitro* assays and animal models, we demonstrate that the response of the bacteria to these interactions with polymers is complex and it can often result in the upregulation of (unwanted) phenotypes. However, this response is bacteria specific, and can also result in the downregulation of important virulence factors, including the production of toxins. Finally, we demonstrate that these polymers minimise the pathogenic burden and reduce the ability of bacterial pathogens to colonise *in-vitro* and *in-vivo* models.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P351

Genomic Sequencing of an obligate bacterial parasite of nematodes

Jamie Orr^{1,2}, Peter Cock¹, Vivian Blok¹, Keith Davies²

¹The James Hutton Institute, Dundee, United Kingdom. ²The University of Hertfordshire, Hatfield, United Kingdom

Abstract

Pasteuria penetrans is a spore forming obligate bacterial microparasite which acts as a natural antagonist of plant parasitic nematodes of the genus *Meloidogyne*. *Pasteuria penetrans* engages in suppression of its host via inhibition of root invasion and sterilisation of the adult female. *Pasteuria spp.* are highly fastidious, this is primarily driven by attachment to the nematode cuticle. Thus, understanding attachment specificity is crucial to the effective application of *Pasteuria spp.* as biocontrol agents. Enzymatic and lectin assays suggest a role for both spore surface exposed collagens and N-acetylglucosamine in attachment. However, the genetic and molecular basis for this process has not yet been elucidated.

Using multiple displacement amplification and PacBio sequencing we have assembled a draft genome estimated to be 86% complete, with minimal contamination. This sequencing reveals a reduced genome, with a similarly reduced metabolic profile. Ten putative spore attachment proteins have been identified which bear a strong structural resemblance to the collagenous BclA fibres of *Bacillus anthracis*. Each of these BclA-like proteins carries at least one predicted N-acetylglucosamine binding site. We will attempt to investigate the role of these proteins in attachment to the nematode host via spore surface expression of globular C-terminal domains in non-parasitic *Bacillus subtilis*.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P352

Characterization and targeting of methylcitrate synthase in *Pseudomonas aeruginosa*

Andre Johan Wijaya, Stephen Dolan, Xavier Chee, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

The methylcitrate cycle (MCC) is an essential propionate degradation/detoxification pathway in many pathogenic micro-organisms, including *Pseudomonas aeruginosa*. We found that disruption of the second enzyme in the MCC, methylcitrate synthase (PrpC), abolished *P. aeruginosa* growth on propionate-containing media. This is important because propionate is highly-abundant in many infection scenarios, especially those involving multiple microbial species (e.g., many long-term chronic infections), so inhibition of the MCC could lead to cell death in these circumstances. Our goals are to understand in detail the mechanism of the MCC in *P. aeruginosa* and exploit this for combating infections. X-ray crystallography and enzyme kinetics were utilized to characterize the structure and activity of PrpC. Computer-aided drug design (CADD) based on our protein structure was also employed in the attempt to find a lead inhibitory compound. Predicted small molecule “hits” from this screen were tested *in vitro* using intact cell assays and enzymatic kinetics to measure their effectiveness. Promising small candidate molecules also underwent biophysical analyses (thermal shift and isothermal titration calorimetry (ITC)) to confirm binding. The structure of the PrpC from *P. aeruginosa* was solved *via* molecular replacement to 1.5 Å resolution. Three out of 40 CADD-predicted compounds based on these structural data were able to specifically inhibit *P. aeruginosa* growth in the presence of propionate. Current work is aimed at understanding the detailed MoA of these, and other hits obtained.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

53

Development of Flaviviridae pseudotyped viruses with global epidemic potential

Kamilla Toon^{1,2}, Robert Francis¹, Kirsty MacLellan-Gibson¹, Emma Bentley¹, Yasuhiro Takeuchi^{1,2}, Nicola Rose¹, Giada Mattiuzzo¹

¹National Institute for Biological Standards and Control (NIBSC), Potters Bar, United Kingdom. ²University College London (UCL), London, United Kingdom

Abstract

Rapid diagnostics to monitor outbreaks and evaluate treatment options are crucial in responding to viral outbreaks. Replication-defective viral pseudotypes, which contain the core of one virus and the envelope proteins from a heterologous virus, have proved to be advantageous research tools as they do not require high biocontainment laboratories. Furthermore, replacing structural proteins in the genome of the vector system with a reporter gene allows for a rapid detection of infection. Flaviviridae is a family of viruses with outbreak potential and strong public health importance, which includes Dengue virus, Zika virus, Japanese encephalitis virus and West Nile virus among others. We have investigated Flaviviridae envelope protein expression and incorporation onto two common vector systems: retrovirus and vesicular stomatitis virus (VSV). Flaviviridae envelope proteins that naturally bud through the endoplasmic reticulum are difficult to display onto retroviral or VSV cores which instead exit from the plasma membrane. We followed localization of viral envelope protein in vector producer cells by flow cytometry and confocal microscopy. We evaluated how varying the expression level of these proteins impacts their localisation in different cell compartments. Furthermore, we are investigating the role of ER resident protein in the trafficking of the flaviviruses envelope. Together with a systematic analysis of the characteristics of Flaviviridae pseudotypes such as quality and yield of different systems, this research could lead to more standardized protocols for pseudotype production enabling faster development of assays essential for outbreak preparedness.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P354

Investigating Phenotypic Diversity in Fungal-Host Interaction during *Cryptococcus neoformans* Titan cell formation

Xin Zhou, Elizabeth R Ballou

Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom

Abstract

Cryptococcus neoformans is a worldwide opportunistic human fungal pathogen causing life-threatening pneumonia and meningitis in immunocompromised patients. During the earliest stage of host lung infection, *C. neoformans* undergoes an unusual yeast-to-Titan morphogenetic transition. Titans are large (>11µm) cells with increased drug resistance and altered Pathogen Associated Molecular Pattern (PAMP) exposure. These features drive Titan resistance to host immunity and are thought to mediate pathogenesis. However, our screen of clinical isolates found wide variation in the capacity of pathogenic strains to Titanise. The *C. neoformans* wild-type Titanising strain H99 elicits meningitis in mice, while the non-Titanising clinical isolate Zc1 causes pneumonia. However, the relative impact of Titan vs. yeast morphology, as well as other underlying differences in virulence between the two strains, remains undescribed. Here we applied our *in vitro* Titan induction protocols to investigate yeast vs. Titan phenotypes and underlying mechanism. We report that H99 Titans displayed altered distribution of important PAMPs, with increased chitin and decreased chitosan and mannan. Additionally, when co-incubate with macrophages, H99 titans showed reduced uptake, consistent with previous reports *in vivo*. We also observed that engulfed Titans and daughters had increased survival rates compared to un-induced H99 yeast. Surprisingly, induced Zc1 cells that failed to Titanise also exhibited changes in PAMP exposure, and induced cells of either isolate were more likely to escape phagocytes via non-lytic vomocytosis. Together, our work suggests that Titan induction has broad implications for host-fungal interactions and pathogenesis and begins to form a framework through which these questions can be addressed.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P355

Towards the identification of novel host or bacterial-associated biomarkers to predict sepsis

Heather M. Chick, Llinos G. Harris, Yuqin Wang, William J. Griffiths, Thomas J. Humphrey, Thomas S. Wilkinson

Swansea University, Swansea, United Kingdom

Abstract

Sepsis represents one of the leading causes of death worldwide. In the U.K. alone, up to 27% of ICU admissions are due to severe sepsis. Current biomarkers exploit key inflammatory components (such as IL-6, C5a, PCT, etc) that often lack strong discriminatory power to identify the causative agent and so inform antimicrobial therapy. In addition, a lack in definitive bacterial virulence factors associated with complex host responses contribute to poor target identification. This project uses an *ex vivo* whole blood model and a collection of sequenced microorganisms cultured from clinical infections to identify important host and bacterial biomarkers for sepsis.

Phylogenetic analysis of 291 sequenced *S. epidermidis* strains from four clinically defined groups including: commensals (127), catheter infections (40), prosthetic joint infections (99), and bacteraemia (25) showed dramatic diversity of sequence types in all clinical groups. Gene-by-gene analysis revealed that genes involved with metabolism, antibiotic resistance, and biofilm formation were significantly associated with pathogenic group. Isolates from all clinical groups could grow in serum.

Host pathogen profiling in whole blood with reference strains, including *S. epidermidis* (1457 and RP62A), *S. aureus* (SH1000) and *E. coli* (K12 and B strain) showed significantly lower IL-6 responses to Gram positive than Gram negative organisms, with concomitant higher phagocytosis of the former. Mechanisms underlying these observations using fully optimised flow cytometry reveal important cellular changes in monocyte and neutrophil populations.

These results identify biomarkers associated with host and pathogen and have implications for directed, personalised, precision medicine for the 21st Century.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P356

Structure-guided identification of a pathogen with pandemic potential without classical gain-of-function experiments

Jamie Kelly¹, Nurshariza Abdullah¹, Stephen C. Graham², Jamie Birch¹, Daniel Gonçalves-Carneiro¹, Vassiliy Bavro³, Tim Mitchell⁴, Robin Thompson⁵, Katrina Lythgoe⁵, Nicola Logan⁶, Margaret Hosie⁶, Brian Willett⁶, Michael P Heaton⁷, Dalan Bailey¹

¹The Pirbright Institute, Surrey, United Kingdom. ²University of Cambridge, Cambridge, United Kingdom.

³The University of Essex, Essex, United Kingdom. ⁴The University of Birmingham, Birmingham, United

Kingdom. ⁵University of Oxford, Oxford, United Kingdom. ⁶University of Glasgow, Glasgow, United

Kingdom. ⁷US Department of Agriculture, Nebraska, USA

Abstract

Morbilliviruses cause devastating diseases in farmed animals, humans and wildlife, across the globe. Although they infect a broad-range of mammals including small and large ruminants, household pets (dogs and cats) and indeed humans, very little is known about the factors that determine the specific host-range of each virus. Although morbilliviruses can be controlled by vaccination (and indeed eradicated, e.g. rinderpest virus), there are well-founded fears that if one morbillivirus is removed from an ecosystem, others may invade the "vacated niche", especially when vaccination ceases and herd immunity wanes.

All morbilliviruses infect their hosts via protein-protein interactions with one of two host receptors: CD150 or Nectin-4. To examine this interaction in greater detail we developed a number of viral haemagglutinin-based entry assays that allowed us to investigate host-range determinants without the need for "live-virus" experiments.

We identified that viral entry, particularly the use of non-cognate CD150/nectin-4, is a critical point of restriction for zoonotic transmission of specific morbilliviruses. Using structural analysis and routine molecular biology approaches we subsequently identified gain-of-function mutants, with apparently expanded host-ranges, which could correlate to pathogens with zoonotic potential.

Our techniques represent a bio-safe alternative to classical, and often controversial, gain-of-function experiments. We hope to use our data to better inform genetic surveillance of morbilliviruses in the field, across the 'One Health' spectrum.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P357

Fingering the cell: the role of a novel capsid protein zinc finger domain in Bluetongue Virus entry

Weining Wu, Han-Chen Chiu, Polly Roy

London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Bluetongue virus (BTV), a member of Orbivirus genus of the family *Reoviridae*, is an agriculturally and economically significant insect-borne virus that causes serious illness and death in sheep and other domestic and wild ruminants in many parts of the world. The cell entry mechanism of BTV, a large non-enveloped capsid virus, is a highly coordinated process requiring the two viral outer capsid proteins VP2 and VP5 to undergo multiple conformational change. In this study, we used our recent high-resolution structure of the complete virus particle to determine how VP2 and VP5 facilitate cell entry. A series of site-specific mutations were made in predicted key residues of VP2, the receptor-binding protein and VP5, the membrane-penetration protein by reverse genetics and their effects on virus uptake and replication were investigated. While the biological data were generally consistent with the functional predictions of the capsid structure, certain residues appeared to be critical for entry. Most importantly, we found that a novel zinc finger identified in VP2 plays an essential role in BTV entry and that a histidine cluster found in VP5 is responsible for sensing pH following virus entry leading to membrane penetration. These data provide novel insights into cell entry by a large, multi-layered non-enveloped virus.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P358

Host response to polymicrobial infection in lung epithelium

Yuan Ji, Albert Bolhuis, Malcolm Watson

University of Bath, Bath, United Kingdom

Abstract

Lower respiratory tract infection is a leading cause of morbidity and mortality. Airway epithelial cells recognise intruding pathogens through pattern recognition receptors such as Toll-like receptors, and subsequently secrete proinflammatory cytokines to activate an immune response. This study is aimed at investigating the host response to multiple microorganisms and the manipulation of this by pathogens.

Host response to microbial infection was assessed by incubating *B. cenocepacia* organisms with cultures of the human airway epithelial cells CALU-3 and 16-HBE. Epithelial barrier integrity, as assessed by transepithelial electrical resistance measurement, was compromised by co-incubation with bacteria. ELISA analysis showed that the transmissible strain of *B. cenocepacia* J2315 potently induced IL-8 production in airway epithelial cells, whereas this effect was substantially reduced by *S. aureus* MRSA252 and MSSA NCTC 6571 cell-free supernatants. The inhibitory factor(s) in *S. aureus* supernatant were characterised as heat-stable, nonproteinaceous, small (<3 kDa) molecules. Although IL-1 β and TNF α co-stimulation upregulated IL-8 production to the same level as J2315, this response could not be diminished by *S. aureus* supernatant. Co-treatment with TLR antagonists suggested that TLR4 but not TLR5 might be involved in the reduction of host response to *B. cenocepacia* by *S. aureus* supernatant. The molecular mechanism of this inhibition is currently under investigation.

These findings may reveal an immune evasion strategy of *S. aureus*, but may also be applied to alleviate the tissue damage resulting from excessive production of proinflammatory cytokines in chronic lung inflammation.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P359

Modulation of *Pseudomonas aeruginosa* pathogenicity in urinary tract infections by sex hormones

Hussain Ebrahim¹, Rachel Floyd², Jo Fothergill¹

¹Clinical Infection, Microbiology and Immunology, Institute of Infection & Global Health, University of Liverpool, Liverpool, United Kingdom. ²Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

Abstract

Background: Urinary tract infections (UTIs) cause high morbidity and mortality rates around the world. *Pseudomonas aeruginosa* causes persistent UTI infections because it forms biofilms and can be resistant to multiple antibiotics. Previous studies suggest that sex hormones such as estradiol may modulate biofilm formation and dysregulate innate immune responses in females. Hence, this study aims to understand the role of hormones in UTI pathophysiology.

Methods: To investigate the effect of estradiol, testosterone and progesterone on biofilm growth and architecture, 15 UTI *P. aeruginosa* isolates were cultured statically for 48 hours in the presence of either 10nM testosterone, estradiol or progesterone. Crystal violet (CV) staining was performed to assess biofilm biomass. In addition, confocal microscopy was carried-out by staining *P. aeruginosa* biofilms with the Baclight® kit. The resulting 3D images were processed using Imaris and Comstat. Expression of a panel of virulence genes was determined by qPCR following exposure to 10 nm estradiol and testosterone. Whole Genome sequencing of 9 isolates was performed and data were compared using Panseq.

Results: Assessment of data produced by both of the CV biofilm assay and confocal microscopy reveal hormone and *P. aeruginosa* isolate-dependent interactions. Whole genome sequencing showed diversity across the UTI isolates.

Conclusion: Our results suggest that sex hormones may modify gene expression, biofilm formation and architecture of *P. aeruginosa*. This may suggest that patients may have variable susceptibility to persistent infections based on their hormonal profile. Therefore, further research is warranted to investigate host-pathogen interactions and the role of sex hormones.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P360

Exploring the effects of oestrogen on *Candida albicans* and its subsequent impact on antifungal immunity

Pizga Kumwenda, Robin May, Rebecca Hall

Institute of Microbiology and infection School of Biosciences, University of Birmingham,, Birmingham, United Kingdom

Abstract

Candida albicans is a commensal of the vagina and is the predominate cause of vulvovaginal candidiasis (VVC). About 75% of otherwise healthy women suffer from VVC at least once in their life time. Recurrent infections occur in approximately 40 - 50% of women with nearly 5 – 8% suffering about four times annually. Topical and oral antimycotic agents are available to treat uncomplicated infections and control recurrent infections. In the vagina, *C. albicans* is exposed to physiologically changing concentrations of hormones to which the pathogen must adapt to in order to survive. Elevated oestrogen levels are associated with *C. albicans* colonisation of the vagina and symptomatic infection. Although it is known that oestrogen facilitates the proliferation and morphogenesis of *C. albicans*, we have less knowledge of the role this adaptation has on the host-pathogen interaction. Here we investigate how adaptation of *C. albicans* to physiologically relevant concentrations of oestrogen impacts on the antifungal innate immune response. Adaptation of *C. albicans* to physiological (10^{-10} M) and supraphysiological (10^{-5} M, 10^{-7} M) concentrations of oestrogen attenuated macrophage phagocytosis. This inhibition in phagocytosis rates did not correlate with changes in carbohydrate exposure. Elucidating the mechanism behind this process will help improve our understanding on how oestrogen facilitates progression of VVC. In addition, new insights into possible therapeutic interventions and better preventive methods of *C. albicans* infections such as VVC could be realised.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P361

Insights into Amoebic Gill Disease: Challenges in *Paramoeba* culture

David MacPhail

University of the West of Scotland, Paisley, United Kingdom. Fish Vet Group, Inverness, United Kingdom.
Benchmark Animal Health, Sheffield, United Kingdom

Abstract

Amoebic Gill Disease (AGD) is a major problem in the aquaculture industry, as it is responsible for substantial losses of farmed Atlantic salmon in various worldwide locations, including Scotland, Ireland, Norway, North America, and Australia. The disease is caused by *Paramoeba perurans* colonisation of the gill tissues, where this free-living and opportunistic parasite compromises the gills through the resulting development of hyperplastic lesions and lamellar fusion. These structural changes result in a reduction in the functional surface area of the gill tissues.

AGD research has many challenges, as the aetiological agent was only recently identified as *Paramoeba perurans*, and there are currently no known axenic cultures of the amoebic pathogen. Bacterial contamination of *Paramoeba* cultures is a challenge to all AGD researchers, and this contamination affects the ability to perform high-throughput screening of compounds, in effort to find potential treatment for the disease. The contamination also affects nucleic acid extractions, to identify potential inhibitory drug targets.

Herein, we describe the challenges and optimisation of *Paramoeba* cultures, in addition to identifying suitable antibiotics to control the bacterial contamination. Optimisation of the cultures will assist with molecular studies and high-throughput drug screening, as well as understanding the dynamics and importance of amoebae-bacteria relationships.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P362

Characterisation of new environmental phages targeting the *Escherichia coli* LamB outer membrane porin.

Ziyue Zeng, George Salmond

University of Cambridge, CAMBRIDGE, United Kingdom

Abstract

Bacteriophages are viral parasites of bacteria. A successful infection starts with the adsorption of the bacteriophage to a specific receptor on the host cell surface. Most bacteriophages are thought to have a narrow host range but this can be extended in certain cases. One strategy for extending host range is to first express a known functional phage receptor protein in bacteria previously non-susceptible to the bacteriophage, thereby enabling adsorption and potential infection by viruses that target the specific receptor. To investigate the feasibility of this approach, a plasmid (pMUT13) encoding the *Escherichia coli* LamB porin, the receptor for bacteriophage lambda, was transferred into three different enterobacterial genera, namely *Citrobacter*, *Yersinia*, and *Serratia*. Over 100 environmental bacteriophages were isolated that infected these pMUT13-containing strains, and some phages were shown to infect their respective hosts in a LamB-dependent way. The host ranges of the environmental bacteriophages were cross-tested across the heterologous genera and surface adsorption kinetics investigated. Unlike bacteriophage lambda, which is a member of the *Siphoviridae*, these newly-isolated LamB-dependent phages were more commonly members of the *Myoviridae*, based on transmission electron microscopy. The phages were characterised in terms of bacterial host promiscuity and genome sequences, and their capacity for horizontal transfer of host genes investigated.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

363

Elucidation and modulation of innate immune responses during mucormycete infections

Herbert Itabangi¹, Ignacio Ignacio Insua², Bradley Pollard¹, Mark Probert¹, Zoe Reading¹, Xin Zhou¹, Joao Correia¹, Paula Seoane¹, Gordon Brown³, Carol Munro³, Jason King⁴, Elizabeth Ballou¹, Francisco Fernandez Trillo², Kerstin Voelz¹

¹Institute of microbiology, school of Biosciences, University of Birmingham, Birmingham, United Kingdom. ²School of chemistry, University of Birmingham, Birmingham, United Kingdom. ³Aberdeen Fungal Group, MRC Centre for Medical Mycology, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom. ⁴Department of Biomedical Science, the University of Sheffield, Sheffield, United Kingdom

Abstract

Mucormycosis is a life-threatening mold infection with overall mortality rates of 50%, yet fatality reaches 100% in patients with disseminated disease, prolonged neutropenia, or brain involvement. The disease is caused by a group of filamentous fungi, the mucormycetes, and particularly targets patients with immunosuppression due to diabetic ketoacidosis (DKA), iron overload, severe trauma, neutropenia, corticosteroid treatment, or organ transplantation. These predisposing conditions are linked to defects in key aspects of the innate immunity, particularly defects in phagocytic effector functions by macrophages and neutrophils, suggesting phagocyte activity is crucial to disease control. Yet, we currently have a very limited understanding of the interaction between mucormycete spores and phagocytes. Recently, we showed that the early events during phagocyte-spore interaction determine disease outcome. Here, we report that, during the early stages of infection, mucormycetes spores modulate macrophage functions including phagocytic uptake, phagosome acidification and cytoskeletal organisation via a secreted factor. We investigate the influence of mucormycete host-cell interaction through a series of infection biology and chemistry techniques that reveal new insights into the molecular mechanisms underlying fungal pathogenesis.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P364

Evaluation of *Galleria mellonella* as an *in vivo* model for identifying virulent strains of the food-related yeast *Saccharomyces cerevisiae*

Rosa de Llanos¹, Allen Flockhart¹, Anindita Nandi¹, Amparo Querol²

¹Edinburgh Napier University, Edinburgh, United Kingdom. ²Instituto de Agroquímica y Tecnología de los Alimentos, Valencia, Spain

Abstract

The history of yeast association with human society is synonymous and synchronous with the evolution of bread, beer and wine as global food and beverage commodities. However, the incidence of infections by food yeast species, such as *S. cerevisiae* has increased in recent decades due to the growing proportion of immunocompromised patients among human population. *S. cerevisiae* have recently been added to the list of opportunistic pathogens according to European Food Safety Authority information, as emerging opportunistic pathogen of low virulence.

The aim of this project was to investigate whether the invertebrate host model *G. mellonella* is an adequate *in vivo* model to evaluate food-yeast pathogenicity. We investigated the virulence of clinical and food strains of *S. cerevisiae*, which were previously tested for their potential pathogenicity in murine models. Different infection conditions varying in yeast inoculum (10^6 - 10^7 - 10^8 cells/larva), and temperature (30-37°C) were tested in 72-hour triplicate assays. A range of end-points including, melanisation, fungal burden, larval death, and alteration of haemocyte density were determined.

Our results showed inter-strain differences in the virulence towards *G. mellonella* larvae, with a dose-dependent pattern effect on the killing and health status of the insect. All strains were able to kill *G. mellonella* larvae at both 30°C and 37°C. We observed a good correlation among strains regarded as virulent in murine models resulted in low survival, increased fungal burdens and low haemocyte density. We conclude that *G. mellonella* can be an alternative model to study food-yeast pathogenicity.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P365

Use of reverse genetics technique to study the early pathogenesis of Peste des petits ruminants virus

Muni Selvaraj¹, Mana Mahapatra¹, Pippa Hawes¹, Richard Kock², Satya Parida¹

¹The Pirbright Institute, Woking, United Kingdom. ²The Royal Veterinary College, London, United Kingdom

Abstract

Across the developing world, Peste-des-petits ruminants virus (PPRV), places a huge disease burden on agriculture, in particular affecting small ruminant production and in turn increasing poverty in many developing countries. The current understanding of PPRV pathogenesis has been mainly derived from the closely related rinderpest virus (RPV). There are few studies that have focused on the late stages of pathogenesis of PPRV in the field and very little is known about the processes underlying the early stages of pathogenesis. It is believed that PPRV replicates mainly in the respiratory epithelium before disseminating throughout the host. We **hypothesize** that PPRV infects immune cells of the respiratory mucosa, but not respiratory epithelial cells and then migrates to the tonsil and local lymphoid organs for primary replication, after which virus enters the general circulation and secondary replication occurs in the epithelium of respiratory and gastro-intestinal tracts. The application of reverse genetics techniques provides a tool to gain a better understanding of the molecular factors underlying virus host range and pathogenesis. Recently we have established reverse genetics system for PPRV and using this we have engineered a GFP tagged PPR virus (rMorrocco 2008 GFP). Further, in the biosafety containment, we have infected targeted animals (goats) with this GFP tagged virus and following this virus in the body of infected goats in 6 hourly interval we could demonstrate that the virus primarily replicates inside the pharyngeal and palatine tonsils and then causes viremia and secondary replication.

Models for Understanding Host-Pathogen Interactions

Zone D

Presentations: Tuesday and Wednesday Evening

P366

THE FUTURE BURDEN OF BLADDER CANCER IN GHANA DUE TO SCHISTOSOMA HAEMATOBIIUM INFECTION.

Ama Afrah, Prof. Richard K. Gyasi

University of Ghana, Accra, Ghana

Abstract

Bladder cancer is the seventh most devastating cancers among men whilst it ranks seventeenth among all women's cancers globally with over 300,000 new cases reported each year. In the developed countries, bladder cancer is attributed to smoking, among other risk factors whilst in developing countries it is mostly due to microbiologic infections. The incidence and mortality rates of bladder cancers among people living with *Schistosoma haematobium* (*S.h.*) infection is high.

Schistosomiasis is the second devastating parasitic disease in terms of socio-economic and public health impact. Schistosomiasis is a neglected tropical disease affecting poor communities lacking basic infrastructures. Children are particularly susceptible to the infection and are unable to develop their full potential. Globally, infection rate had been successfully reduced but an estimated number of over 240 million people in Africa are infected with *S.h* with 800 million people at risk of infection. The introduction of control programs had resulted in decline in bladder cancer in people that had the schistosoma ova. Control programs in some countries using health education, treatment of infected persons, improved sanitation had seen some success but there are some setbacks especially in developing countries. In China and Japan however combination of strategies (one-health) that took into consideration host parasite-interaction had resulted in complete and sustained elimination of the parasites.

A recent survey by Departments of microbiology and Pathology, University of Ghana had revealed pre-cancer stages of urinary bladder among school going children aged 8-14%.

Emerging Model Systems
Zone D
Presentations: Tuesday and Wednesday Evening

P367

Development of *Crithidia fasciculata* as a system for cell surface antigen expression

Tom Miller, Catarina Gadelha

University of Nottingham, Nottingham, United Kingdom

Abstract

Kinetoplastid protozoa include organisms of huge medical relevance, with some species causing serious human diseases (e.g. Leishmaniasis, African trypanosomiasis and Chagas' disease). These systems are often expensive to grow in vitro, require animal models, and can't be cultured in large volumes because of intrinsic quorum sensing mechanism. But some non-human infective kinetoplastid relatives can be grown easily and cheaply, and have extensive shared biology, which includes the processing of molecules that localise to the cell surface membrane. In addition to being useful model organisms to study kinetoplastids in general, these could potentially be good systems for transgenic technologies such as the recombinant expression of parasite surface antigens for the development of novel treatments and diagnostic tools. We have chosen *Crithidia fasciculata* as a monogenic, insect only kinetoplastid parasite which can be cultured without serum, reach high cell density, has short doubling time (<5h), and its genome has been fully sequenced and annotated. We have developed a *C. fasciculata* single marker system that expresses T7 polymerase and the tetracycline repressor (TetR) protein. This enables tetracycline-regulated expression of recombinant transgenes at high levels. We will present progress on using this cell line for the recombinant expression of *Trypanosoma brucei* surface antigens, and the development of transgenics for *Crithidia*. This could be a useful system for recombinant expression of eukaryotic proteins, but also has potential application in functional genomics and novel model system biology.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P368

Metagenomic Insight into the diversity of Indigenous Microbial Community of Potable Spring Water of Sikkim

Ashish Singh, Saurav Das, HK Tiwari

Sikkim University, Gangtok, India

Abstract

Water quality of a region directly corroborates with the health index of people. People in the Himalayan hills mainly depend upon the spring water for potability. To determine the microbial ecology of the spring waters of Sikkim, the variable region of 16S rRNA has been sequenced using illumina MiSeq. Metagenomic study of the springs from four districts of Sikkim; East, West, South and North revealed, bacteria is the dominant domain having 97.68%, 95.05%, 98.56% and 95.04% of abundance respectively. Phylum wise annotation showed the east and north district are mostly dominated by bacteroidetes (18.43%, 30.15%), whereas West and South district is dominated by Planctomycetes (23.45%) and verrucomicrobia (26.19%). The diversity of proteobacteria and firmicutes was consistent in east, west and south district with an average abundance of 10-15%. An exception was found in north district where proteobacterial (15.03%) diversity was higher than the firmicutes (1.5%). Genus wise distribution showed the abundance of *plactomyces*, *flavobacterium*, *cytophaga*, *prothecobacter*, *acinetobacter*, *clostridium*, *azosprilum* in the springs of east, west and south with an exception of *geobacillus* which was only found in the springs of the south. North on the antonymous showed totally different sets of microbial diversity. North district showed abundance of *isophaera*, *flectobacillus*, *pelomonas*, *chitinophaga*, *ochromonas*, *candidatus* along with *flavobacterium*, *cytophaga*, *prothecobacter* and *planctomyces*. This is a first report from the state and it largely enhances our knowledge about the microbial structure of potable spring water of Sikkim. Based on the report different strategic treatment procedures can be opted to improve water quality.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P369

Diversity and Distribution of Thermophilic Bacteria in Hot Springs of Pakistan Arshia Amin, Iftikhar Ahmed & Wen-Jun Li

Arshia Amin¹, IFTIKHAR AHMED², Wen Jun Li³

¹Pakistan Biosafety Association, Islamabad, Pakistan. ²National Culture Collection of Pakistan (NCCP), Bioresource Conservation Institute (BCI), PGRI Building, National Agricultural Research Centre (NARC), Islamabad, Pakistan. ³Sen Yat Sen University Guangzhou China, Guangzhou, China

Abstract

Chilas and Hunza areas, located in the Main Mantle Thrust and Main Karakoram Thrust of the Himalayas, host a range of geochemically diverse hot springs. This Himalayan geothermal region encompassed hot springs ranging in temperature from 60 to 95 °C, in pH from 6.2 to 9.4, and in mineralogy from bicarbonates (Tato Field), sulfates (TattaPani) to mixed type (Murtazaabad). Microbial community structures in these geothermal springs remained largely unexplored to date. In this study, we report a comprehensive, culture-independent survey of microbial communities in nine samples from these geothermal fields by employing a barcoded pyrosequencing technique. The bacterial phyla Proteobacteria and Chloroflexi were dominant in all samples from Tato Field, Tatta Pani, and Murtazaabad. The community structures however depended on temperature, pH, and physicochemical parameters of the geothermal sites. The Murtazaabad hot springs with relatively higher temperature (90–95 °C) favored the growth of phylum Thermotogae, whereas the Tatta Pani thermal spring site TP-H3-b (60 °C) favored the phylum Proteobacteria. At sites with low silica and high temperature, OTUs belonging to phylum Chloroflexi were dominant. Deep water areas of the Murtazaabad hot springs favored the sulfur-reducing bacteria. About 40% of the total OTUs obtained from these samples were unclassified or uncharacterized, suggesting the presence of many undiscovered and unexplored microbiota. This study has provided novel insights into the nature of ecological interactions among important taxa in these communities, which in turn will help in determining future study courses in these sites.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P370

Microbe-mucopolysaccharide interaction fuels the sediment microbial nitrogen cycle

Dale Harriet^{1,2}, Joe Taylor³, Martin Solan², Phyllis Lam², Michael Cunliffe^{1,4}

¹Marine Biological Association, Plymouth, United Kingdom. ²University of Southampton, Southampton, United Kingdom. ³University of York, York, United Kingdom. ⁴University of Plymouth, Plymouth, United Kingdom

Abstract

Sediment nitrogen cycling is a network of microbially mediated processes that plays a key role in regulating ecosystem productivity. Many sediment invertebrates secrete protein/polysaccharide-rich mucopolysaccharides that may be an important source of organic carbon and nitrogen to sediment microorganisms. At present, we have a limited understanding of how mucopolysaccharides impact total sediment microbial communities or the specific microbial groups that drive nitrogen cycling. To address this, sediment was incubated with and without *Hediste diversicolor*-derived mucopolysaccharide. Changes in the concentration of dissolved inorganic nitrogen, and in the microbial community structure, were assessed. The addition of mucopolysaccharide supported a more abundant bacterial community with a distinct community structure. We also observed an increase in the abundance of bacterial and archaeal ammonia oxidisers in the presence of mucus, and a concomitant increase in nitrite and nitrate concentrations. *H. diversicolor* mucopolysaccharide appears to enhance sediment nitrification rates by stimulating nitrifying microbial groups. We propose that invertebrate mucopolysaccharide secretion should be considered a specific functional trait when assessing the contributions that specific invertebrates make to microbial sediment ecosystem function. To further expand on this research, we have now begun to examine the contribution that the *H. diversicolor* microbiome can make to sediment microbial communities, as well as the potential impact of gut passage. The inclusion of these generally understudied invertebrate traits will improve our mechanistic understanding of invertebrate-microbe interactions to achieve more accurate models and predictions of the global nitrogen cycle.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P371

Investigating correlations between endophytic bacteria halotolerance and salt stress mitigation in their host plants

Gareth Raynes, Kerrie Farrar

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Salinity stress caused by high levels of salt (>40mM NaCl) in soils limits crop growth, thereby lowering yields and reducing land use efficiency. It is estimated that 20% of irrigated agricultural land globally is contaminated by high salinity levels, with that number steadily increasing annually. A growing global population combined with diminishing land availability has the potential to cause severe food shortages unless action is taken to alleviate the situation.

Endophytic bacteria inhabit plant tissues without causing disease or impacting negatively upon the host plant. Certain endophytic bacteria have been shown to ameliorate the effects of salinity stress in the host plant, enabling normal or near-normal growth under salt stress conditions. Using a combination of *in vitro* and *in planta* techniques, this study aims to investigate whether halotolerant endophytic bacteria are able to confer salt tolerance on a host plant. Bacteria selectively isolated from plants living in high salinity coastal environments were evaluated for growth on salt supplemented agar.

Brachypodium distachyon inoculated with these strains were subjected to 0mM, 100mM and 200mM NaCl treatments in the national plant phenomics centre (NPPC) at Aberystwyth. Plant growth was monitored over X weeks to evaluate the effects of the endophytes on the host plant salinity tolerance. Outcomes of this study will include a greater understanding of the roles of bacterial endophytes on plant salinity tolerance, and potential mitigation strategies for crop plants utilising inoculations of salt tolerant endophytic bacteria.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P372

Developing a model system and experimental 'toolkit' to investigate the biology and ecology of marine chytrid-diatom interactions

Davis Laundon^{1,2}, Glen Wheeler², Thomas Mock¹, Michael Cunliffe^{2,3}

¹University of East Anglia, Norwich, United Kingdom. ²The Marine Biological Association of the UK, Plymouth, United Kingdom. ³Plymouth University, Plymouth, United Kingdom

Abstract

Chytrids are zoosporic fungi that have been reported as saprotrophs and microalgal parasites in fresh water and marine ecosystems. Spatiotemporal metabarcoding surveys of marine fungi have revealed a massive diversity of as yet uncharacterised chytrid taxa, some of which are correlated in abundance with diatoms. Chytrid parasites therefore have the potential to greatly impact biogeochemical cycling in marine systems. At present, there is a limited understanding about the fundamental physiological and biological basis of chytrid-diatom interactions – including infection processes and host response. We aim to bridge this knowledge gap by establishing a morphologically and genetically characterised chytrid 'BioBank' of chytrid-diatom parasites. From the 'BioBank', novel model parasite-host systems for cell physiology will be developed. In tandem with environmental metabarcoding of fungi and cultural isolation of phytobenthic diatom communities, we are developing a 'toolkit' for investigating aquatic chytrid biology, including quantitative live-cell imaging, fluorescent labelling of subcellular structures, cryopreservation and experimental culturing techniques. Using these tools, we have been able to map and quantify the life cycle of the model saprotrophic chytrid *Rhizoclostridium globosum* JEL800, optimise protocols for lipid, chitin, and plasma membrane visualisation and have developed an optimised protocol for the cryopreservation of zoospores. The results of these studies will form an invaluable 'toolkit' in the investigation of parasitic marine chytrids and allow us for the first time to explore the biology of these enigmatic and ecologically-important fungi.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P373

Biodegradation of *o*-xylene by newly isolated consortium from Iraqi wastewater and its potential use in bioremediation.

Thana Noor

Teesside University, Middlesbrough, United Kingdom

Abstract

Abstract: the wastewater contaminants with hydrocarbons hazardous, like *o*-xylene, considered as one the major health risk issue. Therefore, the modern applications of microorganisms with the aid of molecular techniques establish an accurate and alternate method to clean-up hazardous hydrocarbons in contaminated environments.

Objectives: the objectives of the current study were to isolate the wastewater-inhabiting indigenous hydrocarbon-degrading bacteria from Rustumihia WWTP /Iraq and in vitro and to evaluate the efficiency of the *o*-xylene biodegradability by selecting the most efficient microbial isolates.

Materials and Methods: bacteria were isolated from four different treatment sites (combining phase, activated sludge, settlement tanks and river discharge plant based in Baghdad using (MSB) agar. All the isolates were categorised based on their 16S rRNA gene sequencing applying the molecular analysis. The catabolic gene detected via PCR and primer set of xylE sequence. The gas chromatography-flame ionisation detector (GC-FID) estimated in liquid culture.

Results: 45 different indigenous bacterial strains isolated and recognised as tolerating *o*-xylene; 15 stains categorised as the best *o*-xylene degrading bacteria. According to the 16S rRNA gene sequence analyses, 15 strains classified as new for *o*-xylene degradation processes. Catabolic gene xylE detected. The ecological indices identified based on diversity and richness results. The GC-FID results revealed the ability of mixed consortium to decrease *o*-xylene concentration in (MSB) liquid culture by 43%. Following results highlighted the adaptability of microbes to the high concentration of *o*-xylene under stressful growth conditions.

Conclusions: this study viewed as an important step towards the development of a bioremediation strategy in contaminated area.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P374

Bacterial community structure and diversity in different paddy soil compartments

Xiaoyu Wang, Jing Wang, Ke Ma

China Agricultural University, Beijing, China

Abstract

Different soil compartments in paddy soil showed obvious differences in soil physicochemical properties. However, the microbial differences among paddy soil compartments remains unknown. In the present study, we cultivated the same rice plant in 20 different paddy soils which were collected from 20 cities in China. After 60 days cultivation, the surface soil, bulk soil (non-rooted affected) and rhizosphere soil (rooted affected) were sampled. The bacterial community diversity and structure were analyzed at both DNA and RNA level by high-throughput sequencing. Chao1 index was significantly higher in bulk soil and rhizosphere soil than that in surface soil. There were no significant differences in Simpson and Shannon index among three soil compartments. The results of principal coordinate analysis (PCoA) showed that bacterial community structure was distinct from each other among three soil compartments. The cluster of surface soil significantly separated from those of bulk soil and rhizosphere soil. In the phylum level, the relative abundances (RAs) of *α-Proteobacteria*, *Cyanobacteria* and *Verrucomicrobia* were significantly higher and the RAs of *Firmicutes* and *Chlorobi* were significantly lower in surface soil than those in bulk soil and rhizosphere soil, separately. Many differences were found in the genus level among three soil compartments. For example, the RA of *Geobacter* was higher in rhizosphere soil than surface soil and bulk soil. The highest RAs of *Methylosinus* and *Methylosarcina* were detected in surface soil, whereas the highest RAs of *Methylococcus*, *Methylomonas* and *Methylomicrobium* were detected in rhizosphere soil. Additionally, we found some differences between DNA and RNA levels.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P375

First Insights into the Microbial Diversity of the RSPB Marshside Saltmarsh

Priyanka More¹, Terry McGenity², André Antunes¹

¹Biology Department, Edge Hill University, Ormskirk, United Kingdom. ²School of Biological Sciences, University of Essex, Colchester, United Kingdom

Abstract

Saltmarshes are threatened coastal intertidal ecosystems that provide a wide range of benefits: supporting vegetative and infaunal diversity, avoiding coastal erosion by protecting against waves and sea level rise, reducing global warming, recycling of elements, bio-remediating, and providing recreational value [1]. They are seen as important hotspots of biodiversity with extensive information available on their fauna and flora, yet their microbial communities remain poorly characterized. Furthermore, few studies seemed to have focused on cultivation-based approaches, resulting in a limited number of microbial isolates originating from such environments.

Our study provides the first microbial data on the saltmarsh ecosystem at RSPB Marshside, a nature reserve on the Ribble estuary near Southport (England). We have analysed water samples obtained across the wide span of mudflat and low marsh, with collection of physico-chemical data (temperature, pH, salinity, and concentration of selected ions), followed by a culture-dependent survey of their microbial diversity and their preliminary characterisation. We have successfully isolated over 150 microbial strains spread across different taxa, which include a few likely new species and a couple of groups that have not been previously reported in saltmarshes (e.g. *Anaerobacillus*). Also, we have isolated representatives of genera known to be relevant in biotechnology (e.g. *Halomonas*) or involved in plant growth promotion (e.g. *Planococcus*). Our efforts showcase the substantial untapped biodiversity of this site, as well as their potential future application.

[1] Shepard et al. (2011). *PloS one*, 6(11), e27374.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P376

Identification and Characterization of Bacterial Strains Associated with Oak Symptomatic Trees in Northern Iran

Meysam Bakhshi Genjeh¹, Masoud Shams-Bakhsh¹, Heshmat Rahimian²

¹Tarbiat Modares University, Department of Plant Pathology, Tehran, Iran, Islamic Republic of. ²Sari Agricultural Science and Natural Resources University, Department of Plant Pathology, Sari, Iran, Islamic Republic of

Abstract

Oak decline is complex disease characterized by periodic occurrences of decline and death of oaks over widespread areas. The possible cause of this syndrome could be the combination of several abiotic and biotic factors such as climate changes and various plant pests and pathogens including bacteria, fungi/oomycetes and insects. Oak trees compose a big portion of Iranian forests and oak decline is the major threat to the forest conservation. However, there are limited studies on the aetiology and possible role of bacteria in the disease syndrome worldwide. In order to characterize causative agents, we sampled oak trees with stem bleeding and canker symptoms in Mazandaran and Golestan provinces of Northern Iran. Bacterial isolates were characterized by biochemical and physiological tests, protein electrophoresis, DNA fingerprinting (rep-PCR, Eric and Box primers), sequencing of 16S rRNA and MLSA (Multilocus sequencing analysis) for housekeeping genes (*gyrB*, *infB*, *atpD*). Association of a complex community of the genus *Brenneria* spp. (*B. goodwinii*, *B. roseae*, *Brenneria* sp., *B. nigrifluens*) and *Gibbsiella* spp. with symptomatic trees were confirmed. Due to the presence of several potentially pathogenic associated agent(s) with the oak decline, identification of the principal agent(s) is of major concern. The further of the present research is to the comparison of the taxonomic and genomic criteria of the bacterial strains of healthy and symptomatic oaks in order to identify the most effective microbial communities responsible for the oak decline and their possible interactions in the development of the syndrome.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P377

Arctic microbes – The phylogenetic and functional diversity of prokaryotes at Colour Peak

Michael Christopher Macey¹, Mark Fox-Powell², Claire Rachel Cousins², Jennifer Pratscher³, Eric Baptiste⁴, Stephen Summers^{5,6}, Susanne P. Schwenzer¹, Victoria Pearson¹, Karen Olsson-Francis¹

¹The Open University, Milton Keynes, United Kingdom. ²University of St Andrews, St Andrews, United Kingdom. ³Heriot-Watt university, Edinburgh, United Kingdom. ⁴Institut de Biologie Paris Seine, Paris, France. ⁵Nanyang Technological University, Nanyang, Singapore. ⁶Singapore Centre of Environmental Life Sciences Engineering, Nanyang, Singapore

Abstract

Colour Peak is a region of continuous permafrost in the Canadian arctic known for its perennial cold springs (the temperature does not rise above 7 °C (Perreault et al., 2007)). The springs are highly saline, sulfur-rich and anoxic. These springs are therefore considered analogous to the sub-surface oceans of the icy moons Enceladus and Europa, and aqueous environments on Mars. Therefore, the phylogenetic and functional diversity of prokaryotes which persist in this environment is important for understanding the survivability and metabolism of potential life within these extra-terrestrial environments.

Cultivation-dependant and cultivation-independent techniques were applied in order to assess the diversity of prokaryotes present in sediment collected from a hyper-saline cold pool at Colour Peak. The microbial community at the site was characterised through the Illumina sequencing of 16S rRNA gene amplicons and with a series of metagenomes from DNA extracted from the sediments. The metagenomes were mined and the sequences binned into genomes in order to fully assess the diversity of the phylogenetic groups and metabolic pathways present. Combined with the isolation and characterisation of several aerobic and anaerobic isolates from Colour Peak, this work characterises the microbial community of this analogue site and furthers our understanding of survival under this range of selection pressures.

Perreault, N.N., Anderson, D.T., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the prokaryotic diversity in cold saline perennial springs in the Canadian high Arctic. *Appl Environ Microbiol* **73**: 1532–1543.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P378

Microbial traits in chlorinated Drinking Water Distribution Systems

Carolina Calero, Joseph B. Boxall, Víctor Soria-Carrasco, Isabel Douterelo

University of Sheffield, Sheffield, United Kingdom

Abstract

Background

Despite the presence of disinfectant residual, Drinking Water Distribution Systems (DWDS) are diverse microbial ecosystems. The majority of microorganism in DWDS live attached to internal pipe surfaces forming biofilms. Biofilms can affect the quality of drinking water if they are mobilised to the bulk water. The objective of this research was to study the structural and functional characteristics of biofilm and planktonic communities to better understand the microbial ecology of DWDS.

Methods

Biofilm and water samples were monitored in a real DWDS. Key water physico-chemical parameters were analysed and biofilm samples were obtained from coupons that were inserted along the pipes and left to develop over one year. DNA was extracted using a modified phenol/isoamyl alcohol protocol and then sequenced using an Illumina high-throughput. Sequencing reads were analysed using MG-RAST Metagenomics Analysis Server.

Results

Differences in the microbial community between biofilms and planktonic samples were observed. Bacteria were the most abundant microorganisms in both types of samples. However, there was a higher relative abundance of Eukaryotes, Archaea and Viruses in bulk water samples than in biofilms. Functional analyses revealed that most genes were related to metabolism, cellular processes, signalling and information storage and processing. Differences in functional traits between habitats were evident for genes related to biofilm development and mechanisms of antimicrobial resistance.

Conclusion

This research has significantly improved our understanding of microbial communities in DWDS, which will allow the design of effective management strategies aimed at minimizing the risks associated with microbial presence and activity.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P379

Characterising surfactant-expressing bacteria isolated from hydrocarbon-contaminated soils.

Raji Bamanga, Yusuf Deeni, Andrew Spiers, Scott Cameron

Abertay University, Dundee, United Kingdom

Abstract

We are investigating characteristics associated with oil degradation amongst bacteria isolated from clean and hydrocarbon contaminated soils from Nigeria and the UK. Our current focus has been to identify bacteria expressing surface-active agents or surfactants following isolation on *Pseudomonas* selective (PSA-CFC) and non-selective nutrient media. Of four sites sampled, a total of 1209 colonies were tested using the drop collapse assay, and 110 were found to express surfactants reducing aqueous liquid surface tensions as assessed by quantitative tensiometry to between 24.7 mN.m^{-1} and 26.7 mN.m^{-1} . From these, we chose six strains showing significant surface tension reducing abilities and six strains expressing no surfactants under the conditions tested here for further analysis from each site. We undertook a range of growth and behaviour-based assays which, when investigated by Hierarchical cluster analysis (HCA) demonstrated that this collection of 48 strains showed considerable phenotypic diversity, however, there was no indication that the strains could be differentiated by site, with HCA grouping the strains into five clusters of 6 – 15 strains from 2 – 4 sites per cluster. Our current work is focussed on identifying these strains by limited 16S sequencing, and screening them for outstanding hydrocarbon-degrading capacity, and heavy metal resistances (often associated with hydrocarbon contamination). We intend then to select several strains and obtain draft genome sequences, which will allow us further to investigate the ability of these strains to colonise soils subjected to long-term hydrocarbon contamination and to bioremediate.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P380

Sieving Soil, Destroying Diversity?: The Change in Fungal Community Within Crop Protection Product Degradation Studies

Pasky Miranda¹, James Moir¹, Irene Bramke², Robin Oliver², Thorunn Helgason¹

¹University of York, York, United Kingdom. ²Syngenta, Bracknell, United Kingdom

Abstract

Efficacy of Crop Protection Products (CPP) is determined by their availability in the soil, which is mediated in part by microbial degradation. Fungi are hypothesised to be important in the breakdown of CPPs yet their role is poorly understood. Guidelines laid down by the Organisation for Economic Co-operation & Development (OECD) require soil used for tests to be sieved and moisture adjusted before use. In addition soil samples are regularly stored for periods of up to 12 weeks before use in trials due to sampling regimes. Studies at Syngenta have found a significant link between these preparatory techniques and the rate of degradation of CPPs.

To investigate the impact of soil processing on the fungal communities cores were taken during the spring, summer and autumn seasons from agricultural and pasture sites. To test the effect of soil processing on fungal diversity, DNA was extracted from the cores before they were processed in accordance with OECD guidelines and put into a closed bottle CPP degradation test (test OECD307) environment for a month before a second DNA soil extraction. Fungal community structure and composition were determined via fungal ITS barcoding. We will present data comparing unprocessed and processed communities, demonstrating the full impact which processing, seasonality, and soil location has upon the native fungal community, in addition to the results of a second experiment examining the nutrient drivers of the community shift.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P381

A Metagenomic analysis of bacterial diversity in varying fallow ages of shifting agriculture prevalent in North-Eastern India.

Aishiki Banerjee, Santa Ram Joshi

North-Eastern Hill University, Shillong, India

Abstract

The fallow phase in shifting cultivation cycle allows the soil to regenerate its vitality and restore stability. Predominantly known as *jhum* in the tribal belts of North-East India, the increase in population pressure has resulted in the decrease in land availability and hence, decreases in the fallow ages under the shifting agriculture. The length of the fallow period provides valuable information about how the effects of slashing and burning followed by cropping involved in this unique agriculture practices have an impact on the belowground microbial population. A metagenomic approach was targeted to analyze the changes in bacterial population that varied along the fallow phases of 3 to 15 years from a total of 9 different sites across North-East India. The Illumina MiSeq platform was used to generate the metagenomic data of the DNA extracted from the soil samples. MG-RAST and UPARSE pipelines were used to analyze the paired-end reads obtained and study the diversity indices related to the fallow fields. Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli, Clostridia, and Verrucomicrobia were predominant in all the sites. Pairwise comparisons of α -diversity revealed significant variations in bacterial diversity among different-aged fallows. The metagenomic variance observed among the fallow fields provided basic information that can be used to answer specific questions on the length of the fallow phases and soil health existing in this type of agricultural practice.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P382

Harry Smith Vacation Studentship Summer 2017 Research. How can the revival of dormant Actinobacteria unlock their potential?

Eleanor Furness, Dr. Arwyn Edwards, Aliyah Debbonaire

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Actinobacteria are abundant, prolific producers of natural products including antibiotics. The Resuscitation-Promoting Factor (Rpf) proteins are a cross-reactive family of proteins found in actinobacteria which revive dormant vegetative cells, enhancing cultivation. Culturability is fundamentally linked to natural product retrieval, therefore the use of Rpf in antibiotic discovery is a valid endeavour. Here, both naturally secreted Rpf from *Micrococcus luteus* and recombinant Rpf from an *Escherichia coli* expression system were used in amendments of soil collected from the Aberystwyth University campus. Incubations of active Rpf vs. inactivated Rpf and recombinant Rpf vs. an empty vector control were set up at 4, 5 and 6 hour periods. Dilution and plating using triplicates of each condition followed. The results were both visibly apparent and statistically significant. Plate counts from recombinant Rpf amendments were highly significant (2-way ANOVA $f=15.556$, $p=0.002$) with a 56.9 % increase in counts compared to the controls. The number of morphotypes present on the amended plates were also highly significant compared to non-amended; for naturally secreted Rpf amended a 56.8 % increase in morphotype number (2-way ANOVA $f=10.756$, $p=0.007$) compared to controls was observed and for recombinant Rpf amended, a 23.8 % increase (2-way ANOVA $f=11.000$, $p=0.006$) compared to controls. This increase in both cultivation rate and diversity confirm that Rpf should be considered an invaluable tool for improving antibiotic discovery. This is especially true in the amendment of soils from extreme environments where successful culture of previously uncultured actinobacteria could expose much needed novel bioactive metabolites.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P383

Grassland microbial communities respond to soil drainage and phosphorus availability

Jessica Graca^{1,2}, Giulia Bondi², Rachel Creamer³, Fiona Crispie⁴, Raul Cabrera-Rubio⁴, Paul Cotter^{4,5}, Karen Daly², Achim Schmalenberger¹

¹University of Limerick, Limerick, Ireland. ²Teagasc, Wexford, Ireland. ³University of Wageningen, Wageningen, Netherlands. ⁴Teagasc, Moorepark, Fermoy, Ireland. ⁵APC Microbiome Institute, Cork, Ireland

Abstract

Soil microbes play a central role in the biological phosphorus (P) cycling where they act as sink and source of P. However, microbial responses to changes in grassland P status remain largely elusive. We hypothesised that intensive management of grasslands with a high P index will sustain distinctive belowground microbial communities when compared to low P index grasslands. In addition, we compared the effect of available P alongside soil drainage as a microbial community determining factor. Twenty grassland soils were selected, based on drainage class (well or poorly drained) and available P content (high and low P, determined via Morgan's), resulting in four soil groups (n=5).

Bacterial communities were clearly affected by both, soil drainage and available P. This was evident from the denaturing gradient gel electrophoresis DGGE fingerprints as well as from the sequence based alpha- and beta-diversity (next generation sequencing, NGS). Relatively higher proportions of Acidobacteria were detected in poorly drained soils and Firmicutes were more abundant in well drained and low P soils ($p < 0.05$). Distinct DGGE profiles from fungi and AM fungi were found in poorly drained soils with low P status. Sequencing revealed that Ascomycota and Zygomycota were less abundant in low P soils while Basidiomycota were more abundant (NGS). Fungal alpha-diversity was less affected by drainage and P than the bacterial. Beta-diversities from this study suggest that bacterial and fungal communities were affected by P and drainage status with environmental factor soil pH being more influential on the bacterial diversity.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P384

The influence of temperature on subsurface bacterial community structure in the South Wales coalfield

Sara M E Rassner¹, Andre Soares¹, Arwyn Edwards¹, Gareth Farr², Andrew C Mitchell¹

¹Aberystwyth University, Aberystwyth, United Kingdom. ²British Geological Survey (Wales), Cardiff, United Kingdom

Abstract

The South Wales coalfield mine waters exhibit a large temperature range (10-21°C, average 13.4°C) and are currently the focus of trials to extract geothermal energy from disused mines for heating local communities. However, these mine waters are also home to diverse microbial communities and the impacts of temperature and temperature changes on their community structure are unknown.

We examined the importance of water temperature, as well as pH, conductivity, dissolved oxygen, geochemistry (70 elements using AAS & ICP-MS) and season, for community structure of subsurface microbial communities in mine water sampled at 12 sites across the South Wales coalfield in April, August and December. For comparison, samples were similarly collected from a natural hot spring from an underlying formation. Subsurface mine waters and their microbial communities were accessed at point of egress at high-flow mine adits.

Analysis of 16S Illumina MiSeq sequencing data reveals complex interactions between environmental drivers (e.g. temperature), season and location, with some sites displaying little variation in community structure over the course of a year and other sites exhibiting large shifts in community structure from one season to another. Although most communities were dominated by β - and ϵ -proteobacteria, water chemistry strongly influenced the relative abundance of specific taxa.

The extensive subsurface habitats of the South Wales coalfield harbour diverse microbial communities adapted to varied but distinct temperatures and geochemical conditions. With the renewed commercial and bioengineering interest in the coalfield, we urgently need to develop an understanding of its microbial subsurface communities.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P385

Characterisation of microbial populations in highly radioactive storage facilities in Sellafield, UK

Sharon Ruiz Lopez¹, Lynn Foster¹, Jonathan Lloyd¹, Katherine Morris¹, Nick Cole²

¹University of Manchester, Manchester, United Kingdom. ²Sellafield Ltd, Warrington, United Kingdom

Abstract

The use of nuclear energy has been of great importance to the United Kingdom, with Sellafield being the largest nuclear site used for both power production and more recently reprocessing activities. The main pre-reprocessing storage pond at the Sellafield site is the Fuel Handling Plant (FHP), which is responsible for receiving, storing and mechanically processing spent nuclear fuel from Magnox and Advanced Gas-cooled Reactor stations from across the UK. The aim of this study is to characterize microbial communities of the FHP to create a microbial database consisting of density and diversity of microorganisms involved to better understand the biological complexity of the pond. The findings are discussed in relation to microbial survival to extreme environments and microbial adaptation to operational changes observed during the six months of analysis, and how microorganisms may impact on the management of the pond. The presence of microorganisms in water samples was studied by using molecular techniques e.g. Illumina high throughput 16S rRNA gene sequencing and the microbial density was determined by real-time quantitative PCR (Q-PCR). Organisms identified were predominantly Proteobacteria. The presence of Hydrogenophaga solely in the main ponds suggests the metabolism of hydrogen in the ponds which could be generated by radiolysis of water. Future plans involve further genetic characterisation of the identified communities which will be correlated with the complex biogeochemistry of the pond system. These studies will lead to a better understanding of the factors supporting colonization of the pond, the potential impact of the microbial community on fuel handling operations and the biochemical mechanisms of adaptation to this extreme environment.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P386

The response of a vitamin B₁₂-dependent mutant of *Chlamydomonas reinhardtii* to B₁₂ starvation and coculture with B₁₂-producing bacteria.

Freddy Bunbury

University of Cambridge, Cambridge, United Kingdom

Abstract

Requirement for vitamin B₁₂ is widespread among phylogenetically unrelated algae, suggesting that dependence evolved multiple times. Only prokaryotes are capable of synthesising B₁₂, and therefore are likely to co-occur with B₁₂-dependent algae. *Chlamydomonas reinhardtii*, a naturally B₁₂-independent alga, was shown to become B₁₂-dependent after roughly 500 generations of evolution in high B₁₂ conditions. Here I compare the response to B₁₂ of this novel *Chlamydomonas* auxotroph (*metE7*), with the naturally B₁₂-dependent and closely related alga, *Lobomonas rostrata*. *metE7* has a higher requirement, reduced uptake, and storage capacity for B₁₂, and undergoes a more severe starvation response involving a reduction in protein, and photosynthetic pigments, and an accumulation of energy storage compounds such as starch and triacylglycerides. However, an inability to induce non-photochemical quenching, as occurs under other nutrient starvation conditions, results in overproduction of reactive oxygen species and subsequent cell death. The B₁₂-producer *M. loti*, although capable of fully supporting *L. rostrata*, does not support *metE7* to the same extent, suggesting that *metE7* may be less well adapted for symbiosis. Nonetheless *metE7* is capable of inducing *M. loti* to increase B₁₂ biosynthesis, indicating that perhaps long term coevolution between partners is not necessary for the establishment of a regulated symbiosis.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P387

The geomicrobiology of cobaltiferous environments

Laura Newsome, Jonathan R Lloyd

University of Manchester, Manchester, United Kingdom

Abstract

Cobalt is becoming increasingly important due to its uses in modern “e-tech” technology. The majority of cobalt resources are produced as a by-product of nickel and copper mining, but relatively little is known about the microbiology, geochemistry and mineralogy of cobaltiferous environments, or the impact of biogeochemical cycling on the distribution of cobalt within a deposit.

Here we collected samples from a range of cobalt-rich deposits, with the aim of understanding the natural biogeochemistry of cobalt, and to consider whether new bioprocessing techniques could be developed to improve resource recovery. The samples included iron and manganese-rich laterites, sulphides and arsenides. For each sample the mineralogy and geochemistry were characterised, and the bacterial and fungal microbial communities were profiled by 16S rRNA and ITS sequencing using the Illumina MiSeq platform. Sediment microcosms were set up for selected samples, with electron donor added to stimulate the natural microbial community, with the aim of observing cobalt behaviour after the development of microbially-reducing conditions.

Results showed the cobalt content of the samples ranged from ~0.2% wt in iron oxide laterites from Brazil to ~4.6% wt in cobalt arsenides from Canada. The bacterial community in the laterite was dominated by Burkholderiales and Actinobacteria, and the diversity decreased with depth below ground level. Sequences associated with sub-oxic to anoxic metabolic functions were present at all depths. In biostimulated sediments the cobalt was released to solution or to a sorbed phase, suggesting the development of anoxic conditions may be beneficial to the recovery of cobalt.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P388

Genes of past, present and future: does legacy pollution contribute to antibiotic resistance in industrialised estuaries?

Kiri Rodgers¹, Iain McLellan¹, Roderick Williams¹, Andrew Hursthouse¹, Rebecca Tonner², Tanya PershPershka², Charlse Knapp², Fiona Henriquez¹

¹University of the West of Scotland, Paisley, United Kingdom. ²University of strathclyde, Glasgow, United Kingdom

Abstract

The ability to compare different pollution conditions among stratified layers in estuarine sediments allow us to determine key contributing factors affecting antibiotic resistance (AMR) in microbial communities. This is a unique approach of investigating resistance traits will generate a wealth of information about how our past industrial actions may impact public and environmental health now and/or the near future.

Sediments have been collected from the Clyde, Scotland, at various depths and have been analysed for physicochemical properties (pH, conductivity, organic matter, particle size), potential pollutants (metals and polycyclic aromatic hydrocarbons) as well as prokaryotic and eukaryotic biome.

Our research has found that potentially toxic elements (e.g. Cr and Pb) increase with depth, indicative of historical uses. To date, our analysis correlates with an increase in efflux pump genes in prokaryotes(1-4). The growth difference of Acanthamoeba on the other hand suggests the Cu may aid the prevalence of Acanthamoeba and the speed at which it grows whilst Mg has an inhibitory effect.

Our results strongly indicate that environmental pollution is responsible for the development of AMR and that protists (e.g. Acanthamoeba) are also affected by environmental conditions. Acanthamoeba are also known to harbour bacteria which may also affect the emergence of antimicrobial resistance.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P389

Diversity of bacteria in the rhizosphere of wheat during a crop cycle

Indre Navickaite, Claudio Avignone-Rossa, Emma Laing

University of Surrey, Guildford, United Kingdom

Abstract

The rhizosphere is a complex soil environment associated with living plant roots and colonized by symbiotic and free-living microorganisms. Microbe-plant and microbe-microbe interactions within the rhizosphere are considered to be major contributors to plant growth and survival. However, while rhizosphere microbial community diversity and activity are important to plant development, they remain poorly understood. Here we have analysed bacterial diversity, both composition and relative abundance, in the rhizosphere of wheat at three distinct wheat growth stages: stem elongation (or jointing), flowering, and ripening. The bacterial community analysis was performed using microbial amplicon sequencing of the hypervariable V3-V4 regions of the 16S rRNA gene. Overall, the 'core' microbiome, dominated by Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes and Firmicutes phyla, was identified in all samples. However, significant differences in the abundance of specific phyla between growth stages were observed. For example, Acidobacteria, Actinobacteria, Firmicutes and Chloroflexi abundance were highest at the stem-elongation stage and significantly decreased during ripening. Conversely, Proteobacteria, Bacteroidetes and Gemmatimonadetes, were found to be more abundant at flowering and ripening stages compared to stem elongation stage. Our results suggest active microbial community turn over during the course of wheat development which may correlate to plant age and/or nutrient requirements.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P390

Climate change effects on the microbiology of Arctic soils

Alexandra Burkitt¹, Andrew F.S. Taylor², Thorunn Helgason¹

¹The University of York, York, United Kingdom. ²The James Hutton Institute, Aberdeen, United Kingdom

Abstract

Arctic soils are a major store of global carbon. Increased microbial activity due to climate change may increase the rate of decomposition, causing stored carbon to be released as CO₂. Microbial activity could be increased directly, or indirectly via changes in vegetation or soil biota. The diversity and functioning of soil microbial communities is also likely to change. Understanding how soil microbial communities react to increasing temperatures and changing plant communities is therefore vital.

This study uses soil samples taken from a large, multiyear heating experiment in Svalbard. Open topped chambers were used to heat plots in a factorial design, with different levels of grazing by geese. Grazing is an important control on vegetation and soil in polar regions, and could mediate the effects of temperature increases on soil biota due to changes in carbon dynamics. This is a unique dataset from a long-term field study and provides data on microbial diversity in this endangered habitat.

DNA was extracted from mixed soil root samples and microbial and total eukaryote communities were identified via MiSeq sequencing. Above ground plant diversity was also measured in the field. We will present data showing if a) increased temperature changes the soil microbial community, b) grazing changes the soil microbial community, and c) there is an interaction between the effects of grazing and temperature. Interactions between diversity, relative abundance and community structure of different above and below-ground taxa will also be presented.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P391

Iron and sulphur biogeochemistry in the South Wales coalfield groundwater is dependent on specialised co-dominant Proteobacterial taxa

André Soares^{1,2,3}, Sara Rassner^{1,3}, Arwyn Edwards^{2,3}, Gareth Farr⁴, Andrew Mitchell^{1,3}

¹Department of Geography and Earth Sciences (DGES), Aberystwyth University, Aberystwyth, United Kingdom. ²Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, United Kingdom. ³Interdisciplinary Centre for Environmental Microbiology (ICEM), Aberystwyth University, Aberystwyth, United Kingdom. ⁴British Geological Survey (BGS) in Wales, Cardiff, United Kingdom

Abstract

The South Wales coalfield is now a mostly inactive mining region spanning 2200km² and makes up a large-scale complex hydrogeological network reaching depths of -60m to -1800m below surface from east to west. Characteristically iron-rich, pH neutral groundwater outflows feed the hydrographical basin at rates up to 3000L/s, varying in temperature and geochemistry. Thus far, multivariate, applied approaches towards a wider perception of the underlying biogeochemistry in this region are still lacking. In this study, triplicate samples of 1L of groundwater were collected across 13 sites in the South Wales coalfield in April, August and December 2016, filtered and had DNA extracted. The v3v4 region of the 16S rRNA gene was then amplified and sequenced using Illumina MiSeq next-generation technology. 70,937 sequence variants (SV's) corresponding to 9,656,492 reads (68.3% of trimmed, demultiplexed reads) were found following DADA2 processing. While Proteobacteria were found to generally dominate all sites, recently-discovered phyla Parcubacteria, Omnitrophica and Woesearchaeota followed in abundance. Different β - and ϵ -proteobacterial taxa defined most of the iron- and sulfur-rich community profiles, respectively. Genera *Sideroxydans* and *Gallionella* (β -Proteobacteria, Nitrosomonadales, Gallionellaceae) co-dominated iron-rich sites albeit both being known as aerobic iron-reducing taxa. In sulfur-rich sites however, family Helicobacteraceae composed the vast majority of the community profiles.

This taxonomic database represents the first large-scale effort towards an in-depth understanding of iron and sulphur biogeochemical cycling in the South Wales coalfield. The temporal and geographical functional dynamics of specialised planktonic microbial communities inhabiting its groundwater are assessed and pose promise for further high-end metagenomics ventures.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P392

Rapid high-throughput technique for phototrophic biofilm quantification using plate reader measurements

Maria Papadatou, Maria Salta

University of Portsmouth, Portsmouth, United Kingdom

Abstract

Quantification of biofilm cells is fundamental for a host of research and industrial applications. Nevertheless, there is lack of a standard technique applicable to different quantification studies. That results in particular readouts that do not always allow for comparisons between different approaches. The selection of quantification technique and the sequential interpretation of results may then be hampered. This necessitates the development of a straightforward and accurate method that will provide reproducible and expeditious results as to greatly improve biofilm biomass estimation in complex samples.

The direction of the current study on biofilm-forming marine microalgae was to deliver a rapid high-throughput quantification protocol using *in situ* chlorophyll measurement as a proxy for biofilm biomass, in order to provide insights on designing new tools for antifouling testing. Laboratory and field experiments were conducted testing monoculture model species and natural complex samples respectively, looking at the auto-fluorescence signals of the chlorophyll a-containing biofilms. Fluorescence was measured by applying real-time and non-destructive detection methods including fluorescence microscopy and plate readers. Subsequently, biofilm surface coverage was quantified by digital image analysis. Both field and laboratory -based experiments were successful in quantifying biofilms directly attached on a surface without the need for chlorophyll extraction, a time-consuming and destructive method. The developed technique can therefore be used as a fast-track method for the assessment of phototrophic biofilms on antifouling coatings and other artificial surfaces where biofilm formation is of interest.

Microbial Diversity and Interactions In the Environment

Zone D

Presentations: Tuesday and Wednesday Evening

P393

Antifungal, antioxidant and plant growth promoting characterisation of endophytic fungi of aromatic plant, *Ocimum basicilium*

Kanika Chowdhary¹, Satyawati Sharma²

¹DST-SERB post doctoral fellow, Indian Institute of Delhi, CRDT, DELHI, India. ²Senior Professor Indian Institute of Technology, Delhi, DELHI, India

Abstract

Endophytic microbes are hosted inside plants in a symbiotic relationship and considered as agriculturally beneficial. In the present work fungal endophytes associated with aromatic plant *Ocimum basicilium* L. were investigated for plant growth promotion and biocontrol potential. Anti-plant pathogenic activity of fungal endophytes was tested against *Sclerotinia sclerotiorum*. 75 endophytic fungi were recovered through culture-dependent approach. Fungal identification was performed both microscopically and by rDNA ITS sequencing. These fungal isolates were screened for *in vitro* plant growth promotion, antioxidant activity, stress tolerance ability and suppressible fungal disease potential. *Curvularia lunata* (Sb-6) and *Colletotrichum lindemuthianum* (Sb-8) inhibited 86% and 72% mycelia growth of *S. sclerotinia* on Sabouraud dextrose agar medium at 7.4 pH. Both isolates tested positive for plant growth promoting properties i.e. siderophore, IAA, HCN and ammonia production. Both the potent isolates survived imposed stressful conditions like pH range of 2-12, temperature range of 4-60 °C and high salt concentration at 10% NaCl. Their small - scale fermentation was carried out on sterilised oatmeal grain medium. EAF extract of *C. lunata* and *C. lindemuthianum* suppressed *S. sclerotinia* conidial germination at IC₅₀ values of 0.514± 0.02 and 0.913± 0.04 mg/ml respectively. Total phenolic content of Sb-6 was higher (18.6 ± 0.06 mg GAE/g) than Sb-8 (8.2 ± 0.07 mg GAE/g) as determined by Folin-Ciocalteu oxidation method. Therefore, fungal endophytes of *O. basicilium* are highly promising bio-resource agent, which can be developed further for sustainable agriculture.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P394

Understanding the importance and function of conserved RNA structure of Human coronavirus HKU1 ORF1a-1b frameshift signal involved in translation

SAAD MUTLK¹, Ian Jones²

¹Mr, Reading, United Kingdom. ²Prof, Reading, United Kingdom

Abstract

Human coronaviruses are spherical or pleomorphic enveloped viruses. The genome is a single stranded, positive-sense RNA and have the largest genomes among known RNA viruses. These viruses have the ability to infect humans and cause diseases that include both mild upper respiratory infections (Common cold) and severe acute respiratory syndrome (SARS). RNA structure has an important role in virus replication cycle and pathogenicity at both the transcription and translation stages of virus replication. A part of this project has an aim to understand the RNA secondary structure of the human coronavirus HKU1 ORF 1a-1b frameshift signal and to investigate how these structures affect transcription and translation by using site direct mutagenesis.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P395

Polycipiviridae: a proposed new family of polycistronic picorna-like RNA viruses

Ingrida Olendraitė¹, Nina I. Lukhovitskaya¹, Sanford D. Porter², Steven M. Valles², Andrew E. Firth¹

¹University of Cambridge, Cambridge, United Kingdom. ²Center for Medical, Agricultural and Veterinary Entomology, Gainesville, USA

Abstract

Solenopsis invicta virus 2 is a single-stranded positive-sense picorna-like RNA virus with an unusual genome structure. The monopartite genome of approximately 11 kb contains four open reading frames in its 5' third, three of which encode proteins with homology to picornavirus-like jelly-roll fold capsid proteins. These are followed by an intergenic region, and then a single long open reading frame that covers the 3' two-thirds of the genome. The polypeptide translation of this 3' open reading frame contains motifs characteristic of picornavirus-like helicase, protease and RNA-dependent RNA polymerase domains. An inspection of public transcriptome shotgun assembly sequences revealed five related apparently nearly complete virus genomes isolated from ant species and one from a dipteran insect. By high-throughput sequencing and *in silico* assembly of RNA isolated from *Solenopsis invicta* and four other ant species, followed by targeted Sanger sequencing, we obtained nearly complete genomes for four further viruses in the group. Four further sequences were obtained from a recent large-scale invertebrate virus study. The 15 sequences are highly divergent (pairwise amino acid identities of as low as 17% in the non-structural polyprotein), but possess the same overall polycistronic genome structure, which is distinct from all other characterized picorna-like viruses. Consequently, we propose the formation of a new virus family, Polycipiviridae, to classify this clade of arthropod-infecting polycistronic picorna-like viruses. We further propose that this family be divided into three genera: Chipolycivirus (2 species), Hupolycivirus (2 species) and Sopolycivirus (11 species, with members infecting ants in at least 3 different ant subfamilies).

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P396

Healthy honey bees – analysis of the virus population to assess rationale vector control

Luke Woodford¹, David Evans¹, Alan Bowman², Fiona Highet³

¹University of St Andrews, St Andrews, United Kingdom. ²University of Aberdeen, Aberdeen, United Kingdom. ³SASA, Edinburgh, United Kingdom

Abstract

Varroa destructor is an ectoparasitic mite which causes serious losses of honey bee colonies globally. The mite acts as a vector for a range of pathogenic viruses, most important of which is Deformed Wing Virus (DWV). Overwintering colony losses, accounting for 25-50% of colony losses annually, are associated with high levels of *Varroa*-DWV infestation. Effective miticide treatments are available to improve colony health. However, treatment is rarely coordinated or used rationally, meaning controls are not implemented to maximise their efficacy. This study uses coordinated treatment of *Varroa* in a geographically isolated environment (the Isle of Arran). The aim is to show that rational, coordinated treatment is beneficial, using known characteristics of the DWV virus population as an indicator of colony health. A high level of a near-clonal virus population indicates poor colony health, whilst low levels of a diverse population of DWV is characteristic of healthy colonies with low/no *Varroa* levels. The study area contains ~50 colonies and 25 beekeepers. Sampling and virus analysis – strain diversity and viral loads – was conducted before and after treatment. Changes in virus diversity were quantified by genetic methods, including NGS analysis. The first year of the three-year study is now complete. In parallel studies, we are exchanging colonies between apiaries containing very high and low infestations of *Varroa* to ascertain the temporal changes in the virus population upon acquisition of the mite, or application of miticides. These control studies will inform our development of rationale *Varroa* control strategies for UK beekeepers.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P397

Investigating membrane viral bending proteins in coronavirus replication.

Entedar ALSAADI¹, Prof. Ian M. Jones¹, Dr. Benjamin W. Neuman²

¹University of Reading, READING, United Kingdom. ²Texas A&M University, Texarkana, USA

Abstract

The coronavirus nsps 3, 4, and 6 are thought to have fundamental functions in the rearrangement of the membranes that are required for the establishment of the viral replication–transcription complexes. Nsp3, nsp4 and nsp6 may also have roles in the creation of double-membrane vesicles although their more precise role is not understood. The coronavirus envelope protein by contrast has defined functions in virus assembly and release. It may also induce membrane curvature in ERGIC leading to scission of budding virions. Thus, nsp3, nsp4, nsp6 and E potentially all contain membrane-modifying peptides. To search for such peptides, parameters such as amino acid conservation and amphipathic helix prediction were used. Peptides were synthesised and tested for membrane-modifying activity in the presence of GUVs from both *Mouse Hepatitis Virus* and *Middle Eastern Respiratory Syndrome Virus*. MHV-E cytoplasmic domain and other peptides from nsp3, nsp4 and nsp6 were found to change the size and shape of vesicle membranes in a manner consistent with membrane insertion. Select peptides from nsp4 and nsp6 caused pore formation in GUVs. To assess the roles of peptides identified with membrane-modifying activity *in vivo*, MHV E protein was expressed in insect cells using the baculovirus expression system and the active peptide sequence mutated. Mutant expression levels were modified compared to wild type with evidence of a redistribution within the expressing cell. These results suggest that the MHV-E post transmembrane region can modify the membrane *in vitro* and *in vivo* and that peptides from nsps can modify and cause membrane permeability.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P398

Functional characterization of human astrovirus ORFx

Valeria Lulla, Susanne Bell, Andrew Firth

University of Cambridge, Cambridge, United Kingdom

Abstract

Human astroviruses, which belong to genus *Mamastrovirus* within the *Astroviridae* family, are small non-enveloped viruses with a (+)ssRNA genome. Their genome contains three overlapping open reading frames (ORF1a, ORF1b and ORF2). Previously, using comparative genomics, we identified a conserved fourth ORF (ORFx) in some genogroups of astroviruses, which appears to be subject to purifying selection and therefore is likely to encode a functional protein product, termed Px. ORFx overlaps the 5' region of the capsid-encoding ORF2 and is predicted to be translatable via ribosomal leaky scanning. To evaluate the significance of ORFx in the context of virus infection, a set of mutant genomes was created and analyzed for virus titer and stability of the introduced mutations. ORFx knockout viruses were strongly attenuated and reverted to wild-type phenotype after a few passages via pseudo-reversions. Using molecular virology and biochemical approaches, we are investigating possible function(s) for the ORFx protein.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P399

Exploring translation efficiency and protein accumulation through the use of synthetic biology to design novel UTRs for transient overexpression of proteins in plants

Hadrien Peyret, George Lomonosoff

John Innes Centre, Norwich, United Kingdom

Abstract

The most successful transient expression systems for the overproduction of proteins in plants are deconstructed viral vectors which include different genetic components from different (usually) viral sources in order to maximise protein yield. Work in this area has led to the development of the CPMV-*HT* expression system and its associated pEAQ vectors. Recently, we have set about creating an expression system that maximises yield of recombinant protein based on completely synthetic components designed from first principles rather than deploying what nature has given us. In this way, we can hope to free our expression system of the restrictions that were placed on viral genetic components by evolution, in order to truly tailor our vectors for industrial applications.

We have begun creating new components for a novel expression system, and the early work has focused on creating novel synthetic untranslated regions (UTRs) to use as translational enhancers and RNA stabilisers. This work seeks to understand what makes a “good” UTR from the perspective of protein accumulation. Results show that while designing novel 5’UTRs for efficient protein overexpression is surprisingly easy, the same cannot be said for 3’UTRs, in which RNA secondary structure seems to play a far more important role. The ultimate goal of this project is to eventually create a completely novel expression system based on rationally-designed components. Along the way, insight is being gained into the function of different viral genetic components and challenging our view of the role and importance of some of these.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P400

Investigating the role of the Chikungunya nsP3 macro domain in virus replication and its interactions with host innate immune pathways.

Grace Roberts, Nicola J Stonehouse, Mark Harris

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a re-emerging human alphavirus. Previously confined to Asia and Africa due to its mosquito vector, recent events have allowed the virus to spread further across the globe resulting in severe, large-scale outbreaks in naïve populations.

CHIKV encodes for four non-structural proteins (nsP1-4) that are essential for virus replication. The role of nsP3 is poorly understood, although it is known that nsP3 comprises three domains. This project focuses on the N-terminal macro domain, a domain found in proteins of all species and shown to bind RNA and DNA, mono- and poly-ADP-ribose and in some cases to exhibit ADP-ribose hydrolase activity. Our aim is to understand the role of the nsP3 macro domain in CHIKV replication.

Initially we generated a range of mutations in the macro domain binding pocket. These produced a range of phenotypes, many resembled wildtype but some exhibited significantly increased or decreased levels of replication. When compared to published biochemical analyses of the macro domain, our data suggests there is an unknown function, unrelated to RNA/ADP-ribose binding or hydrolase activity, that is required for CHIKV replication.

More recently, we have investigated interactions between the nsP3 macro domain and the NFκB pathway. We have found that the NFκB pathway is not activated upon CHIKV infection and that expression of nsP3 in cells is able to reduce NFκB activation even in the presence of a stimuli such as TNFα. We are currently working to determine the mechanism of action of this effect.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P401

The Chikungunya virus nsP3 alphavirus unique domain exhibits cell and species-specific functions, and is required for both genome replication and virus assembly.

Yanni Gao, Andrew Tuplin, Mark Harris

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a re-emerging arbovirus causing fever, joint pain, skin rash, arthralgia, and occasionally death. Antiviral therapies or safe, effective vaccines are urgently required.

Much of the biology of CHIKV is poorly understood, in particular the functions of the non-structural protein 3 (nsP3). Here we present the results of a mutagenic analysis of the central alphavirus unique domain (AUD) of nsP3. Informed by the published structure of the Sindbis virus AUD and an alignment of the amino acid sequences of multiple alphaviruses, a series of mutations in the AUD were generated in the context of a CHIKV sub-genomic replicon. This analysis revealed that the AUD plays an essential role in CHIKV RNA replication, and the mutants exhibited both species- and cell-type specific phenotypes. In particular, two basic residues (R243 and K245) were shown to be required for the ability of the purified AUD to bind CHIKV 3'UTR, mutation of these residues rapidly reverted in mosquito cells suggesting that one role of the AUD is to bind the virus RNA during genome replication. The phenotypes of the mutant panel were also analysed in the context of infectious CHIKV. Results indicated that the AUD was also required for virus assembly and release.

We propose that the AUD may be a pleiotropic protein domain, functioning in both genome replication and virus assembly, as well as playing a role in counteracting host defences in both mammalian and mosquito cells.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P402

Identification of external stabilising factors associated with the production of poliovirus VLPs

Helen Fox¹, Johanna Marsian², Mohammad Bahar³, Claudine Porta⁴, Inga Kruse², Elizabeth Fry³, David Stuart³, George Lomonosoff², Andrew Macadam¹

¹NIBSC, South Mimms, United Kingdom. ²John Innes Centre, Norwich, United Kingdom. ³University of Oxford, Oxford, United Kingdom. ⁴The Pirbright Institute, Pirbright, United Kingdom

Abstract

Polio virus-like-particles (VLPs) produced in recombinant expression systems would provide an ideal vaccine for the post-eradication world if they were stable. We have previously shown that native antigenic forms of VLPs of all three serotypes can be stabilised by introduction of suitable amino acid substitutions. We have now identified a number of key additional factors that affect the stability of VLPs and could therefore be crucial during large-scale production.

In poliovirus particles produced by infection of mammalian cells, a pocket in VP1 is naturally occupied by one of a range of fatty acids, whose identities are not completely known, and this (pocket) factor is known to play a role in the stability of the capsid. VLPs made in a plant recombinant system appear to lack pocket factor and are less thermostable than expected; we have investigated whether this is true for particles produced in other cells and whether addition of a synthetic pocket factor analogue such as Pleconaril or GPP3 can improve stability.

The stability of polio VLPs made in plant and mammalian recombinant systems is significantly improved by addition of cation chelators during extraction. The concentration at which metal cations, that may be present in recombinant systems, destabilise the VLPs has been identified and it is clear the effects differ between serotypes. The ratio of native:non-native VLPs in preparations also depends on the recombinant system used, temperature and extraction method.

(Part of a WHO funded consortium also including the Universities of Reading and Leeds - PI David Rowlands).

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P403

Exploring the role of Chikungunya virus nsP1 protein in virus replication

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 antiviral therapeutics, combined with increasing global spread, has facilitated the re-emergence of CHIKV in recent years. A key approach to limiting CHIKV virus infection would be to target viral replication. Our research is focused on the CHIKV encoded nsP1 methyltransferase, encoded by ORF-1 of the virus genome, and aims to investigate roles of nsP1 during different stages of the virus replication cycle.

We have previously shown that substituting the second nsP1 methionine for alanine (M²⁴>A²⁴) significantly enhances sub-genomic replicon genome replication, while production of infectious CHIKV virions is blocked. Interestingly, these phenotypes appear to be host-cell dependent and we are currently utilising a reverse genetics approach to investigate mechanisms by which they function. *In vitro* biochemical assays will be used, to allow us to better understand why and how mutations at this methionine residue produce the resulting mutant phenotypes. Additional mutations have been introduced to this residue, in order to further dissect its role and that of nsP1 during CHIKV replication. In addition, with extension of this research towards a structural approach, we have expressed recombinant nsP1 in a bacterial system - with the aim of using X-ray crystallography to further understand the structural and mechanistic bases for the observed mutant phenotypes and nsP1 function.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P404

Disruption of RNA structure within the 5'UTR of Chikungunya virus RNA attenuates viral genome replication

Henna Khalid¹, Marietta Müller¹, Catherine Kendall¹, Alain Kohl², Andres Merits³, Andrew Tuplin¹

¹University of Leeds, Leeds, United Kingdom. ²MRC - University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. ³University of Tartu, Tartu, Estonia

Abstract

Chikungunya virus (CHIKV) is a positive sense RNA virus within the alphavirus genus, presenting significant morbidity to patients and with no currently available vaccine or anti-viral therapy. CHIKV transmission occurs through *Aedes* infected mosquitoes, with its increasing geographical distribution partly reflecting expanding host vector range.

Using a combination of thermodynamic, phylogenetic and biochemical SHAPE mapping methods, we previously identified a novel RNA stem-loop structure SL47, within the 5' untranslated region (5'UTR) of the CHIKV genome, adjacent to the *nsp1* start codon. Using a sub-genomic replicon system and full-length CHIKV infectious virus, we investigated the role of this RNA structure in CHIKV genome replication and translation.

Our studies demonstrate that nucleotide substitutions that disrupt base pairing within the heteroduplex of the stem-loop, in either the context of full-length infectious virus or sub-genomic replicon system, significantly attenuate CHIKV replication - in both Huh7 mammalian hepatoma and *Aedes albopictus* C6/36 cell lines. Comparative investigations using a replication deficient sub-genomic reporter system, encoding a GDD>GAA substitution in the polymerase active site, suggest that SL47 plays a role in genome replication rather than initiation of translation. Using a compensatory mutant which restores SL47 heteroduplex base-pairing but changes nucleotide sequence also restores virus replication to wild-type levels.

Studies to isolate escape mutants to determine the basis of restored CHIKV replication are ongoing.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P405

ANTIVIRAL ACTIVITY OF DIFFERENT SPECIES OF FICOLINS AGAINST HEPATITIS C VIRUS (HCV) AND OTHER EMERGING VIRAL INFECTIONS

Yemisi Adedeji, Alexander Tarr, Jonathan Ball, Christopher Mason, Patrick McClure

University of Nottingham, Nottingham, United Kingdom

Abstract

Hepatitis C virus (HCV) encodes two envelope glycoproteins, E1 and E2, which are found on the surface of the viral lipid envelope. These glycoproteins facilitate entry into the host. They have been shown to possess exposed regions that are targets for host immune recognition. Ficolins are polymorphic liver-expressed pattern recognition receptors (PRRs) that contribute to the innate surveillance of virus infections, recognising carbohydrates such as N-acetylglucosamine. Three ficolin genes have been identified in humans; *FCN1*, *FCN2* and *FCN3* which encode M-ficolin, L-ficolin and H-ficolin, respectively. L-ficolin has been demonstrated to bind to HCV virions and inhibit infection. However, HCV persists in the presence of this protein, suggesting that the virus may adapt to avoid recognition by host immune effector.

Three ficolin-encoding genes have been identified in all non-human primates, while two are found in mice (*fcn-a* and *fcn-b*). However, the antiviral activity of non-human ficolins is unknown. In this study, we showed an interaction between ficolins of mouse origin and HCV glycoproteins E1 and E2. These interactions inhibit virus entry. A competitive assay confirmed that this interaction was via the fibrinogen domain by competition with other saccharides. Neutralisation of HCV entry by mouse ficolin was more potent than primate ficolin and human L-ficolin. This is the first time the neutralisation of HCV is been assessed by other species of ficolin and may provide a better insight into their divergent evolution in mammals. The role of the different species of ficolins might serve as a potential therapeutic anti-viral agent.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P406

The Effect of Immune-Related Gene Downregulation on Deformed Wing Virus Replication in Honeybees

Katharin Balbirnie, Olesya Gusachenko, David Evans

University of St Andrews, St Andrews, United Kingdom

Abstract

Deformed wing virus (DWV) is a single stranded, positive-sense RNA virus of the *Iflavirus* family. DWV is a leading cause of honeybee population decline; as honeybees are pollinators of many economically valuable crops, it is important to study the pathogenesis of this virus and understand the host-pathogen interactions involved.

Previous studies have shown that DWV infection can influence the expression of immune-related genes in honeybees. The aim of this study was to determine if these genes play a role in the development of DWV infection.

Short interfering RNAs (siRNAs) targeting selected immune-related genes were synthesised *in vitro* and injected into honeybee pupae, which were further challenged with DWV. After 24 hr incubation with the virus, pupae were sacrificed and analysed by RT-qPCR to determine if the decrease in the expression of targeted genes had an effect on virus titre. Our results indicate that altering expression of some honeybee immune-related genes results in a change in DWV accumulation rates in infected pupae. We also show that downregulation of Dicer – one of the major components of the anti-viral response system in honeybees - correlated with higher virus titres in injected pupae and reduced survival of honeybee larvae challenged with DWV via feeding. These results confirm that Dicer can play a role in the suppression of DWV infection.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P407

Bats as a source of zoonotic spillover: Investigating virus in enteric bat samples from Viet Nam

Áine Niamh O'Toole^{1,2}, Carlijn Bogaardt^{1,2}, Lu Lu^{1,2}, Jordan Ashworth^{1,2}, Mark Woolhouse^{1,2}

¹University of Edinburgh, Edinburgh, United Kingdom. ²VIZIONS Consortium, Edinburgh, United Kingdom

Abstract

Bats, belonging to the order Chiroptera, are the natural reservoir hosts for an array of zoonotic viruses. Aspects of bat ecology, behaviour and physiology; such as migrating great distances, roosting in close association in large numbers and variation in metabolic rate and core body temperature during sustained flight, make them a unique concern for viral zoonotic transfer.

As part of the Viet Nam Initiative on Zoonotic Infections (VIZIONS) project, 169 enteric bat samples were collected from two sites in the Dong Thap province in Viet Nam, ~100km apart, and Illumina sequenced in the Sanger Institute.

Based on host mtDNA sequence present in these enteric samples we identified *Scotophilus kuhlii* as the host species for > 97% of the 196 samples, with remaining samples of the *Myotis*, *Murina* or *Pipistrellus* genera. Significant quantities of *Alphacoronavirus*, *Rotavirus* and *Mamastrovirus* reads were identified in the enteric bat samples using Kraken. We confirmed significant mixing and jumping of *Alphacoronavirus* between the two locations, using two independent analyses: Bayesian Tip-associated Significance (BaTS) testing (Parker et al, 2008), which confirms no significant clustering of virus with respect to location, and host-state reconstruction analysis, which predicted a mean of 17 host-jumps between the two locations. These findings suggest that 100km is negligible for *Scotophilus kuhlii*, in terms of viral transfer.

References

Parker, J., Rambaut, A. and Pybus, O. G. (2008), 'Correlating viral phenotypes with phylogeny: Accounting for phylogenetic uncertainty', *Infection, Genetics and Evolution* 8(3), 239–246.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P408

Characterization of the stimulators of protein-directed ribosomal frameshifting in Theiler's murine encephalomyelitis virus

Sawsan Naphthine, Andrew Firth, Ian Brierley

Cambridge University, Cambridge, United Kingdom

Abstract

Programmed -1 ribosomal frameshifting (-1 PRF) is a gene expression mechanism whereby a proportion of translating ribosomes are stimulated to shift -1 nt at a specific site and continue translating in the new reading frame. Sites of -1 PRF normally comprise an X_XXY_YYZ shift site followed by a 5–9 nt spacer and then an RNA secondary structure. Such intra-mRNA stimulators lead to a fixed ratio of frameshift and non-frameshift products. Recently we identified two cases of PRF where the stimulator involves a virus protein binding to the mRNA 3' of the shift site: one in arteriviruses (order *Nidovirales*) and one in coronaviruses (order *Picornavirales*). Despite there being hundreds of known cases of RNA-structure stimulated PRF, these comprise the only known cases of protein-stimulated PRF. In coronaviruses, viral protein 2A binds an RNA stem-loop beginning ~ 14 nt 3' of the shift site. Cellular levels of 2A build up strongly between 4 and 6 h.p.i., and this correlates with a switch in frameshifting efficiency from negligible levels before 4 h.p.i. to $\sim 70\%$ after 6 h.p.i., thus downregulating enzymatic protein synthesis at late timepoints. Here we investigate protein-stimulated PRF in the coronavirus Theiler's murine encephalomyelitis virus (TMEV). We showed that $\sim 55\%$ -efficient PRF can be achieved in a cell-free system in the presence of recombinant 2A. Next we performed an extensive mutational analysis of the frameshift stimulators using *in vitro* translations, electrophoretic mobility shift assays, *in vitro* ribosome pausing assays, and virological studies of wild-type and mutant viruses.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P409

STUDIES ON THE CORRELATION OF GENOTYPING AND CHALLENGE TEST FOR FMDV TYPE O, A AND SAT2 OF FIELD ISOLATES AND VACCINAL STRAINS IN EGYPT.

Mohamed Abousenna

Central Laboratory for Evaluation of Veterinary Biologics, CLEVB, Cairo, Egypt

Abstract

Background: Foot and mouth disease (FMD) is a highly infectious disease in cloven- hooved animals, The regular vaccination is widely used to control, eradicate, reduce transmission and prevent FMD infection.

Methods: Detection the protective level of vaccinated calves with inactivated polyvalent FMD vaccine against FMDV Vaccinal strains and different circulating FMDV field isolates using challenge test depend upon genotyping and Identity percentage of different serotypes of FMDV types A,O and SAT2, the challenge test and titration of the used serotypes (O/EGY/6/2011, O/EGY/23/2014, A/EGY/1/2012, A/EGY/31/2014, SAT2/EGY/2/2012 and SAT2/EGY/24/2014) were carried out.

Results: it was found that the protection level of the vaccine in vaccinated calves using challenge test against O/EGY/6/2011 and O/EGY/23/2014 viruses at the 28th day, was 100% while the protection against O/EGY/23/2014 was 80% . The protection level of the vaccine in vaccinated calves using challenge test against A/EGY/1/2012 and A/EGY/31/2014 viruses at the 28th day, was 100% for each. the protection level of the vaccine in vaccinated calves using challenge test against SAT2/EGY/2/2012 and SAT2/EGY/24/2014 viruses at the 28th day, was 100% for each.

Conclusion: All these results revealed the correlation between genotyping and challenge test which should be considered in predicting of the protection for FMD vaccines thus it can be useful in selection for supplied vaccine and detection if the vaccine should be updated or not.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P410

Replication Inhibition by a Glycolysis Inhibitor is a General Feature of Alphaviruses, Operating After Entry/Fusion and Before Assembly

David Ulaeto^{1,2}, James Findlay¹, Rennos Fragkoudis²

¹CBR Division, Dstl Porton Down, Salisbury, United Kingdom. ²The Pirbright Institute, Pirbright, United Kingdom

Abstract

Glycolysis inhibitors have previously been shown to differentially inhibit viruses from different families, and to be potent inhibitors of both Semliki Forest virus and Sindbis virus replication. In this study we show the activity of the potent anti-glycolytic compound 2-deoxyglucose extends to ACP3 Old and New World arthralgic and encephalitic alphaviruses. Fluorescence microscopy and flow cytometry demonstrate a substantial reduction in fluorescence intensity of a ZS-green recombinant virus, suggesting a reduction in levels of translation from the viral genome, while counter-staining for double-stranded RNA indicates that virus entry and uncoating, and a degree of RNA replication, take place. Single step growth curves indicate that production of progeny virus is almost entirely abolished, even with late addition of drug to the culture. Rescue experiments with a panel of glycolysis intermediates suggest restriction of glycolysis-dependent energy supply is an important component of the mechanism. Our findings, with a drug that has already passed through clinical trials as an anti-cancer agent, suggest 2-deoxyglucose has potential as a candidate therapeutic for alphavirus disease, and that the glycolysis pathway may be a source of drugable targets for alphavirus and potentially some other viral diseases.

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P411

Inactivation of the Infectious Bronchitis Virus ADRP Domain results in loss of pathogenicity.

Sarah Keep, Erica Bickerton, Paul Britton

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Infectious Bronchitis Virus (IBV) is a gammacoronavirus that is the aetiological agent of Infectious Bronchitis; an economically significant respiratory disease of poultry. IBV has a large positive sense RNA genome of which the replicase gene comprises the 5' two thirds, encoding 15 non-structural proteins (nsps). Of these, nsp 3 is the largest, coding for a multi-functional protein containing several putative domains. One such domain, conserved amongst coronaviruses is the ADRP domain, named due to its ADP-ribose-1''-phosphate phosphatase activity. For alpha and betacoronaviruses mutations within the active site have been demonstrated to result in attenuation. Crystal structures of the ADRP domain from two strains of IBV, the pathogenic strain M41 and a pathogenic strain Beaudette have previously been characterised, with a significant difference in the binding cleft identified. Whilst the domain from M41 contains a triple glycine (Gly-Gly-Gly) motif found to be essential for the binding of ADP-ribose, Beaudette contains a glycine to serine mutation (Gly-Ser-Gly) abolishing ADRP activity. To investigate the role of this domain in pathogenicity, recombinant IBV (rIBV) Beau-R-G-ADRP, containing a Serine to Glycine mutation, and M41-K-S-ADRP containing the reverse mutation were constructed. The pathogenicity of rIBVs was investigated in chickens; for both rIBVs a reduction in clinical signs was observed in comparison to M41 infected birds. Ciliary activity, a marker for pathogenicity, was comparable to a pathogenic and mock infected birds. Whilst the restoration of the triple glycine motif is not sufficient to confer pathogenicity to the avirulent Beaudette, the Gly-Ser-Gly configuration is attenuating for M41.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P412

Recombinant empty capsids of a type O vaccine isolate of Foot and Mouth Disease Virus

Hemanta Maity¹, Silvia Loureiro¹, Neil O'Flynn¹, Bryan Charleston², Ratchanee Atthi³, Somkiet Sripisuth³, Ian Jones¹

¹University of Reading, Reading, United Kingdom. ²The Pirbright Institute, Guildford, United Kingdom.

³Bureau of Veterinary Biologics, Pakchong, Thailand

Abstract

Foot and mouth disease (FMD) is a highly contagious and economically important disease of cloven footed animals. Current vaccination campaigns use inactivated cell culture derived virus which are often difficult to produce and have limited stability on storage. A number of recombinant strategies have been reported for FMDV including individual structural proteins, empty capsids and recombinant viral vectors. Previously we showed that empty capsids of FMDV can be assembled in insect cells and that they induce protective titres of antibody following purification and immunisation. We have applied this technology to a current O serotype vaccine isolate to assess the potential for rapid vaccine generation following an outbreak.

We expressed the P1 structural protein region of the type O serotype fused to the virus protease 3C in a baculovirus vector in which the levels of P1 and 3C were adjusted to allow synthesis, cleavage, and assembly of empty FMDV particles. Following isolation of a recombinant baculovirus, processed P1 was observed in the infected insect cells and banded material typical of empty capsids was visualised in peak fractions from sucrose velocity gradients. Interestingly, the same genetic construct also produced similar empty capsids when transferred into a yeast expression vector and transformed into *Saccharomyces cerevisiae*. Our data suggest that empty FMDV capsids form for a current vaccine strain offering an alternative to traditional culture. Moreover, the observation of capsid assembly in yeast suggests an economically attractive production method suitable for many developing regions of the world.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P413

Construction of a reverse genetics system for Avian Nephritis Virus, and characterisation of recovered virus

Conor Haydon¹, Pippa Hawes¹, Venugopal Nair¹, Eric Dykeman², Reidun Twarock², Ashley Roberts³

¹The Pirbright Institute, Pirbright, United Kingdom. ²University of York, York, United Kingdom.

³University of Glasgow, Glasgow, United Kingdom

Abstract

Astroviruses are small, positive-sense non-enveloped RNA viruses that are prevalent globally, infecting a wide range of birds and mammals. Avian astroviruses cause a diverse range of pathologies, including enteritis, hepatitis, nephritis, and mortality, resulting in reduced growth rates and large economic impacts on the poultry industry. There are currently limited control measures for astroviruses; vaccines developed against chicken astroviruses thus far only provide partial protection, and elimination of environmental contamination is difficult to achieve. New control strategies are urgently needed as increasingly pathogenic avian astroviruses are emerging. Since understanding of avian astroviruses in general is limited, we sought to create a reverse genetics system in order to further investigate the avian astrovirus; Avian nephritis virus (ANV). Genomic RNA of ANV G-4260 was isolated, cloned and sequenced, then assembled into pUC19. The ANV cDNA was placed under the control of the T7 promoter, with a polyA and hepatitis delta ribozyme sequence, followed by a T7 terminator site positioned downstream to generate poly A tailed transcripts for translation. Expression of the construct in BSR T7 cells produced intracellular virus which could be serially passaged in CK cells, producing infectious ANV. Presence of reverse genetics system derived virus was confirmed by confocal microscopy using an ANV-positive chicken serum, electron microscopy of thin-sectioned cells and RT-qPCR. Thus, we have established a reverse genetics system for the avian astrovirus, ANV G-4260, which will allow for further investigation of this virus in subsequent work.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P414

Identification of membrane rearrangements induced by infectious bronchitis virus non-structural proteins

Nicole Doyle¹, Hasan Alrashedi², Pippa Hawes¹, Benjamin Neuman³, Helena Maier¹

¹The Pirbright Institute, Pirbright, United Kingdom. ²University of Reading, Reading, United Kingdom.

³Texas A&M University, Texarkana, USA

Abstract

Infectious bronchitis virus (IBV), an avian gammacoronavirus, is an important pathogen that causes substantial animal welfare problems and economic losses to the global poultry industry. Positive-strand RNA viruses, such as coronaviruses, induce cellular membrane rearrangements during replication to form replication organelles, which are thought to allow for efficient viral RNA synthesis. IBV replication has been shown to induce the formation of double membrane vesicles (DMVs), zippered ER and tethered vesicles, known as spherules. Although these membrane rearrangements are virally-induced, it is not yet understood which viral proteins are responsible for this action and this is therefore the main focus of these studies. In this project, we have compared the membrane rearrangements induced when expressing different viral non-structural proteins (nsps) from two different strains of IBV. Fluorescently tagged forms of nsp3, nsp4 and nsp6 were expressed in cells singularly or in combination and the effects on cellular membranes investigated using electron microscopy. In contrast to previously studied coronaviruses, which require the presence of more than one nsp, we have shown that nsp4 alone is necessary and sufficient to induce membrane pairing. We have also noted a difference in the membrane pairing ability between the two strains of IBV used in this study. Using these methods we are able to begin to understand the specific viral proteins involved in IBV-induced membrane rearrangements.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P415

Detection and characterization of homologues of human hepatitis viruses and pegiviruses in rodents and bats in Vietnam

Dung Nguyen¹, Cuong Nguyen², Tue Ngo², Juan Carrique-Mas^{2,1}, David Bonsall¹, Anh Pham², Juliet Bryant³, Guy Thwaites^{2,1}, Stephen Baker^{2,1,4}, Mark Woolhouse⁵, Peter Simmonds¹

¹University of Oxford, Oxford, United Kingdom. ²Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam. ³Centre International de Recherche en Infectiologie, Lyon, France. ⁴The London School of Hygiene & Tropical Medicine, London, United Kingdom. ⁵University of Edinburgh, Edinburgh, United Kingdom

Abstract

Rodents and bats have been recognised as important sources of zoonotic virus infections in other mammals, including humans. Surveys have expanded our knowledge on viral diversity in a wide range of rodent and bat species, as well as on the origins, evolution and host ranges of the diverse viruses they harbour. In this study of pegivirus and human hepatitis-related viruses, liver and serum samples from Vietnamese rodents and bats were examined by PCR and sequencing. Nucleic acid of homologues of human hepatitis B, C, E viruses was detected in 2 (1.3%) of 157 bat, 38 (8.1%) and 14 (3%) of 470 rodent liver samples, respectively. A high detection frequency (42.7%) of hepacivirus-like viruses was observed in bamboo rats (*Rhizomys pruinosus*). Contrastingly, pegivirus RNA was only evident in 2 (0.3%) of 638 rodent serum samples. The obtained complete or near complete genome sequences of HBV, HEV and pegivirus closely related to those previously reported from rodents and bats. However, the rodent hepacivirus-like complete coding region sequences were substantially divergent from their closest matches among currently classified variants and potentially represent new species in the *Hepacivirus* genus based on sequence distances in the regions for classification. The existence of highly divergent, host species-associated variants of the hepaciviruses detected in the current study contrasted with a much broader host range infected with HEV-like viruses, indicative of their lower host species specificity and potentially greater zoonotic potential. The transmission routes and potential to emerge in humans of the identified viruses remain to be determined.

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P416

Selection and Characterization of Rupintrivir-Resistance Norwalk Virus Replicon Cells in vitro

MITSUTAKA KITANO, MYRA HOSMILLO, IAN GOODFELLOW

University of Cambridge, Cambridge, United Kingdom

Abstract

Background— Human noroviruses (HuNoV) are a major cause of human epidemic nonbacterial gastroenteritis. A HuNoV replicon system has been widely applied in the evaluation of antiviral compounds against HuNoV infection. Here we report, for the first time, the isolation of replicon cells with reduced susceptibility to protease inhibitor.

Methods – Rupintrivir, an irreversible inhibitor of the human rhinovirus (HRV) 3C protease, was used in this study. Norwalk replicon cells were cultured in the presence of increasing concentrations of rupintrivir with G418 up to 45 days. The effects of rupintrivir on the reduction of the replicon were evaluated by qRT-PCR. Cell-based fluorescence resonance energy transfer (FRET) assay was utilized to investigate whether the identified substitutions were involved in susceptibility to rupintrivir.

Results— We successfully isolated replicon cells with reduced susceptibility to rupintrivir following several passages in the presence of rupintrivir. In addition, sequence analysis revealed that these replicon cells contained amino acid substitutions of A105V and I109V in the protease. The application of a cell-based FRET assay demonstrated that these substitutions were involved in enhanced resistance to rupintrivir. Furthermore, we elucidated that recombinant murine norovirus with a single I109V substitution in the protease also showed reduced susceptibility to rupintrivir in cell culture.

Conclusion – This study demonstrated that a combination of approaches, HuNoV replicon system and a cell-based FRET assay, can characterize antiviral sensitivity and emergent resistance mutants.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P417

Characterisation of broadly cross-reactive monoclonal antibodies to the N-terminus of FMDV capsid protein VP2: tools to understand VP2 function

Amin Asfor¹, Nathalie Howe¹, Santina Grazioli², Emiliana Brocchi², Toby Tuthill¹

¹The Pirbright Institute, Ash Road, Pirbright, Woking, United Kingdom. ²Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via Bianchi 9,, Brescia, Italy

Abstract

Foot-and-mouth disease virus (FMDV) is responsible for a highly contagious disease of livestock that causes significant financial losses. The virus exists as seven immunologically distinct serotypes with additional antigenic variability even within serotypes. FMDV is a member of the picornavirus family of non-enveloped viruses which also includes human pathogens such as poliovirus and rhinovirus. In these human viruses, the internal N terminus of capsid protein VP1 is conserved and has been shown to be transiently exposed to the surface of the virus in a process termed “breathing”. During cell entry the VP1 N-terminus becomes irreversibly externalised and is involved in membrane interactions together with the pore forming capsid protein VP4. This allows the virus to penetrate the cell membrane and deliver the viral genome into the cytoplasm. Interestingly in FMDV, sequence conservation and structural studies suggest the VP2 N-terminus (VP2N) may have a role equivalent to the VP1 N-terminus in poliovirus and rhinovirus. Five novel monoclonal antibodies (mAbs) specific to VP2N have been characterised as tools to understand the function of FMDV VP2N in capsid breathing and cell entry. In addition, the mAb epitopes are located within the highly conserved first 15 amino acids of VP2N making them broadly cross-reactive and promising candidates for the development of serotype-independent diagnostic assays.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P418

Characterising birnavirus replication in vitro using a split-GFP infectious bursal disease virus (IBDV)

Alice Gray, Andrew Broadbent

The Pirbright Institute, Woking, United Kingdom

Abstract

Birnaviruses are economically important veterinary viruses that infect birds, insects and fish, causing production losses in aquaculture and to the poultry industry. Fluorescently tagged reporter viruses are important tools for studying the pathogenesis of viral diseases. Using reverse genetics, we have made the first ever reporter birnavirus by tagging a small sub-unit of Green Fluorescent Protein, GFP11, to a protein of infectious bursal disease virus (IBDV) to make a split GFP virus (IBDV-GFP11). When DF-1 cells were transiently transfected with the rest of the GFP molecule (GFP1-10) and subsequently infected with IBDV-GFP11, the tag completed the molecule and the full-length GFP fluoresced green. Infected cells had multiple green foci in the cytoplasm. When cells were fixed and stained with an antibody against the IBDV VP3 protein, which coats the genome and binds the polymerase, there was a high degree of co-localisation, suggesting these foci are the sites of viral replication. When movies were taken of the infected cells at 18 hours post-infection for a period of 2 minutes or 2 hours, the foci were not found to be trafficking within the cell, however from still images taken from 8 hours to 24 hours post-infection, the average diameter of the foci increased while the average number of foci per cell decreased, consistent with the merging of two small adjacent foci into a single larger focus as they grow in size. Taken together, the IBDV-GFP11 virus will be a useful tool to further elucidate birnavirus-host cell interactions.

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P419

The protein interactions of the Chikungunya virus nsP3

Raymond Li, Andrew Tuplin, Mark Harris

University of Leeds, Leeds, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a single stranded positive sense RNA virus from the *Togaviridae* family (*Alphavirus* genus), its vectors include the *Aedes albopictus* and *Aedes aegypti* mosquitoes which can spread the virus to humans during blood meals. CHIKV infection in humans causes an acute infection resulting in fever, nausea, muscle pain and debilitating joint pain which can persist after the infection has been cleared. Recent outbreaks of the virus have occurred worldwide and there are no antivirals or vaccines to combat the virus. CHIKV expresses four non-structural proteins that function in unison to facilitate viral genome replication, however the exact function of the non-structural protein 3 (nsP3) is currently unknown.

In order to elucidate the function of the nsP3 we set out to identify cellular proteins with which it interacts. To achieve this an infectious clone of CHIKV with a Strep-tagged nsP3 has been developed, this virus replicates to similar levels as wildtype CHIKV in human cells. Strep-tagged nsP3 can be efficiently purified from infected cell lysates and we are currently using mass spectrometry to identify nsP3 interacting partners. We expect that this analysis will reveal previously unknown functions of the viral protein and could serve as targets for development of vaccines & antiviral treatments in the future.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P420

Investigating the prevalence of picornavirus in Polish rodent populations

Joseph Chappell, Theocharis Tsoleridis, Jerzy Behnke, Rachael Tarlinton, C. Patrick McClure, Jonathan K Ball

University of Nottingham, Nottingham, United Kingdom

Abstract

The Picornaviridae are a diverse family of non-enveloped, positive sensed RNA viruses, known to infect a wide range of mammal species, often resulting in mild to severe disease. Rodents serve as reservoir to a large number of these viruses, some of which are thought to be capable of zoonotic transmission into humans. Bank voles are the natural reservoir of Ljungan virus (Parechovirus B), which has been associated with diabetes, intrauterine death and foetal malformation in humans. Ljungan has been detected in Fennoscandia, western and central Europe, but has yet to be detected in eastern Europe, which this study aims to redress.

Samples were collected from 294 bank voles caught across three locations in north-eastern Poland. The initial screening process used two-step reverse transcription PCR to screen liver tissue, using degenerate pan-parechovirus primers targeting the 5' UTR. 97 (33.2%) samples were found to be PCR positive, with mature individuals showing a statistically significantly lower incidence of infection than junior or adolescent individuals. Sequencing of the 205bp products positively identified a picornavirus isolate, however, it was difficult to differentiate between Ljungan, hunnivirus and rosavirus, as they share a highly conserved 5' UTR. To resolve this, further regions of the genome are currently being targeted with both Sanger and Illumina Deep sequencing, which will allow an accurate identification and eventual characterisation of the genome. These findings represent the first detection of a rodent-borne picornavirus in Eastern Europe, extending the known distribution from western Europe and Fennoscandia.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P421

Comparative infection dynamics of Zika virus in New and Old World non-human primates

Claire Ham¹, Debbie Ferguson¹, Jo Hall¹, Adrian Jenkins¹, Elaine Giles¹, Nicola Rose¹, Roger Hewson², Stuart Dowell², Sarah Kempster¹, Neil Almond¹, Neil Berry¹

¹NIBSC, Herts, United Kingdom. ²PHE Porton, Salisbury, United Kingdom

Abstract

Zika virus (ZIKV) represents an emergent global pathogen, although the full pathogenesis spectrum in susceptible hosts is not fully understood. To further understanding of its host range and infection dynamics we undertook a series of studies in different primate species, comparing Old World Indian rhesus macaques (*Macaca mulatta*) and Mauritian cynomolgus macaques (*Macaca fascicularis*) with New World red-bellied tamarins (*Saquinus labiatus*). Virus kinetics and tissue bio-distribution were determined in separate time-course studies in monkeys inoculated sub-cutaneously with the South American/Puerto Rican PRVABC59 strain, euthanised at 3, 42 and 100 days post infection (p.i.). Productive infection was established in all cases. Red-bellied tamarins exhibited rapid virus uptake with a significant viraemic spike in blood 1-2 days p.i. to ($\sim 10^6$ ZIKV RNA copies/ml). Analysis of a range of tissue-types recovered *post-mortem*, indicated widely disseminated ZIKV infection in each species determined by qRT-PCR and RNAScope at both peripheral and central anatomical sites. Virus was widely disseminated 3 days p.i., detectable in multiple tissues especially brain, reproductive tissues and lymphoid organs. We identified some species-specific differences, notably that tamarins were highly susceptible to ZIKV inoculation and displayed rapid, early virus sequestration in lymphoid tissues accompanied by low, persisting levels of ZIKV RNA in multiple skin sites and nerve supply tissues. These findings inform the comparative long-term sequelae of ZIKV infection in multiple primate species, demonstrating a more persisting infection than hitherto recognised with implications for New World sylvatic transmission and potential persistence of ZIKV reservoirs in primate populations in Zika-endemic regions.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P422

Type I IFN responses are less pronounced following infection with a very virulent IBDV strain compared to attenuated or classical strains *ex vivo* and *in vivo*

Katherine Dulwich^{1,2}, Efstathios Giotis², Michael Skinner², Andrew Broadbent¹

¹The Pirbright Institute, Woking, United Kingdom. ²Imperial College London, London, United Kingdom

Abstract

Infectious Bursal Disease Virus (IBDV) is an immunosuppressive birnavirus causing substantial economic losses to the poultry industry worldwide. Despite its preferred tropism for B cells, only one study has investigated B cell gene expression during IBDV infection, in a retrovirus-immortalised cell line. Other studies have utilised fibroblast cells, dendritic cells and tissue from the bursa of Fabricius (BF) or spleen to investigate the response to infection. To study the interaction of IBDV with its target cell population, we infected a recently described chicken *ex vivo* B cell culture model with an attenuated IBDV strain (D78) and a very virulent strain (UK661). IFN-stimulated genes (ISGs) Mx1 and IFIT5 were up-regulated in chicken primary B cells infected with D78 to a greater extent than UK661 at 18, 24 and 48 hours post infection by RTqPCR ($p < 0.001$). Following microarray analysis, we confirmed that many ISGs were more significantly up-regulated following D78 infection than UK661. In addition, we identified key genes involved in B cell activation and signalling (TNFSF13B, CD72 and GRAP) that were down-regulated following infection, which may contribute to immunosuppression. There was no difference in peak virus titre in BF tissue extracted from birds infected with either a classical strain F52/70, or UK661, although a panel of pro-inflammatory and type I IFN-related genes were expressed to a significantly lower level in UK661 compared to F52/70 infected birds. These observations confirm our *ex vivo* findings suggesting UK661 down-regulates antiviral responses more than other IBDV strains, which may contribute to its enhanced virulence.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P423

Generation of antibodies against foot-and-mouth disease virus capsid protein VP4 to study virus entry

Jessica Swanson^{1,2}, Anusha Panjwani¹, Rennos Fragkoudis¹, David J. Rowlands², Nicola J. Stonehouse², Toby Tuthill¹

¹The Pirbright Institute, Woking, United Kingdom. ²University of Leeds, Leeds, United Kingdom

Abstract

The picornavirus family includes significant pathogens such as the animal virus foot-and-mouth disease virus (FMDV), human rhinovirus (HRV) and enterovirus 71 (EV71). VP4 is a small and highly conserved internal capsid protein, which is transiently exposed during a process called virus breathing.

Previous studies have shown that antibodies raised against peptides corresponding to the N-terminus of VP4 have the ability to neutralise HRV and EV71. In this study, we compared the antibody responses induced against FMDV VP4 peptides displayed by conjugation to keyhole limpet hemocyanin (KLH) or by presentation on hepatitis B virus core VLPs. The antibodies were characterised using ELISA, model membrane assays and classical virology to test the ability of the antibodies to recognise VP4 during virus breathing, disrupt VP4 function in model membranes and neutralise virus infectivity.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P424

What factors control alternative proteolysis pathways of the foot-and-mouth disease polyprotein?

Danielle M Pierce, Joseph C Ward, Morgan R Herod, David J Rowlands, Nicola J Stonehouse

University Of Leeds, Leeds, United Kingdom

Abstract

Foot-and-mouth disease affects cloven hoofed animals, and is caused by the picornavirus foot-and-mouth disease virus (FMDV). The FMDV genome contains a single open reading frame, which is translated into one polyprotein that is cleaved by viral proteases to produce viral structural and non-structural proteins. Processing of the polyprotein occurs at three main junctions, generating four primary products; L^{pro}, P1, P2 and P3. These precursors undergo subsequent proteolysis to generate the 'mature' proteins. Cleavage is thought to occur both *in cis* (intra-molecular proteolysis) and *in trans* (inter-molecular proteolysis).

Here, we have used *in vitro* assays to investigate alternative P3 cleavage pathways and the factors controlling differential *cis* and *trans* proteolysis. Coupled transcription/translation pulse-chase experiments in the absence of viral replication suggest that cleavage of P3 may be mediated *in cis* at early times when the relative abundance of precursor is low. This is supported by the observation that cleavage appears to be unaffected by dilution or increasing concentration of viral RNA. Similar outcomes have been observed with other picornaviruses, further supporting our observations. However, the addition of 3C^{pro} *in trans* induced rapid cleavage and the appearance of alternative precursors. These data are consistent with steady-state polyproteins generated after transfection of eukaryotic cells. We are establishing *in vitro* replication of FMDV in cell-derived lysates to investigate which factors help orchestrate differential proteolysis. This system, previously used for other picornaviruses, will allow us to probe the requirements for processing of viral proteins in the presence of replication.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P425

Lipid Metabolism in the Replication of Infectious Bronchitis Virus

Ben Noble, Nicole Doyle, Helena Maier

The Pirbright Institute, Woking, United Kingdom

Abstract

Infectious Bronchitis Virus (IBV) is a coronavirus that naturally infects poultry and is an economically important pathogen for the poultry industry. Like all positive-sense single stranded RNA viruses (+ssRNA), IBV causes membrane rearrangements within the cells it infects to form a range of membranous structures, some of which are novel to IBV infection. It is becoming increasingly clear that lipids play an integral role in the life cycle of this group of viruses, however there is little understanding of the lipid composition of IBV induced membrane rearrangements or the function of lipids in virus replication. The aim of this study is to determine the role of lipid metabolism in IBV replication and assess changes in the localisation of lipids following infection with IBV. Lipid metabolism inhibitors including the fatty acid synthase inhibitor C75 and the cholesterol transport inhibitor U18666A were used to treat DF1 cells at different points during IBV BeauR infection and viral titre was determined by plaque assay. We found a drop in viral titre in cells treated with these inhibitors. Confocal microscopy was used to investigate the changes in localisation of lipids in DF1 cells infected with Beau R. IBV, like other +ssRNA viruses, was shown to rely on normal cellular lipid metabolism in order to replicate, which may provide a new target area for future therapeutics.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P426

Development of *Aedes aegypti* mosquito cell lines stably expressing Zika Virus proteins

Rommel Gestuveo^{1,2}, Margus Varjak¹, Alain Kohl¹

¹MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. ²University of the Philippines Visayas, Iloilo, Philippines

Abstract

Recent outbreaks of Zika Virus (ZIKV) infection in the Americas have led to a global public health alarm. Causing febrile illness similar to other arboviral infections, it was recently associated with cases of microcephaly in neonates of mothers infected during pregnancy. With the threat of superinfection or co-infection with other arboviruses transmitted by the same *Aedes* mosquito vector, effort has been made in studying the molecular interaction of ZIKV and its vector. We constructed plasmids that encode for different V5-tagged ZIKV proteins and zeocin resistance marker to construct cell lines based on *Aedes aegypti* Aag2 cells. The produced cell lines had stable expression of ZIKV proteins as shown by protein half-life and immunoprecipitation assays. Confocal microscopy was able to show protein localization and distribution within the developed mosquito cell lines. Further experiments were designed to assess the functionality of the cell lines. To simulate superinfection, the cell lines were infected with Semliki Forest Virus (SFV) or ZIKV at low multiplicities of infection (MOI) to know the effect of ZIKV proteins on viral replication. RNA interference activity of the cell lines was also evaluated to determine the role of ZIKV proteins in evading mosquito immunity.

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P427

Human interactome of MERS-CoV accessory proteins

Joshua Lee, Lisa Stevens, Andrew Davidson, David Matthews

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 aim to identify cellular proteins that interact with the MERS-CoV accessory proteins ORF3, ORF4a, ORF4b and ORF5 and investigate the role of these interactions in viral infection. 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 and confirmed the close interactions between MERS-CoV accessory proteins and proteins involved in host innate response pathways. Interestingly, ORF4A, ORF4B and ORF5 have a large number of highly confident host protein interactors involved in nuclear transport, suggesting their possible role in nucleocytoplasmic transport modulation. Consistent with previous studies, we have also identified interactions between ORF4a and host proteins that bind to double stranded RNA and play roles in the innate immune response. Our results demonstrate the involvement of MERS-CoV accessory proteins with host proteins involved in innate immune response and with the limited treatment measures available currently, targeting these accessory proteins may serve as a potential therapeutic strategy.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P428

Environmental sampling, a simple surveillance tool for foot-and-mouth disease

Emma Brown¹, Claire Colenutt¹, Jenny Maud², David Paton¹, Bishnu Adhikari³, Noel Nelson^{4,1}, Jose Gonzales⁵, Keith Sumption², Simon Gubbins¹

¹The Pirbright Institute, Woking, United Kingdom. ²European Commission for the Control of Foot-and-Mouth disease (EuFMD), Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy.

³Food and Agriculture Organization of the United Nations Representation in Nepal, United Nations Building, Kathmandu, Nepal. ⁴Met Office, Exeter, United Kingdom. ⁵Wageningen BioVeterinary Research, Lelystad, Netherlands

Abstract

Foot-and-mouth disease virus (FMDV) is a viral disease of cloven-hoofed animals characterized by formation of vesicles around the mouth and on the feet. The main transmission route of FMDV is through direct contact, yet indirect transmission such as via fomites and aerosols have been shown to play a role in the spread of the disease.

Environmental sampling is a non-invasive, relatively cheap sampling tool that can be applied with limited technology and expertise. We present sampling methods developed and validated in experimental conditions whereby live virus and virus genome were recovered for up to 7 days from a contaminated environment. The methods were then assessed in field conditions, in Nepal which is endemic for FMD. A total of 226 environmental samples were taken from a variety of surfaces, including from the floor, wall, tether posts, food/water troughs. FMD viral RNA was successfully detected, in 72 samples, by RT-PCR. Samples were taken from small holdings, a milk collection point and a goat market. Sites were selected where apparently healthy, clinically affected or recovered livestock, were or had been present or their products had been present.

Results show that environmental sampling can be used as a simplistic surveillance tool for FMD with the potential to confirm the presence of disease before clinical signs can be confirmed and after recovery of animals. In an FMD free country, these methods could contribute to rapid detection of disease and the implementation of control measures, and also help inform on re-stocking time after an outbreak.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P429

Selection of thermally-resistant mutants of enterovirus 71 (EV71) for future application as a genome-free VLPs vaccine.

Mona Shegdar, Oluwapelumi O. Adeyemi, Elisabetta Groppelli, Nicola Stonehouse

University of Leeds, Leeds, United Kingdom

Abstract

Mona Shegdar, Oluwapelumi O. Adeyemi, Elisabetta Groppelli, Nicola. J. Stonehouse.

School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, UK.

Enterovirus 71 (EV71) is a picornavirus which causes hand, foot and mouth disease (HFMD) among children and infants. It is usually associated with mild infection, but similarly to poliovirus (PV), EV71 can also cause serious neurological complications and can have significant mortality. Several vaccine candidates are in clinical trials. However, there is a drive towards development of safer vaccines such as those that are based on virus-like particles (VLPs). VLPs can have thermal instability which therefore can have implications for their use in vaccine development. This study aims to identify thermally-stable EV71 mutants by applying our established protocols with PV. The capsid proteins will be used to generate VLPs as the basis of a future genome-free EV71 vaccine candidate.

There are several EV71 strains, this work is focused on two strains - B2 and C. The temperature at which > 99.99% of each virus was inactivated was determined. This temperature was used in thermally stressing experiments to select structurally-stable EV71 mutants from the viral quasi-species. The structural protein precursor will be over-expressed a heterologous expression system e.g. in yeast, along with the viral protease 3CD in order to produce EV71 VLPs, which will be characterised in term of their antigenicity and structure.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P430

Novel antibody binding determinants of serotype O Foot-and-mouth disease virus

Mana Mahapatara, Sasmita UPADHYAYA, Amin Asfor, Satya Parida

The Pirbright Institute, Woking, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV) displays various epitopes on the capsid outer surface. Five neutralising antigenic sites have been identified in serotype O FMDV using murine mAbs. In addition, there is evidence of the existence of other, yet unidentified epitopes, which could play a role in antibody-mediated protection. However, the relative importance of different epitopes in FMD vaccine induced-protection has not been ascertained to date. Comparison of the ability of bovine antisera to neutralize a panel of serotype O FMDV identified three novel putative sites at VP2-74, VP2-191 and VP3-85, where amino acid substitutions correlated with changes in sero-reactivity. The impact of these positions was tested using site-directed mutagenesis to effect substitutions at critical amino acid residues within an infectious copy of serotype O FMDV. Using reverse genetics technique a series of recombinant viruses were generated out of which two recombinant viruses, (1) by substituting the critical amino acid residues of the five neutralising antigenic sites of a FMDV type O cDNA clone (5M), and (2) by adding two additional substitutions at position VP-74 and VP2-191 (M6), are noteworthy. Serological characterisation of 5M and M6 viruses revealed 56% and 74% reduction in VNT titre reflecting the significance of these residues in the antigenicity of the virus. However it is possible that more unidentified epitopes may exist as 100% reduction in neutralization was not observed. Work is ongoing in our laboratory to identify additional capsid amino acid residues that could have an impact on the antigenic nature of the virus.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P431

Viral shedding, genetic stability and mucosal immune response to Rotarix® in a cohort of vaccinees in the UK.

Ledicia Botas-Perez¹, Jane L Mitchell¹, Martin Fritzsche², Mark D Preston², Miren Iturriza-Gomara³, Nicola J Rose¹

¹Division of Virology, National Institute for Biological Standards and Control (NIBSC), The Medicines and Healthcare Products Regulatory Agency, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, United Kingdom. ²Division of Technology, Development and Infrastructure, National Institute for Biological Standards and Control (NIBSC), The Medicines and Healthcare Products Regulatory Agency, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, United Kingdom. ³Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool L69 7BE, United Kingdom

Abstract

A two-dose, live-attenuated G1P[8] rotavirus (RV) vaccine was implemented in the 2013 UK programme. We are investigating viral loads and genetic stability of Rotarix® shed in stool post vaccine doses, in the context of mucosal protection by measuring faecal anti-RV secretory IgA (sIgA) levels.

Sequential faecal samples from twelve infants were collected throughout their vaccination period. Viral RNA (vRNA) shedding was assessed in 17-45 samples from each infant using a vaccine strain-specific qPCR. Genetic stability of vaccine virus has been assessed at 5-6 time-points in two infants for the genes encoding VP3, VP4, VP6, VP7, using specific primers and Nextera® XT DNA v2 kit on an Illumina® MiSeq or RNA ScriptSeq® v2 kit to sequence directly from vRNA to assess all 11 genes. Anti-RV sIgA levels are being evaluated as a proportion of the total faecal sIgA by ELISA.

Vaccine strain in stool was detected at 10^3 - 10^9 copies/g. All infants shed detectable virus after dose 1 and 11/12 after dose 2. Viral loads and duration of shedding fluctuated between infants and with duration of shedding, but at the peak of shedding were within the range of wild-type infections (10^2 - 10^{10} copies/g of stool), suggesting active replication in all infants.

NGS data suggest the emergence of SNPs, a consequence of viral replication during shedding, with the highest number of SNP loci being present in the VP4 encoding gene. The accumulation of mutations in the vaccine strain is being investigated based on shedding load and duration.

Virology Workshop: Positive Strand RNA Viruses

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P432

The Short end of the Stick: Truncating mutations of the S-fragment reveal insights into the role of the 5' untranslated region in FMDV replication

Joseph C Ward¹, Lidia Lasecka-Dykes², Sarah Gold², David Kealy¹, Morgan R Herod³, Toby Tuthill¹, David J Rowlands¹, Nicola J Stonehouse¹

¹University of Leeds, Leeds, United Kingdom. ²Pirbright Institute, Pirbright, United Kingdom. ³University of Leeds, Leeds, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV) is a single-stranded positive-sense RNA virus in the picornavirus family. It is the causative agent of foot-and-mouth disease, an important pathogen of cloven-hoofed animals. The unusually long 5' untranslated region (5' UTR) includes five highly structured domains. The functions of two of these, the *cre* and the IRES, are well understood but little is known of the roles of the remaining three elements. These include a 360 nucleotide region, the S fragment, located at the 5' terminus and predicted to fold into a single stem loop, a poly(C) tract of varying length and a region comprising up to 4 tandemly repeated pseudoknots. Many of these structures have no prescribed function but are assumed to be involved in viral replication or translation.

We have used FMDV replicons and recombinant viruses to investigate features of structural elements within the 5' UTR. Using selective 2'-hydroxyl acylation analysed by primer extension (SHAPE), we provide experimental support for the predicted structure of the S-fragment. In addition, extensive mutagenesis of both replicons and infectious virus has illuminated the role of this element in replication. We demonstrate that the length of the stem-loop is more important than the specific sequence and that the effects of some mutations are cell type dependent. We have also investigated the binding of host proteins to the S-fragment and how truncating mutations alter these interactions.

Together these studies throw further light on the enigmatic roles of unusual features present in the 5' UTR of FMDV.

Virology Workshop: Positive Strand RNA Viruses
Zone D
Presentations: Thursday Evening and Friday Lunchtime

P433

Identification of dengue virus immunodeficiency pathway antagonists in *Aedes aegypti* cells

Louisa Elizabeth Wallace, Kevin Maringer

University of Surrey, Guildford, United Kingdom

Abstract

Dengue virus (DENV) is the most significant arthropod-borne virus (arbovirus) of humans with *Aedes aegypti* being its major mosquito vector for transmission. There are currently no specific therapeutics and the existing DENV vaccine exhibits limited efficacy. Therefore, vector control remains the best approach to manage the spread of disease.

By studying molecular barriers of arbovirus transmission in disease vectors may allow these to be exploited to prevent human disease. One major barrier is the mosquito innate immune response, which includes the immunodeficiency (IMD) pathway, an NF- κ B-inducible pathway implicated in *Ae. Aegypti* antiviral immune responses. Our laboratory previously demonstrated that DENV-2 infection does not induce IMD signalling in the *Ae. aegypti* Aag2 cell line, recapitulating *in vivo* data from other research groups. Moreover, infection with DENV-2 reduces subsequent IMD activation by classical immune stimuli such as heat-inactivated bacteria. This project aims to identify the DENV-2 protein(s) responsible for this antagonism by establishing an RT-qPCR-based screening platform in which IMD signalling is stimulated in cells transfected with plasmids expressing DENV-2 proteins individually or in combination. Once identified, the IMD antagonist(s) can be used to enhance our understanding of *Ae. aegypti* antiviral immunity by investigating its interaction and localisation with its cellular targets. Ultimately, we will use our first-in-field CRISPR knockout mosquito cell lines to confirm the relevance of these interactions during viral infection.

These insights may contribute to the development of genetically modified transmission-incompetent mosquitoes, which will ultimately reduce the global burden of dengue disease.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P434

Pneumococcal vaccine impacts on the population genomics of non-typeable *Haemophilus influenzae*

David Cleary¹, Vanessa Devine¹, Denise Morris¹, Karen Osman¹, Rebecca Gladstone², Stephen Bentley², Saul Faust³, Stuart Clarke¹

¹University of Southampton, Southampton, United Kingdom. ²Wellcome Trust Sanger Institute, Hinxton, United Kingdom. ³University Hospital Southampton Foundation NHS Trust, Southampton, United Kingdom

Abstract

Background: Non-typeable *Haemophilus influenzae* (NTHi) are recognised to cause significant human disease and there is evidence that the epidemiology can be altered as a consequence of the introduction of pneumococcal conjugate vaccines (PCVs). Here, we examined the impact of PCV13 introduction in a UK paediatric population.

Methods: Nasopharyngeal swabs were collected from children <5 years of age attending outpatient clinics at University Hospital Southampton Foundation NHS Trust during five consecutive winters, October - March, 2008/9 to 2012/13. The phylogeny of 275 NTHi isolates was examined using hierarchical Bayesian Analysis of Population Structure (hierBAPS). Lineage diversity, stability during PCV13 introduction and levels of recombination were also examined.

Results: NTHi were associated with carriage of vaccine serotype *Streptococcus pneumoniae* in the pre-PCV13 era ($p < 0.05$, OR 2.36, 95% CI 1.17-4.75). Following PCV13 introduction, significantly increased carriage of NTHi was observed in two of the three years examined ($p < 0.05$). Genomic analysis revealed a highly recombinogenic, diverse population (Simpsons MLST diversity, 1-D: 0.97 - 0.99) that could be characterised into eleven temporally stable lineages. Increased carriage was not linked to the expansion of a particular lineage. However a significant association of lineage 6 with *S. pneumoniae* in both pre- and post-PCV13 eras, OR of 14.75 (95% CI: 3.14-69.38) and 16.95 (95% CI: 0.93-309.96), was observed.

Conclusion: We have shown that the introduction of PCV13 increased NTHi carriage prevalence in a paediatric population, that the eleven lineages displayed remarkable temporal stability during this period, and there exists lineage specific *S. pneumoniae* associations.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P435

Genomic analysis of two pandemic, high-risk multi drug resistant clones of *Pseudomonas aeruginosa*

James Redfern, Joanna Verran, Elliot Whittard, Roobinidevi Ragupathy, Mark Enright

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Pseudomonas aeruginosa is a Gram-negative microorganism capable of causing severe and life-threatening human infections. It is generally agreed that *P. aeruginosa* has a non-clonal epidemic population, in which successful high-risk clones occasionally arise. Recent whole genome studies and genome-wide association studies (GWAS) have started to highlight the importance of understanding high-risk clonal genomics, particularly in relation to antibiotic resistance profiles. ST235 and ST111 are prominent multidrug resistant, global *P. aeruginosa* clones. This study analysed 260 *P. aeruginosa* genomes (153 of which belong to the ST111 clone and 107 to the ST235 clone). Following maximum-likelihood and Bayesian phylogenetic analysis (BEAST2), genomes were annotated (using PROKKA) and a pan-genome was created using Roary. Antibiotic resistance genes were identified within the pan-genomes. Analysis shows both clone pan-genomes contained a similar number of genes (approx. 15,000). ST111 pan-genome contained easily-identifiable blocks of genes that were present in some sub-populations but absent from others. These contained genes conferring antibiotic resistance and metal resistance as well as type IV secretion system genes. ST235 gene presence/absence was less homogeneous across its pan-genome compared to ST111. Antibiotic resistance genes represented a variety of Mux efflux complexes, beta-lactam resistance, aminoglycoside resistance in addition to chloramphenicol, sulfonamide and fosfomycin resistance. Further analysis is required to understand the evolutionary differences/similarities between the two clones, and the wider *P. aeruginosa* population.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P436

Probing genomic aspects of the multi-host pathogen *Clostridium perfringens* reveals significant pangenome diversity, and a diverse array of virulence factors

Raymond Kiu^{1,2}, Shabhonam Caim¹, Sarah Alexander³, Purnima Pachori⁴, Lindsay Hall¹

¹Quadram Institute Bioscience, Norwich, United Kingdom. ²University of East Anglia, Norwich, United Kingdom. ³Public Health England, London, United Kingdom. ⁴Earlham Institute, Norwich, United Kingdom

Abstract

Background: *Clostridium perfringens* is an important causative agent of animal and human infections, however information about the genetic makeup of this bacterium is currently limited. Thus, we characterised the genomic variation, pangenomic diversity, and virulence traits of 56 *C. perfringens* strains, representing the largest genomic study of this pathogen to date.

Methods: We performed an array of *in silico* analysis on 56 sequenced genomes (including 5 historical isolates sequenced by PacBio), including phylogenetic and pangenome analysis, genome-wide functional annotation, and CRISPR, prophage, toxin and antimicrobial resistance profiling.

Results: Our analysis indicated that *C. perfringens* has an 'open' pangenome comprising only 12.6% of core genes, identified as the most divergent single-species bacterial pangenome currently reported. Profiling virulence-associated factors confirmed presence of well-characterised *C. perfringens*-associated toxins genes including α -toxin (*plc*), enterotoxin (*cpe*) and perfringolysin O (*pfo*). Furthermore, our analysis indicated significant horizontal gene transfer events as defined by presence of prophage genomes, and notably absence of CRISPR defence systems in >70% of the strains. In relation to antimicrobial resistance, tetracycline resistance genes (*tet*) and anti-defensins genes (*mprF*) were consistently detected (*tet*: 75%; *mprF*: 100%). However, pre-antibiotic-era strains (isolated before 1950s) did not encode *tet*, thus implying antimicrobial selective pressures in *C. perfringens* evolutionary history over the past 70 years.

Conclusion: This comprehensive genomic study of *C. perfringens* reveals the full profile of virulence-related genes, and sheds lights on the involvement of prophages in shaping the highly-divergent pangenome. These data suggest future research directions into this medically, and veterinary important pathogen.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P437

Spark your joy: Kon Mari for microbiologists

Ben Thomas

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Bioinformatics is increasingly driving how microbiologists explore and understand microbial ecology. But as a discipline bioinformatics has increasingly forked into a service wing and an intellectual wing: those who wrestle with tools and those who create them.

The terrifyingly bright people in the intellectual wing are engaged in wheeling aerial brain-combat to elucidate as much information as possible from information constrained systems. This bleeding edge of bioinformatics is a Wild-West frontier built on Github; where the six-shooters are algorithms. Those in the service wing (the dystopian bioinformatics underclass) primarily spend time connecting the tools handed to them by the intellectuals in a gossamer web of interfaces often optimistically called a “pipeline”. With a paucity of version control and change management frameworks many of these pipelines encompass various versions of barely understood black-box software cobbled together with the software equivalent of duct tape.

In her book, ‘The Life Changing Magic of Tidying’, Marie Kondo defined her philosophy as essentially framing every aspect of your life with the question: “does it spark joy?”. With a background in Asset and Fund Management (and the designer of the AMPLY pipeline for novel antimicrobial detection from from 'omic data) Ben examines why the zombie ideas at the heart of the global financial crisis of 2008 are stalking modern microbial bioinformatics, a few critical rules for creating your own bioinformatics pipeline and how he managed to spark his own joy.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P438

Colonization of broiler chickens by *Campylobacter jejuni* is initiated by a single bacterial cell

Joseph Wanford¹, Lea Lango-Scholey¹, Harold Nothaft², Christine Szymanski², Christopher Bayliss¹

¹University of Leicester, Leicester, United Kingdom. ²University of Alberta, Edmonton, Canada

Abstract

Phase variation (PV), involving stochastic switches in gene expression, is exploited by the human pathogen *Campylobacter jejuni* to adapt to different environmental and host niches. PV genes of *C. jejuni* modulate expression of multiple surface determinants, and hence may influence host colonization, and propensity to cause human disease. Population bottlenecks can rapidly remove the diversity generated by PV and strict single-cell bottlenecks can lead to propagation of PV states with highly divergent phenotypes.

Using a combination of identification of PV genes from next generation sequencing (NGS) data, high-throughput fragment size analysis and comparison with *in vivo* and *in silico* bottleneck models, we have characterised a strict population bottleneck during the experimental colonisation of broiler chickens with *C. jejuni* strain 81-176. We identified high levels of variation in five PV genes in the inoculum, and subsequently, massively decreased population diversity following colonisation with each bird colonised by a dominant five-gene phasotype. 21 of 22 contained a different dominant phasotype to other birds. These phasotypes were present in the inoculum indicative of random sorting through a narrow, non-selective bottleneck during colonisation.

These results are evidence of the potential for confounding effects of phase variation on *in vivo* studies of *Campylobacter* colonisation factors. And an argument for population bottlenecks as mediators of stochastic-variability in the propensity of *Campylobacters* to survive through the food-chain and cause clinical human disease.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P439

Genomic analysis of a novel *Rhodococcus equi* isolate from a bovine host

Megan. L Paterson¹, Diyanath Ranasinghe¹, Lynn. G Dover¹, Iain. C Sutcliffe¹, Bruno. S Lopes², Vartul Sangal¹

¹Northumbria University, Newcastle upon Tyne, United Kingdom. ²University of Aberdeen, Aberdeen, United Kingdom

Abstract

Rhodococcus equi strains are Gram-positive, obligate aerobic bacteria that are responsible for pneumonia-like infections in foals, with high mortality rates. *R. equi* can also infect a number of other animals and is emerging as an opportunistic human pathogen. A previous study showed the presence of a *paa* operon in a human isolate that may be associated with the 'host jump'. In this study, we analysed the draft genome sequence of a novel *R. equi* isolate, B0269, isolated from the faeces of a bovine host. Comparative genomic analyses with seven other published genomes from equine or human sources revealed a pan-genome comprising of 6,876 genes with 4,141 genes in the core genome. 276 genes were specific to the bovine isolate, mostly encoding hypothetical proteins of unknown function. However, these genes include four copies of *terA* and five copies of *terD* genes that may be involved in stress responses.

Virulence characteristics in *R. equi* are associated with the presence of large plasmids carrying a pathogenicity island, including genes from the *vap* multigene family. A BLAST search of the protein sequences from known virulence-associated plasmids (pVAPA, pVAPB and pVAPN) revealed a similar plasmid backbone on two contigs in bovine isolate B0269; however, no homologues of the main virulence associated genes, *vapA*, *vapB* or *vapN* were identified. In summary, this study confirms that *R. equi* genomes are highly conserved and reports the presence of a novel plasmid in the bovine isolate B0269 that needs further characterisation to understand its potential involvement in virulence properties.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P440

Characterising intragenic promoters and H-NS repression in *E. coli*

Emily Warman, Shivani Singh, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

The histone-like nucleoid structuring (H-NS) protein is responsible for repressing transcription that initiates spuriously 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 show that randomly generated promoters, and promoters within AT-rich genes, 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 non-specific interactions between the promoter and RNA polymerase sigma factor.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P441

Essentiality of pSCL4, A Giant Linear Plasmid of *Streptomyces clavuligerus*.

Lis Algora, Paul Herron

University of Strathclyde, Glasgow, United Kingdom

Abstract

Clavulanic acid is a β -lactamase inhibitor that is commonly used in combination with the β -lactam antibiotic Amoxicillin. It is produced by *Streptomyces clavuligerus*, a bacterium belonging to the phylum *Actinobacteria*. The genome of *S. clavuligerus* includes a linear chromosome and four linear plasmids pSCL1, pSCL2, pSCL3 and pSCL4. With a length of 1.8 Mb, pSCL4 is the largest linear plasmid sequenced to date. It carries 20% of the *S. clavuligerus* coding sequences, none of which are thought to be involved in the primary metabolism of the bacterium and therefore its essentiality is questionable. However, sequencing results showed that the genes encoding the terminal proteins Tap and Tpg were located on pSCL4. These proteins are necessary for telomere replication of linear replicons in streptomycetes. As *tap* and *tpg* are absent from the chromosome, it suggests that *S. clavuligerus* might rely on these plasmid-encoded proteins to maintain the chromosome ends. In this study we have confirmed that *tap* and *tpg* are present as single copies in the genome of *S. clavuligerus* type strains by Southern hybridisation of digested genomic DNA. This indicates an essential role for pSCL4 in provision of the terminal proteins. Further experiments involving deletion of the *tap-tpg* operon from pSCL4 will confirm the role of the plasmid-encoded proteins on maintenance of chromosomal linearity and telomeric sequences. In addition, we are also constructing *S. clavuligerus* mutants complemented with second copies of *tap-tpg* that we predict will leave pSCL4 dispensable for growth of the bacterium.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P442

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

Tom Guest, Gemma Warren, James Haycocks, David Grainger

Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

Vibrio cholerae is an aquatic bacterium and the cause of cholera, an acute and potentially fatal diarrhoeal disease. Throughout human history cholera has been a significant cause of morbidity and mortality and remains so (particularly for children under 5 years) in endemic regions or areas where local infrastructure has been devastated by natural disaster or war, limiting access to clean potable water.

Between outbreaks *V. cholerae* can persist in aquatic environments by colonising alternative hosts (such as fish) and by the formation of biofilms on chitinous surfaces, switching from its motile planktonic form. The production of vibrio polysaccharide (or VPS, an exopolysaccharide) is necessary for the formation of biofilms, and its production is coordinated by a complex regulatory network that links quorum sensing with the bacterial second messenger cyclic-di-GMP. C-di-GMP is known to up-regulate the expression of genes involved in biofilm formation and repress virulence in several bacterial pathogens. In *V. cholerae* the transcriptional regulator VpsT requires c-di-GMP to bind DNA and regulate the expression of *vps* genes.

To better understand the global regulatory role of VpsT, we used ChIP-seq to investigate its distribution across the genome of *V. cholerae* El Tor E7946. Our data suggest that VpsT binds to and potentially regulates additional targets in the genome other than the *vps* genes, possibly expanding the VpsT regulon. These targets include genes encoding proteins involved in motility and adhesion, as well as the degradation of c-di-GMP. We have subsequently investigated the role of VpsT binding at these targets.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P443

Reconstructing microbial genomes from a frozen 5000 year old iceman's microbiome.

Shabhonam Caim, Raymond Kiu, Magdalena Kujawska, Lindsay Hall

Quadram Institute, Norwich, United Kingdom

Abstract

Extracting and assembling individual microbial genomes from metagenomes is challenging, due to the complexity of the data. Availability of computational resources, and also bioinformatics expertise for establishing and applying appropriate processing and analytical pipelines, are also important considerations. Here, we describe a new bioinformatics pipeline to extract and assemble high quality single microbial genomes from complex gut-associated metagenomes. We benchmarked and compared existing methods to determine robustness of our pipeline. To validate our method, we have utilised gut metagenomic data (~700 million paired-end Illumina reads) obtained from a frozen iceman, which is roughly 5000 years old. Our method showed successful extraction and reconstruction of the pathogenic bacterium *Clostridium perfringens*. Further checks for example average nucleotide identity (ANI) determined 98% identity to publicly available genome based assemblies of *C. perfringens*. We also determined that the reconstructed genome showed a 'clean' anti-microbial resistance (AMR) profile i.e. no AMR genes, which highlights the evolution of specific AMR in response to antibiotic usage. Thus our pipeline for single species extraction and assembly from complex metagenomes provides an additional open source tool for genomic assembly and downstream analysis, and may prove useful in other metagenomic contexts.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P444

CRP is a master regulator of lifestyle switching and accessory toxin expression in *Vibrio cholerae*

James Haycocks, Jainaba Manneh, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

Vibrio cholerae is the causative agent of cholera, a disease that affects 4.3 million people each year and results in up to 140,000 deaths (WHO, 2015). 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 coastal waters, where *V. cholerae* can colonise the chitinous surfaces of shellfish, and ii) the human intestinal tract.

In this work, we have studied the regulatory mechanisms that govern switching between these two lifestyles. The cyclic AMP receptor protein (CRP) is a global transcription factor that is responsive to nutrient availability. Given that the two habitats of *V. cholerae* vary significantly in nutrient levels, we reasoned that CRP might act as a regulator for lifestyle switching to support survival in both the human intestine and marine environment.

We used ChIP-seq to map the global binding profile of CRP across the genome of *Vibrio cholerae* El Tor N16961. Our data shows that CRP targets genes involved in biofilm formation (e.g. *cqsA*), chitin metabolism (e.g. *nagE*), and colonisation of the human intestine (e.g. *ompU*) supporting our hypothesis that CRP has a role in *V. cholerae* lifestyle switching. In addition, we have investigated unusual CRP binding at the *PhlyA* promoter, which controls expression of a haemolysin.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P445

TaF: A Web Platform for Taxonomic Profile-based Fungal Gene Prediction

Sin-Gi Park¹, Dong Sung Ryu¹, Hyunsung Lee¹, Hojin Ryu², Yong Ju Ahn¹, Seung il Yoo¹, Junsu Ko¹, Chang Pyo Hong¹

¹Theragenetex, Suwon, Korea, Republic of. ²Chungbuk National University, Cheong-ju, Korea, Republic of

Abstract

Accurate prediction and annotation of gene structures from the genome sequence of an organism enable genome-wide analyses including the identification of targeted genes associated with a trait, transcriptome profiling, comparative genomics, and evolutionary analysis, thus providing the insights into the biological properties of organism. We have recently developed an high accurate fungal gene prediction pipeline and web platform, so-called 'TaF'. TaF is a gene predictor on the base of an *ab initio*, transcriptome and/or protein evidence-drivable prediction methods. TaF searches for protein sequences for each of close relatives with annotated genomes by taxonomic profiling, generates evidence hints for predicting exon-intron boundaries by aligning the searched protein sequences to an assembled fungal sequence, and then predicts gene structures by using protein sequence homology and *ab initio* information. TaF will provide great potential to improve the quality of gene prediction in newly sequenced genomes.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P446

Prevalence and distribution of phase-variable type I restriction modification systems in the *Lactobacillus casei* group

Joseph Ralph¹, Ana Neves², Marco Oggioni¹

¹Department of Genetics and Genome Biology, University of Leicester, Leicester, United Kingdom.

²Bacterial Physiology and Improvement, R&D Microbial Platform, Chr. Hansen A/S, Hørsholm, Denmark

Abstract

Recently a phase-variable type I restriction-modification-system(RMS) in the lactic acid bacterium *Streptococcus pneumoniae* has been shown to influence virulence. This system can switch between six global methylation patterns through the recombination of multiple target recognition domains of the *hsdS*(specificity) genes facilitated by a Cre-recombinase. Here we present an analysis of phase-variable type I RMS in deposited genome sequences of the *Lactobacillus casei* group, including *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. zeae*. Data show the presence of three type I RMS across the group, two phase-variable and one non-phase-variable, the latter is largely conserved and located on plasmids although chromosomal integration is observed. The phase-variable RM loci in the *L. casei* group is composed of one *hsdR*, one *hsdM* and up to three *hsdS* genes in opposite orientation interspaced by a recombinase, the locus is not part of the core genome. While the *hsdR/M* genes show >90% identity in all *L. casei* group strains carrying the locus, *L. rhamnosus* strains contain a different phase-variable system and importantly the *hsdS* genes show significant heterogeneity between strains. Identical *hsdS* genes are shared by one group of closely related *L. casei* strains. This group of strains would be expected to methylate the same chromosomal targets, except the methylase (*hsdM*) shows a conserved frameshift. The distribution of phase-variable type I systems to selected phylogenetic clusters with conservation of the *hsdRM* modules and variation of the phase-variable *hsdS* genes is reminiscent of the situation in *Listeria monocytogenes* and *Streptococcus suis*.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P447

Investigating the within-patient genotypic diversity of *Helicobacter pylori* populations from different niches of the human stomach

Daniel Wilkinson¹, Alan McNally², Benjamin Dickins¹, John Atherton³, Karen Robinson³, Jody Winter¹

¹Nottingham Trent University, Nottingham, United Kingdom. ²University of Birmingham, Birmingham, United Kingdom. ³University of Nottingham, Nottingham, United Kingdom

Abstract

Helicobacter pylori has vast genetic diversity at a global level and diversity has also been observed between family members and within the stomachs of individual patients, with infected individuals thought to harbour a unique and diverse population of *H. pylori* quasispecies. Despite this, *H. pylori* diversity between and within different niches of the human stomach is not yet well understood.

We investigated both within niche and between niche *H. pylori* variability within the same stomach, using whole genome population deep sequencing and single colony whole genome sequencing. Paired biopsies from the antrum (non-acid producing) and corpus (acid producing) regions were obtained from 8 patients selected based on antrum-corpus differences in inflammation, atrophy, activity, intestinal metaplasia, strain virulence and antimicrobial resistance profiles.

Polymorphic alleles were identified most frequently in hypothetical and outer membrane proteins and virulence genes such as *vacA* within population sweeps. The pan-genomes of paired antrum and corpus populations were different but grouped together phylogenetically. This indicated that initial infection of each patient was with a single strain that diversified within different stomach niches, except for one patient that showed evidence of multiple infecting strains with subsequent diversification. Single colonies from the antrum and corpus of the same patient grouped into respective clades and common unique accessory genes were observed between the grouped antral and corpus strains suggesting some common mechanisms for niche adaptation across several *H. pylori* strains. Recombination was observed both within niches and between different niches of the same stomach.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P448

Effectiveness of pneumococcal conjugate vaccines on serotype 6C paediatric carriage

Laurence Baker¹, Jessica Jones¹, Rebecca Gladstone², David Cleary^{1,3}, Denise Morris¹, Stephen Bentley², Saul Faust^{1,3,4}, Stuart Clarke^{1,3,5,4}

¹Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, United Kingdom. ²Infection Genomics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom. ³NIHR Southampton Biomedical Research Centre, University Hospital Southampton Foundation NHS Trust, Southampton, United Kingdom. ⁴NIHR Southampton Clinical Research Facility, University Hospital Southampton Foundation NHS Trust, Southampton, United Kingdom. ⁵Global Health Research Institute, University of Southampton, Southampton, United Kingdom

Abstract

Background: *Streptococcus pneumoniae* is a nasopharyngeal opportunistic pathogen with a high incidence of morbidity and mortality. The seven-valent pneumococcal vaccine (PCV7) lacked sufficient cross-protection against novel non-vaccine serotype (NVT) 6C. The inclusion of 6A in the thirteen-valent pneumococcal vaccine (PCV13) may reduce the burden of 6C carriage.

Methods: 3455 nasopharyngeal samples were collected over 10 years from children ≤ 4 years and cultured on selective media. 1131 putative pneumococci were confirmed by displaying optochin sensitivity. DNA was extracted and sent to the Wellcome Trust Sanger Institute for whole genome sequencing. Serotype 6C was distinguished using the *in-silico* Pneumococcal Capsular Typing tool (PneumoCaT), with sequence types (ST) identified using Short-Read Sequence Typing 2.

Results: PneumoCaT confirmed 61 serotype 6C isolates, the second most prevalent serotype overall, following serotype 11A. Following PCV13 introduction, 6C carriage significantly decreased between 2010/11 and 2015/16 ($p=0.004$), from 9.18% to 1.98% respectively (1.5% annually, CI 0.8-2.1). Fourteen unique sequence types were identified in total. ST1692 and ST1714 were the most frequently observed STs; both ST1692 and ST1714 significantly decreased in prevalence ($p=0.008$, $p=0.03$).

Conclusions: PCV13 appears to have reduced carriage of pneumococcal serotype 6C in paediatric populations since its introduction in 2010. Serotype replacement by novel NVTs may reduce effectiveness of current and future vaccines, hence the rapid evolution of pneumococci in response to vaccine selective pressures makes continued surveillance critical to disease prevention and vaccine efficacy.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P449

Genomic evolutionary history of Legionnaires' disease in Scotland

Bryan Wee¹, Joana Alves¹, Diane Lindsay², Andrew Smith², Ross Fitzgerald¹

¹The Roslin Institute, Edinburgh, United Kingdom. ²Scottish Haemophilus Legionella Meningococcus Pneumococcus Reference Laboratory, Glasgow, United Kingdom

Abstract

The Scottish Microbiology Reference Laboratories have maintained a comprehensive collection of clinical isolates of *Legionella* spp. from around Scotland ever since one of the first recognised outbreaks of Legionnaires' disease in the United Kingdom in 1984. In this study, whole genome sequencing (WGS) was carried out to investigate relationships of both clinical and environmental isolates from this collection in the context of global strains. A total of 900 *L. pneumophila* isolates including 400 from the Scottish collection and 500 publicly available genomes were analysed. Population genomic analyses including phylogenetic reconstruction, examination of gene flow and genome-wide association studies was also performed.

The data show that all major outbreaks in Scotland over the last 30 years were caused by unrelated lineages representative of the global diversity. WGS also revealed clusters of isolates that persist in specific locations over several decades with the potential to cause sporadic human infections. Genome-wide association analyses (GWAS) on this dataset also clearly highlight that genes responsible for serogroup specificity are over-represented in clinical isolates and that the evolution of these genes are strongly influenced by recombination.

This study represents the largest and most diverse population genomics study of *L. pneumophila* to date showing that strains responsible for Legionnaires' disease are drawn from across the species phylogeny. GWAS analysis also highlights the potential role of the *Legionella* lipopolysaccharide in human infection irrespective of clonal lineage, suggesting that specific lipopolysaccharide properties may enhance its ability to cause human disease.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P450

Population metagenomics of the porcine pathogen *Lawsonia intracellularis*

Rebecca J. Bengtsson¹, Bryan A. Wee¹, Rodrigo Bacigalupe¹, Eleanor Watson², Alan L. Archibald¹, J. Ross Fitzgerald¹, Tahar Ait-Ali¹

¹The Roslin Institute, Edinburgh, United Kingdom. ²Moredun Research institute, Edinburgh, United Kingdom

Abstract

Lawsonia intracellularis (LI) is a Gram-negative obligate intracellular bacterium and the aetiological agent of proliferative enteropathy (PE), a common intestinal disease which affects many mammalian species, most notably pigs. The severity of PE ranges from a mild, chronic form, often observed in grower pigs to a severe, acute form in young pigs. The complete genome of LI of 1.7MB has been sequenced and is composed of a circular chromosome of 1.4Mbp and 3 plasmids of 27, 39 and 194Kbp. The dynamics of LI infection have been examined, but currently very little is known about the genetic basis for its pathogenesis. We utilised a metagenomic shotgun approach to directly sequence from faecal and ileum samples of infected pigs and horses to assemble LI genomes. In total, 21 LI assemblies were obtained for isolates from UK, Poland and Sweden. Comparative genomic analysis identified a complement of 1,344 genes that are shared by all strains and very small number of accessory genes observed representing 7% of the genome on average. Reconstruction of the phylogeny based on core single nucleotide polymorphisms revealed very limited diversity across two sub-lineages each comprising strains from multiple geographic locations. Ongoing work involves identification of bacterial traits that may influence the severity of PE in pigs.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P451

Costs of Host Switching and Associated Allelic Variants during Viral Adaptation to Alternating Hosts

Oyeronke Ayansola, Benjamin Dickins

School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom

Abstract

Responding to novelty is a challenge for an evolving population and for a virus a substantial part of its environment is the host that it infects. Host-switching capability may be constrained by fitness trade-offs or by evolutionary or ecological interactions. Understanding these (non-exclusive) factors may improve our understanding of the emergence of new viral diseases.

Here the bacteriophage ϕ X174 is used as a model with laboratory strains of *Escherichia coli* (C) and *Salmonella* Typhimurium (S) as different environments (hosts). We analyzed phenotypes and genotypes arising during evolution when large populations of viruses were switched between hosts for ~720 generations for four consecutive periods. Changes in viral fitness and attachment associated with each host adaptation were measured using qPCR. Instead of a simple fitness trade-off, these measures revealed non-reciprocal interactions: ϕ X174 recently grown on S showed a fitness cost on C; conversely, populations grown on C, were missing a correlated cost on S. To understand the allelic basis of these patterns, deep sequencing of population samples was carried out. Both host-specific and shared alleles were identified and the fitness effects of specific alleles were examined in isolation through targeted mutagenesis.

This study provides a detailed view of the changes that occur during adaptation to novel host. We hope that our work contributes to a better understanding of the general constraints affecting the evolution of parasite populations as they adapt to the complexities of a novel host environment.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P452

Comparison of the Genital Microbiome of Australian Aboriginal Women giving Birth at Term or Preterm

Nicole Dinsdale¹, Natalia Castano-Rodriguez², Julie Quinlivan³, George Mendz³

¹The University of Western Australia, Crawley, Australia. ²The University of New South Wales, Kensington, Australia. ³The University of Notre Dame Australia, Darlinghurst, Australia

Abstract

Background: There are no data on the vaginal and placental microbiomes of pregnant Australian Aboriginal women. In this population the preterm birth rate is higher than in many poor countries. To address this gap in information, a pilot prospective cohort study was conducted at Hedland Health Campus in Western Australia.

Methods: The study included 23 Aboriginal gravidae women of which 6 gave birth preterm. Participants were administered a questionnaire including demographic and clinical data. Mid-vaginal swabs were obtained prior to birth. Placental swabs taken between the amnion and chorion layers were collected shortly after birth. Qiagen kits were used to extract the DNA from the swabs and its purity was verified by standard procedures. The identity of taxa was determined by ultrafast sequencing of the V4 region of the 16S rDNA gene.

Results: Results of statistical analyses suggested differences between the placental and vaginal microbiomes of both groups of women; species richness and evenness were significantly higher in placental samples relative to vaginal samples. The frequency and abundance of *Bradyrhizobium*, *Phenylobacterium* and *Paracoccus* taxa in placental samples of women giving birth preterm were significantly higher than in women giving birth at term. PERMANOVA analyses showed significant differences in the composition of the genital microbiomes of both groups of women.

Conclusions: For the first time, taxa present in the vaginal and placental microbiomes of pregnant Aboriginal women were identified. Comparisons of the microbiomes of women giving birth at term or preterm showed significant differences between their microbiota

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P453

A phylogenomic classification of the probiotic rich genera *Lactobacillus* and *Bifidobacteria* including comparative probiogenomic analysis.

Laura Baker¹, Alison Jack², Sue Plummer², Daryn Michael², Ricardo Del Sol¹, Paul Facey¹

¹1. Swansea University Medical School, Swansea, United Kingdom. ²2. Research and Development Department, Cultech Ltd, Swansea, United Kingdom

Abstract

It is now widely accepted that probiogenomic studies (those that study the genomics of probiotic bacteria) are providing significant insights in to the mechanisms behind probiotic function – not only because it is helping us understand how they confer health benefits but also, because it provides information on the presence of antibiotic resistance genes and virulence factors. However, probiogenomic studies rely heavily on identification of a suitable reference genome. We present an in depth classification of the two important probiotic rich genera *Lactobacillus* and *Bifidobacteria*. Improving on existing single gene phylogenies we offer a Maximum-likelihood, Multi-Locus Sequence Analysis (MLSA) using a concatenation of 400 conserved protein sequences from approximately 1300 *Lactobacilli* and 400 *Bifidobacteria* genomes to resolve the major relationships within this clade. In addition, we present the draft genome sequences of novel probiotic isolates and classify them using our MLSA. Lastly, we offer up an in depth probiogenomic analysis of our isolates and identify the presence of virulence factors, and antibiotic resistance genes.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P454

Assessing Genomics-Based Strategies to Identify Biosynthetic Gene Clusters in Uncharacterised *Streptomyces* spp. Marine Isolates

Eduardo Leao de Almeida, Lekha Margassery, Alan Dobson

School of Microbiology, University College Cork, Cork, Ireland

Abstract

Identifying genes encoding novel bioactive compounds in bacterial genomes while challenging is becoming increasingly important, given the urgent need for novel antibiotic molecules. Hence, optimising genomics-based strategies and the downstream *in silico* analysis, allied with natural products chemistry; is essential if we are to increase our chances of discovering entirely new compounds. To this end we have analysed the genomes of a number of *Streptomyces* spp. strains isolated from marine sponges, which have shown diverse antimicrobial activity, including against methicillin-resistant *Staphylococcus aureus*. The efficiencies of different genome sequencing technologies (including Illumina MiSeq and PacBio) and genome assembly methods were assessed. The antiSMASH 4 software was used to *in silico* benchmark the power of detection of secondary metabolites biosynthetic gene clusters (BGCs) from the different methods we employed. The production of compounds was then confirmed via chemical analysis of production media and *in vitro* bioactivity assays. By benchmarking different genomics-based approaches both *in silico* and *in vitro*, it was possible to indicate the best strategies to be employed in the identification of BGCs, which can serve as guidelines to further explore the biotechnological potential of marine *Streptomyces* spp. isolates. Ultimately, the high quality genomic information provided by the proposed strategies will facilitate the application of molecular biology techniques, such as the heterologous expression of BGCs of interest, with potential further biotechnological applications.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P455

Regulation of mucinase expression in pathogenic *Escherichia coli* strains: CRP has a pic.

Rita Godfrey, Gurdamanjit Singh, Joseph Ingram, Camilla Bathurst, Muhammad Yasir, Stephen Busby, Douglas Browning

Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

The Pic mucinase is a serine protease autotransporter protein that is secreted by the pathogenic Enteroaggregative *Escherichia coli* (EAEC) strain 042. This important protein has been shown to aid intestinal colonization, allowing EAEC 042 to degrade gastrointestinal mucin and utilize it as a growth substrate, and cleave both complement and glycoproteins on various immune cells, enabling modulation of the immune system. Here, using a variety of *in vitro* and *in vivo* techniques, we investigate the promoter controlling *pic* expression in EAEC 042 and show that it is regulated by the CRP and Fis global transcription factors, with CRP activating expression and Fis repressing it. Bioinformatic analysis indicates that Pic is also found in other pathogenic *E. coli* and *Shigella* strains. In most instances, the *pic* promoter is identical to that from EAEC 042, whilst for the uropathogenic *E. coli* (UPEC) strain CFT073, the *pic* promoter region differs markedly, possessing a radically different promoter architecture. In spite of this, CRP- and Fis-mediated regulation is maintained, demonstrating that different promoter organizations can have the same regulatory outcome. As CRP and Fis regulate many genes in response to nutrient availability, we also show that the expression from both the EAEC and UPEC *pic* promoters is maximal during growth in minimal medium and propose that co-regulation by CRP and Fis ensures that *pic* expression and ultimately mucin utilization only occurs under conditions of nutrient limitation.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P456

The Multiple Antibiotic Repressor (MarR) protein has one binding target in the *Escherichia coli* genome

Alistair Middlemiss, James Haycocks

University of Birmingham, Birmingham, United Kingdom

Abstract

In order to respond to environmental stresses bacteria have evolved complex systems of global genetic regulation. One such system involves the multiple antibiotic resistance (*mar*) operon, which is negatively regulated by MarR. Environmental stresses, such as antibiotics, cause MarR to dissociate from the *marRAB* promoter. Currently, the genome-wide DNA binding properties of MarR are unknown. Here, we generated a set of genetic tools to allow 3x FLAG or 8x Myc tagging of a target protein at the N- or C-terminus. These tools were used to tag MarR and chromatin immunoprecipitation was used to show that MarR has a single binding target in the *Escherichia coli* genome.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P457

Pangenome analysis of *Escherichia coli* phylogroups for the identification of phylogroup-specific genes

Sarah Johns¹, André Becker S Saidenberg², Jono Betts¹, Diane G Newell¹, Arnoud H M van Vliet¹, Roberto M La Ragione¹

¹Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey, Guildford, United Kingdom. ²Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil

Abstract

Background: *Escherichia coli* isolates show high levels of genetic and phenotypic diversity, highlighting the need for consistent typing schemes such as the Clermont-scheme for phylotyping, which subdivides *E. coli* into phylogroups A, B1, B2, C, D, E and F. These phylogroups are not randomly distributed, but are more associated with the source of isolation or disease. Here we investigated the linkage between whole genome phylogeny and phylotypes in a large collection of *E. coli* genome sequences.

Methods: 2,999 *E. coli* genome sequences were phylotyped *in silico* with the 4-gene primer sequences from Clermont. Representative groups of 628 isolates were used for pangenome analysis for identification of genes differentially represented in phylogroups (Bonferroni $p < 0.05$).

Results: Within the genome sequences, the individual phylogroups were distributed as follows; A-23.9%, B1-29.9%, B2-15.7%, C-5.1%, D-5.6%, E-11.3%, F-7.0% and unknown-1.5%. Phylogenetic clustering using core genome SNPs showed general clustering of phylotype genomes. Some phylotypes lacked homogeneity, with B1 and C clustering together and B2 showing three distinct subclusters. Pangenome analysis was used to search for genes specific for phylotypes/clusters to assist with more accurate phylotyping, e.g. a three-gene operon encoding a GntR-family regulator, an enolase-like enzyme and a major facilitator superfamily were found in almost all phylogroup F strains, but not in other phylogroups.

Conclusion: Pangenome analysis has the potential to assist further improvements of the Clermont phylotyping scheme and can improve the understanding of *E. coli* pathobiology and the relationship between phylogroups, pathotypes, epidemiology and disease outcome.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P458

Nosocomial Acquisition and Transmission of *Enterococcus Faecium* Revealed by Whole Genome Sequencing: a Longitudinal Cohort Study

Francesc Coll^{1,2}, Theodore Gouliouris^{3,4,5}, Catherine Ludden^{1,2}, Kathy E. Raven³, Beth Blane³, Plamena Naydenova³, Charles Crawley⁵, Nicholas M. Brown^{4,5}, M. Estée Török^{3,4,5}, Julian Parkhill², Sharon J. Peacock^{1,2,3,5}

¹London School of Hygiene & Tropical Medicine, London, United Kingdom. ²Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom. ³Department of Medicine, University of Cambridge, Cambridge, United Kingdom. ⁴Clinical Microbiology and Public Health Laboratory, Public Health England, Cambridge, United Kingdom. ⁵Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

Abstract

Background

Vancomycin-resistant *Enterococcus faecium* (VREfm) is a major cause of nosocomial bacteraemia. Longitudinal studies that integrate epidemiology with bacterial sequencing to investigate the source of acquisition and spread of *E. faecium* in hospital settings are lacking.

Methods

We recruited 174 participants into a prospective longitudinal study on two adult haematology wards at Addenbrooke's Hospital (Cambridge) during six months. A total of 376 stools were obtained from 149 patients (median 3) to determine the carriage rate. Environmental screening of *E. faecium* around the bedspace, bathrooms, communal areas and healthcare worker devices was performed. Multiple colonies of *E. faecium* from stool samples (median 5 per sample) and single colonies from environmental swabs were whole-genome sequenced. A combined epidemiological and phylogenetic analysis was used to delineate acquisition and transmission.

Results

E. faecium and VREfm were commonly carried by haematology patients (85% and 63% respectively) and VREfm contaminated environmental sites (49%). The whole-genome phylogeny of 1,477 *E. faecium* isolates (943 from stool, 534 environmental) resolved 115 subtypes. Of these, 91 were carried by patients and 55/91 were also detected in the hospital environment. We detected 111 instances of *E. faecium* acquisition involving 63% of patients for which strong genetic (78/111) and epidemiological (61/111) evidence further supported transmission. Three of the six patients who developed VREfm bloodstream infection had evidence of nosocomial acquisition.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

Conclusion

The combination of microbiological, epidemiological and genomic analyses revealed that nosocomial acquisition of *E. faecium* is common and that at least half of invasive infections were acquired in the hospital.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P459

A meta-transcriptomics pipeline for the analysis of RNA-seq data sets from multiple host species

Quan Gu, Andrew Shaw, Joseph Hughes, Richard Orton, Massimo Palmarini

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

Abstract

Transcriptomics of systems infected with microbes are key for predicting resistance to specific antibiotics, quantifying gene expression changes, and tracking disease progression. RNA-Seq has become a standard method for analyzing transcriptomes. In this study, we describe a meta-transcriptomics pipeline for processing RNA-Seq data sets from multiple host species. The High Throughput sequence data was generated from the Ion Proton platform. We first used *Kraken* for quality control and contamination detection, followed by a two-step alignment method to align the data against specific host genomes. We also used the Cox-Reid profile-adjusted likelihood method of *edgeR* for estimating read dispersions to improve the performance of the differential gene expression analysis. *Ensembl* genomes were utilized in the pipeline, enabling orthologues to be readily identified through the *Ensembl* framework, facilitating cross species comparisons. Our pipeline was applied to multiple interferon stimulated cells lines from ten host species to identify species-specific interferon-stimulated genes (ISGs) as well as ISGs that are shared between the species. There was a total of 62 core ISGs identified that were upregulated in all nine mammalian species as well as the chicken. We used Approximate Entropy (ApEn) and sequence length to determine whether the core ISGs had a conserved pattern of amino acid usage. However, the core ISGs did not show a significant difference in the distribution of their ApEn compared to non-core ISGs and the ApEn distribution was not significantly different between the species.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P460

The Transcriptional regulator RamA controls OmpC expression in *Klebsiella pneumoniae*

Manasa Bharathwaj¹, James Haycocks², Charles Metcalf¹, Aishwarya Saxena¹, David. C. Grainger², Thamarai Schneiders¹

¹Division of Infection and Pathway Medicine, University of Edinburgh, Edinburgh, United Kingdom.

²Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Abstract

The bacterial outer membrane (OM) functions as the primary protective barrier against antibiotic pressure. Disruptions in OM permeability, due to mutational or regulatory changes as a result of antibiotic challenge are key in maintaining membrane integrity when exposed to multiple stresses. RamA is an intrinsic transcriptional regulator, in *Klebsiella pneumoniae*, which is primarily implicated in reduced antibiotic susceptibility via the up and down regulation of the AcrAB-TolC efflux pump and OmpF porin. Transcriptome profiling experiments demonstrate that RamA controls 77 genes such as those involved in membrane permeability, lipid A synthesis and OM integrity within the *K. pneumoniae* genome. Thus in order to expand on the regulatory role of RamA further, we used CHIP-seq analyses to determine additional target genes of the RamA protein.

HIS and FLAG tags were genetically introduced into wildtype (*Ecl8-ramAHTF*) and *ramA* overexpressing (*Ecl8ΔramRramA-HTF*) strains. The levels of *ramA* gene in the tagged strains of the wild type and *ramA* overexpressor were validated using western blots with the Anti-FLAG antibody. CHIP libraries were generated, validated and subjected to Illumina sequencing as described previously. Bioinformatic analysis was undertaken using Galaxy.

CHIP-analysis identified multiple genes of which one is OmpK36. Subsequent validation of *ompC* levels by QPCR, fluorescence and SDS-PAGE analysis indicates that RamA overexpression directly correlates with increased expression of the OmpK36 porin (up to 3 fold) in RamA overexpressing strains relative to the wild type. Antibiotic assays reveal a RamA-mediated synergistic effect of differential AcrAB, OmpK35 and OmpK36 expression levels in conferring reductions in susceptibility.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P461

The effect of dam methylase on gene expression

Karen Cooke¹, David Lee², Stephen Busby¹

¹University of Birmingham, Birmingham, United Kingdom. ²Birmingham City University, Birmingham, United Kingdom

Abstract

“Epigenetic” mechanisms result in information being carried by DNA, independent of its base sequence. These are widely studied in eukaryotic cells, but less so in bacterial contexts. DNA methylases such as DNA adenine methyltransferase (Dam), methylate DNA at ‘5-GATC-3’ sequences and this is known to impact upon DNA replication, DNA repair, and transcriptional regulation at some virulence promoters.

To investigate global effects of Dam on transcription in *Escherichia coli* K-12, a *lac28::egfp* fusion was inserted at different chromosomal loci and expression was measured using flow cytometry. We found differences in expression at these loci between wild-type and Δdam cells, with some differences depending on growth medium. At many loci, Δdam cell populations exhibited decreased fluorescence and, occasionally, less variation than wild-type cells.

Measurements of levels of methylation, investigated by digestion of plasmid pBR322 with DpnI restriction enzyme, showed nutrient-dependent differences. DNA topology in wild-type and Δdam cells was investigated by chloroquine gel electrophoresis and this revealed a higher degree of supercoiling in Δdam cells in the exponential phase of growth. In further experiments, we focussed on CRP (the catabolite-repression global transcription regulator protein) and found that Dam methylation could affect the action of CRP at a model promoter. Our work implicates Dam methylase as a potent regulator of gene expression in *Escherichia coli* and an important agent of cell heterogeneity.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P462

The impact of guanidine hydrochloride on genetic and epigenetic determinants in *Saccharomyces* species

Laura Petch, Mick Tuite

Kent Fungal Group, School of Biosciences, University of Kent, Canterbury, United Kingdom

Abstract

Phenotypic heterogeneity in the yeast *Saccharomyces cerevisiae* has input from both genetic and epigenetic determinants. In addition to changes in DNA sequence induced by mutagens, there are a variety of 'non-mutagenic' chemicals that result in inherited changes in phenotype. One such agent, guanidine hydrochloride (GdnHCl) can both generate mitochondrial *petite* mutants and also induce the loss of various prions from this yeast species. Prions are novel protein-based epigenetic determinants that undergo self-perpetuating, heritable changes in their conformation. Our study is asking two questions: (1) is the mechanism of *petite* induction by GdnHCl the same as leads to prion loss i.e. by inhibition of the molecular chaperone Hsp104; and (2) can we identify prion-associated traits in other *Saccharomyces* species by GdnHCl-mediated curing? To answer the first question we have used mutants of *S. cerevisiae* lacking either Hsp104 or the related mitochondrial chaperone Hsp78, and find both mutants are able to maintain mitochondrial function. This rules out inhibition of Hsp104 and Hsp78 function as the mechanism of *petite* induction by GdnHCl. Furthermore we find that both GdnHCl and other guanidinium salts (e.g. Gdn thiocyanate) can generate respiratory-deficient *petite* mutants in three different *Saccharomyces* species: *S. bayanus*, *S. mikatae* and *S. kudriavzevi*. This has led us to undertake a detailed comparative analysis of the impact of GdnHCl on the ultrastructure and respiratory functions of mitochondria in these species and the outcomes of this study will be reported as well as our search for prion-related phenotypes in these same *Saccharomyces* species.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P463

Decoding *Fusobacterium nucleatum* genomics and genetics for virulence factor identification and characterization in colorectal cancer host-pathogen interactions

Michael Casasanta, Blake Sander, Ariana Umana, Christopher Yoo, Allison Duncan, [Daniel Slade](#)

Virginia Tech, Blacksburg, USA

Abstract

Fusobacterium nucleatum is a Gram-negative oral pathogen with strong connections to the onset and progression of infectious diseases, including colorectal cancer. Here we present our methods and analysis of the first completed genomes of multiple pathogenic *Fusobacterium* species, as well as the first efficient, selectable, markerless, and complementable gene deletion system in the biomedically significant bacterium *Fusobacterium nucleatum* 23726. Our genomic analysis revealed both previously characterized and newly identified virulence factors, and we showcase the power of our genetic system by developing strains containing multiple gene deletions from ~300bp -12kb. In addition, we show the ability to chromosomally complement tagged versions of *Fusobacterium* proteins. Finally, we combine our newly developed genetic methods with cell biology and microscopy to characterize multiple virulence factor proteins that play significant roles in *F. nucleatum* induced host-pathogen interactions in colorectal cancer.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P464

Antibiotic Resurrection

Conrad Lichtenstein, Gi Mikawa, Frank Massam

Nemesis Bioscience Ltd, Cambridge, United Kingdom

Abstract

Developing new antibiotics (*hardware*) is costly and time-consuming and bacteria evolve quickly under selection pressure acquiring new AMR genes.

Nemesis engineers CRISPR/Cas-based RNA-guided nucleases (RGNs) to make double-strand breaks (DSBs) in target AMR genes, thereby leading to gene inactivation. But DSBs can also lead to plasmid loss and subsequent cell death through plasmid-mediated post-segregational killing (PSK): we have shown that we can protect cells from this direct killing –so avoiding direct selection against Symbiotic delivery. A single multiplex Symbiotic inactivates multiple beta-lactamase (*bla*) resistance genes – so resurrecting sensitivity to beta-lactams.

Symbiotics are delivered by Transmids©: our novel dual delivery transmissible plasmid vectors, Transmids (Tds), provide a single delivery platform. (i) Therapeutic applications will use a formulation of Transmids encapsidated in a bacteriophage coat (ϕ Td) to deliver DNA into bacterial pathogens by phage coat infection, in an infected patient. The ϕ Td is prepared in a helper strain carrying an inducible phage that expresses head&tail proteins to package the Transmid. (ii) Prophylactic applications use probiotic bacteria to deliver Transmids directly to bacteria by plasmid conjugation to clear the microbiome of AMR, in anticipation of auto-inoculating opportunistic infections. We have proved both applications in early in vivo experiments

As companion DNA therapeutics, Nemesis Symbiotics will make existing antibiotics effective again, prevent the spread of AMR genes, and protect the efficacy of new antibiotics. Our technology is also applicable to all antibiotic classes, all known resistance mechanisms, and for inactivation of virulence factors, in all bacteria.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P465

Screening and characterization of novel sporulation genes in *Clostridium sporogenes* using transposon mutagenesis

Syeda Rubab Rizvi^{1,2}, Aleksandra Kubiak^{1,2}, Nigel Minton^{1,2}, Alan Cockayne^{1,2}

¹Centre for Biomolecular Sciences, Nottingham, United Kingdom. ²University of Nottingham, Nottingham, United Kingdom

Abstract

The genus *Clostridium* consists of bacteria that are of high significance level in various aspects of life, from health and cancer therapy to food and biofuel production. Most of the clostridial species produce resilient spores that ensure their survival under harsh environments. However, the knowledge about clostridial sporulation mechanisms is very limited. In conditional transposon mutagenesis, transposon is allowed to jump into random genes by the help of transposase enzymes; thus, giving rise to a pool of mutants that can be screened for desired phenotype and for which the desired genotype can be determined. Therefore, libraries of random mutants with transposon insertion were generated in *Clostridium sporogenes*, a non-pathogenic surrogate to proteolytic *Clostridium botulinum*. In total, 5,061 mutants were screened for sporulation-defective phenotypes by spore assay, in which 28 unique gene mutants showed either some reduction or complete loss of heat resistant spores. The genomic DNA of these mutants was extracted and the chromosome-transposon junction was sequenced in order to identify the affected genes. The identified genes were observed being involved in sporulation stages, cell division, DNA translocation or unidentifiable cellular processes, having direct or indirect impacts on the *C. sporogenes* sporulation. One gene mutant (CLSPO_RS15205) with asporogenous phenotype was also successfully targeted by CRISPR-Cas9. The function of affected genes will be confirmed by gene-complementation. The characterization of growth and sporulation profile of the acquired mutants will aid in better understanding of sporulation mechanisms in *C. sporogenes*. This data will eventually help in spores applications and food safety research.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P466

Advances in pursuing genome closure from short reads through rDNA repeat resolution and streamlining assembly pipelines.

Nicholas Waters^{1,2}, Leighton Pritchard², Florence Abram¹, Ashleigh Holmes², Fiona Brennan³

¹National University of Ireland, Galway, Galway, Ireland. ²James Hutton Institute, Dundee, United Kingdom. ³Teagasc, Johnstown Castle, Ireland

Abstract

The vast majority of bacterial genome sequencing has been performed using Illumina short reads. Because of the inherent difficulty of resolving repeated regions with short reads alone, only ~10% of sequencing projects have resulted in a closed genome. The most common repeated regions are those coding for ribosomal operons (rDNAs), which occur in a bacterial genome between 1 and 15 times, and are typically used as sequence markers to classify and identify bacteria. Here, we exploit conservation in the genomic context in which rDNAs occur across taxa to improve assembly of these regions relative to de novo sequencing by using the conserved nature of rDNAs across taxa and the uniqueness of their flanking regions within a genome. We describe a method, riboSeed, which constructs targeted pseudocontigs generated by iteratively assembling reads that map to a reference genome's rDNAs. These pseudocontigs are then used to more accurately assemble the newly-sequenced chromosome.

We show that this method, implemented as riboSeed, correctly bridges across adjacent contigs in bacterial genome assembly and, when used in conjunction with other genome polishing tools, can result in closure of a genome. riboSeed is a standalone package available through conda, and can be used with the BugBuilder genome assembly pipeline. By removing the main barriers to genome polishing (difficulty in installation, tool selection, and pipeline integration), researchers can easily go from raw reads to high-quality, polished assemblies.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P467

Genomic and Morphological Comparison Between Marine Sponge-Derived Streptomyces and their Terrestrial Counterparts

Eduardo Leao de Almeida, Andrés Felipe Carrillo Rincón, Alan Dobson

School of Microbiology, University College Cork, Cork, Ireland

Abstract

The *Streptomyces* genus is well known for its capacity of producing secondary metabolites with biological activity. Nevertheless, there is still much to be understood regarding these organisms – especially the ones derived from the marine environment. A number of bacteria were isolated from the marine sponge *Haliclona simulans*, and up to 50% of the isolates produced antibiotic activity against clinically important pathogens. Two of the strains determined to belong to the *Streptomyces* genus were selected for further analyses due to their diverse antimicrobial activity as shown in initial screening, including activity against the methicillin-resistant *Staphylococcus aureus*. In addition to morphological differences, phylogenetic analysis also showed they had 96% identity in their 16S rRNA gene sequences. Genomic DNA sequencing was performed using the PacBio technology, which provided high quality *de novo* assembled ungapped scaffolds. Whole genome alignments and 16S rRNA phylogeny allowed us to determine their closest related terrestrial species to be *S. griseus* and *S. albus*; organisms known to produce important antimicrobial compounds such as streptomycin and salinomycin, respectively. *In silico* biosynthetic gene cluster (BGC) prediction using antiSMASH 4 indicated that, despite the presence of highly conserved genomic regions, there is a potential variety of BGCs between the marine and their terrestrial counterparts, perhaps as a consequence of their evolutionary distances and different microbial niches. This study highlights the importance of investigating marine-derived bacteria – especially the *Streptomyces* genus – due to their potential of producing analogs or novel compounds with promising bioactivity, which could possibly replace compounds currently in the market.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P468

Untangling the pangenomes of the *Butyrivibrio* group

Sara Pidcock¹, Fernanda Santos², Sharon Huws³

¹Aberystwyth University, Aberystwyth, United Kingdom. ²Universidade Federal de Viçosa, Viçosa, Brazil.

³Queen's University Belfast, Belfast, United Kingdom

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 to classification has become antiquated with the development of sequencing technologies and downstream bioinformatics analysis. As such, this study aims to investigate the taxonomic relatedness and functional capacity of the ruminal *Butyrivibrio* group using the 72 genomes. Seventy one genomes were obtained via JGI (the Hungate 1000 project), and one additional bacterial strain was sequenced by ourselves. DNA extraction was performed using a FastDNATM SPIN Kit for Soil (MP Biomedicals) and sequencing and downstream analyses were conducted by MicrobesNG. Genomes were annotated via Prokka. Comparative annotation was also conducted via The Seed platform. 16S rDNA were aligned using the Ribosomal Database Project, and a phylogenetic tree created using FastTree. An additional tree was constructed using 40 gene markers. Visualisation was by Interactive Tree Of Life. Pangenome analysis was conducted using Spine/AGEnt/ClustAGE version. Three primary clades were observed, one being the genus *Pseudobutyrvibrio*, another *B. fibrisolvens* strains, and the third being the remaining *Butyrivibrio* species. Pangenome analysis revealed greater diversity within *Butyrivibrio* than *Pseudobutyrvibrio*. These findings emphasise the need for further research into the *Butyrivibrio* group, with future research focusing on enzyme characterisation and exploration of metabolic activity within this group through metatranscriptomics.

Forum: Genetics and Genomics

Zone D

Presentations: Thursday Evening and Friday Lunchtime

P469

Undiscovered evolutionary trends of *Bordetella pertussis*

Jonathan Abrahams¹, Andrew Preston¹, Michael Weigand²

¹University of Bath, bath, United Kingdom. ²CDC, Atlanta, USA

Abstract

Undiscovered evolutionary trends of *Bordetella pertussis*

Background

The bacterium *Bordetella pertussis* is the causative agent of the respiratory disease whooping cough. It is a textbook example of the evolution of pathogenicity through genome reduction. I present evidence that pertussis strains also possess large duplications up to 100kb.

Methods

Due to the inefficiencies of short read sequencing duplications appear in mapped data as regions of double coverage. Using this theory, 854 *Bordetella pertussis* genomes are mapped to the reference genome using Snippy and coverage statistics are generated using the R package CNOGpro. Phylogenetic trees were used to assess the relationship of strains with duplications using Fasttree and plotted using iTOL. Strains were manually grouped based on the presence and, if applicable, the location of the duplication.

Results

19 strains were found to have one or two duplications. Strains were grouped based on the location of their duplications which fell at 3 main loci. These groups were not phylogenetically related, often falling in disparate clades of the phylogenetic tree. This strongly indicates that duplications arise independently in diverse pertussis isolates rather than one specific clade containing the majority of the duplications. In turn this could mean that duplications in pertussis are under a lesser purifying selection pressure than would have been thought before the experiment.

Conclusions

This research reveals an exciting evolutionary quirk of *Bordetella pertussis* which has been thought to almost exclusively evolve by genome reduction. Further work is needed to elucidate the impact of duplications on pertussis.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P470

Impact of Sequential Passage on overall Protein Expression Using Proteomic Analysis

Mohammed Al-Hajouj¹, Ghadah Alsharif¹, David Stead², Ahmed Mirza¹

¹King Abdulaziz University, Jeddah, Saudi Arabia. ²University of Aberdeen, Aberdeen, United Kingdom

Abstract

Urinary tract infection is one of the most prevalent bacterial infections in the world affecting bladder and kidney. Gram-negative bacteria are a major cause of such infections, particularly *Escherichia coli*. *E. coli* is the main cause of 80-90% of community-acquired infection, 40% of nosocomial UTI, and is responsible for 25% of recurrent infections. The field of proteomics has emerged as a great tool to analyze expressed proteins and to identify possible biomarkers associated with a number of pathological states, and to the same extent associated with bacterial pathogenesis. Researchers elsewhere are investigating *E. coli* proteomics profiles to identify possible biomarkers, however, protein profiles could vary due to environmental stress created by subculturing. In this research, impact of sequential passage on overall protein expression was investigated. Urine samples were collected from individuals with recurrent UTI, sequentially subcultured, and analyzed using one and two-dimensional gel electrophoresis and mass spectrometry to identify any significant changes in the protein profile of the bacteria. We observed the effect of passage on the protein profile of this common pathogen. We found there is slightly change of protein expression between the original culture of bacteria compared with the last culture for 8 passage of same bacteria in short time of passaging . Also, we found there is a big differences of generated and expressed protein between the original culture of bacteria compared with the last culture for many passages of same bacteria in long time of passaging for many years.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P471

Predicting antimicrobial efficacy via an empirical scoring function and identifying structural resistance possibilities using triclosan as a case study

Joshua Corkhill¹, Ilias Soumpasis², Samantha Miller¹

¹University of Aberdeen, Aberdeen, United Kingdom. ²Unilever, Bedford, United Kingdom

Abstract

Since its development over three decades ago CADD (computational aided drug discovery) has played a major role in identifying therapeutic small molecules. Despite its success in other fields it has been used sparingly in evaluating potential antimicrobial resistance possibilities towards a compound. Our research addressed whether computational modelling can predict the development of resistant mutants towards an antimicrobial in a structured and semi-quantitative way. In addition, we investigated whether computational modelling can identify structural mutations that cause resistance and predict the susceptibility change.

The method developed correlates predicted binding affinities produced by the scoring function LUDI energy estimate 3 with the experimental MIC's (Minimum inhibitory concentration) of triclosan against *E. coli* mutants with different FabI enzyme structures. A profile was developed using previously published mutants and the wild-type, the model was then used to develop *in silico* mutations in FabI and predict their effect on the MIC. Our results show a positive linear correlation can be observed between the predicted affinities using the LUDI scoring function and the experimentally derived MIC's. In addition, we discovered 2 novel mutations *in silico* (L100A and I200K) and accurately predicted an increase in resistance towards triclosan. Our model showed a great level of accuracy in predicting resistance of the microorganism using changes in the MIC. In conclusion, we believe that CADD methods can have strong potential in screening new antimicrobials for their potential tendency for inducing antimicrobial resistance.

Escherichia Coli: The Model Microbe

Zone D

Presentations: Tuesday and Wednesday Evening

P472

The short chain fatty acid and antimicrobial propionic acid enhances the virulence of Crohn's disease associated adherent-invasive *Escherichia coli*

Michael Ormsby¹, Síle Johnson¹, Lynsey Meikle¹, Robert Goldstone², Anne McIntosh¹, Hannah Wessel¹, Heather Hulme¹, Ceilidh McConnachie¹, Konstantinos Gerasimidis¹, Douglas Morrison¹, David Smith², Donal Wall¹

¹University of Glasgow, Glasgow, United Kingdom. ²Heriot Watt University, Edinburgh, United Kingdom

Abstract

Crohn's disease (CD) is an inflammatory bowel disorder that is characterized by chronic inflammation of the intestine. It is primarily a Western disease and many studies have linked diet and gut microbiome dysbiosis to disease onset in genetically predisposed individuals. However, to date a definitive link between CD, Western diet and intestinal bacteria has remained elusive. Short chain fatty acids (SCFA), such as propionic acid (PA), are natural human intestinal antimicrobials and immune modulators that are also used extensively in Western food production. Here we examine the effect of PA on the pathogenicity of the CD associated microbe, adherent-invasive *Escherichia coli* (AIEC). We show that AIEC is insensitive to the antimicrobial effects of PA and utilises it as an efficient medium for growth. Preadaptation of AIEC to PA significantly increases AIEC adherence to intestinal epithelial cells, acid tolerance and biofilm formation. In addition, PA upregulates genes for bacterial microcompartment-mediated growth on ethanolamine, a carbon and nitrogen source which when utilised offers a competitive advantage in the inflamed intestine. Additionally, we report the implementation of new murine model in which PA concentrations are increased to mimic those found in the human intestine. We observe increased colonization by a PA-adapted AIEC strain and an increase in pro-inflammatory cytokine production over a 21 day period. Overall, our data suggests that exposure to SCFA antimicrobials during food production risks evolving bacteria that are resistant to our natural human intestinal antimicrobials and it is imperative that we understand the long term consequences of this approach.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P473

Metabolic Adaptation in *Escherichia coli* Isolates During Transition from UTI to Bloodstream Infection

Nizar Saeedi^{1,2}, Vikram Sharma¹, Majed Alghoribi³, Simon Jackson¹, Mathew Upton¹

¹Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, United Kingdom. ²Faculty of Applied Medical Sciences, University of Tabuk, Tabuk, Saudi Arabia. ³King Abdullah International Medical Research Center (KAIMRC), Riyadh, Saudi Arabia

Abstract

Background: Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infection (UTI), sometimes leading to bacteraemia. Previous work with paired urine and bloodstream UPEC revealed identical results for molecular typing and virulence gene content. However, different antibiotic susceptibility and metabolic activity profiles indicate phenotypic changes, potentially due to SNPs or epigenetic events. We aim to reveal the mechanisms underlying metabolic adaptation of UPEC to grow in blood, using a quantitative proteomics and genomic analysis.

Materials/methods: We employed classical shotgun proteomics approach to analyse paired blood and urine isolates from multiple strains of UPEC following growth in Lysogeny broth. The Max Quant Environment was used to search against a proteome database corresponding to *E. coli* strains K12 and UTI189. Bioinformatic analysis was performed using Persues software.

Results: A total of 175 proteins were up-regulated (>2 fold) in blood isolates compared to urine isolates, and 148 proteins were down-regulated across ST131 and ST127 strains. Gene ontology analysis of differentially expressed proteins indicated increase in pathways linked to aerobic respiration, peptidoglycan-based cell wall, cofactor binding, and magnesium ion binding. Down-regulated genes in a number of blood isolates were involved in carbohydrate catabolic process and aminobutyraldehyde dehydrogenase activity. Biological processes related to organelle proteins, including the two lipid bilayers, were also altered.

Conclusions: We revealed stable changes in metabolism in blood isolates of UPEC. A deeper understanding of UPEC metabolic adaptation to growth in blood may facilitate identification of novel diagnostic/therapeutic targets to reduce bacteraemia following UTI.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P474

Tunable Zinc Responsive Bacterial Promoters for Controlled Gene Expression

Joseph Ingram, Phil Hill, Dov Stekel, Jon Hobman

University of Nottingham, Nottingham, United Kingdom

Abstract

Background - *E. coli* tightly regulates its internal zinc concentration to a femtomolar level, this is achieved through gene regulation controlled by two transcription factors; Zur and ZntR. ZntR, a zinc induced active activator, regulates ZntA, a P-type ATPase, with a high affinity to Zn^{2+} . ZntR has the potential to be developed into a cost-effective inducible gene expression system.

Methods – A dual reporter plasmid, pGLUX, was created containing both *gfp3* and *LuxCDABE* reporter genes. HiFi assembly was used to clone P_{zntA} into pGLUX (P_{zntA} -GLUX) and transformed into *E. coli* MG1655. Lux and GFP assays were conducted by culturing transformants at 37°C, in Neidhardt's zinc depleted media, with various concentrations of $ZnSO_4$. A Tecan GENious pro recorded OD_{600} , fluorescence, and luminosity readings every 10mins for 16 hr.

Results – P_{zntA} has shown significantly increased promoter activity with the addition of 40 μM $ZnSO_4$, compared to Neidhardt's zinc depleted media. P_{zntA} promoter activity is incrementally increased with increasing concentrations of $ZnSO_4$, up to a concentration of 400 μM , where maximum activity is observed with a 300x increase.

Conclusion – Results show promise for the use of inexpensive zinc compounds, such as $ZnSO_4$, to be used as a cost-effective inducer. P_{zntA} shows increasing promoter activity with increasing concentrations of $ZnSO_4$, allowing for controlled expression level.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P475

Characterising the role of potential accessory factors that facilitate secretion of the EspC autotransporter

Shakib Alhagh, Christopher Penfold, Kim R Hardie

University of Nottingham, Nottingham, United Kingdom

Abstract

Enteropathogenic *E. coli* (EPEC) is one of the major causes of death from infantile diarrheal-related illness in children under 2 years old. EspC is an Extracellular Serine Proteases Autotransporter belonging to the Enterobacteriaceae (SPATE) superfamily. It is secreted via the type Va classical secretion system. This autotransporter protein can act as an enterotoxin, Iron-binding protein and has cytopathic effect on the host cells. The complete mechanism of EspC secretion, role in pathogenesis, interaction with type III secretion system and pathway of secretion are not completely known. In this study, to better understand the molecular pathway of EspC secretion, the role of potential secretion accessory factors was investigated. To do this, plasmids encoding full length EspC or EspC fused to mCherry with or without a functional β -barrel transporter domain were inserted into the AT accessory factor secretory mutants. In order to investigate the localization patterns, florescent microscopy was performed, and these will be presented.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P476

Identifying genetic pathways using high density transposon mutagenesis

Karl Dunne, Jeff Cole, Ian Henderson

University of Birmingham, Birmingham, United Kingdom

Abstract

The discovery of transposon mutagenesis in the mid-20th century resulted in powerful advancement in the field of genetics. Traditional transposon mutagenesis screens have usually been performed on a relatively small scale. However it has become clear that large scale genetic interaction experiments easily enable the identification of gene function and the role these genes play in specific pathways. Recently transposon mutagenesis has been combined with next generation sequencing in order to rapidly screen high density mutant libraries consisting of millions of mutants. It is this development in transposon insertion sequencing that has rapidly enabled the analysis of gene essentiality under a specific condition as well as the identification of all essential genes required for viability in a targeted organism. Here we hijacked this transposon system and applied in it a specific way to enable us to look at novel genetic interactions.

Escherichia Coli: The Model Microbe

Zone D

Presentations: Tuesday and Wednesday Evening

P477

CHARACTERIZATION OF THE MOLECULAR DETERMINANTS INVOLVED IN THE COLONISATION ABILITY OF *ESCHERICHIA COLI* O157:H7 TO THE EXTRACELLULAR MATRIX

Valentin Ageorges¹, Nelly Caccia¹, Ricardo Monteiro¹, Christian Provot², Frédérique Chaucheyras-Durand³, Mickaël Desvaux¹

¹UMR454 MEDIS, Clermont-Ferrand, France. ²Biofilm Control, Saint-Beauzire, France. ³Lallemand Animal Nutrition, Clermont-Ferrand, France

Abstract

Among intestinal pathogenic *Escherichia coli* (InPEC), enterohaemorrhagic *E. coli* (EHEC) O157:H7 are anthropozoonotic agents leading to haemorrhagic colitis and haemolytic-uremic syndrome (HUS). From ruminants (the animal reservoir), EHEC can contaminate some foodstuffs and consequently infect human. While the locus of enterocyte effacement (LEE) is generally considered as a key determinant of EHEC, it is neither sufficient nor systematically present in all EHEC strains. The presence of extracellular matrix (ECM) components along the food chain could increase their colonisation capacity. Numerous proteins can be present at the bacterial cell surface of EHEC and could participate to the bacterial adhesion and biofilm formation at the ECM. This study aimed at identifying and characterizing the respective involvement of cell-surface proteins in the colonisation process of EHEC to some ECM components. Target genes were identified by a proteogenomic approach based on the secretome concept. The involvement of these genes in bacterial adhesion and biofilm formation was characterized following functional genetic analysis. Several organelles and adhesins, either secreted by the Type I (T1SS) to the Type VIII (T8SS), could be identified in EHEC and related enteropathotypes. Following gene knock-out, the ability of the mutants to adhere and/or to form biofilm to ECM components was compared to the wild type strain. Besides the flagella (T3bSS) and pili of the T7SS, some autotransporters belonging to the T5aSS appeared to contribute significantly to specific and/or non-specific colonisation of the ECM by *E. coli* O157:H7.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P478

pH Homeostasis and Indole Signalling in *E. coli*

Ashraf Zarkan, Santiago Caño Muñiz, David Summers

University of Cambridge, Cambridge, United Kingdom

Abstract

E. coli can grow at pH_{out} values from ~ 5.0 – 9.0 but generally maintain the cytoplasmic pH in a narrow range ~ 7.2 – 7.8 . *E. coli* is critically dependent upon pH homeostasis as most proteins have distinct ranges of pH for function. Indole is an aromatic molecule with diverse signalling rules and is produced from tryptophan by the enzyme tryptophanase (TnaA). Indole signalling in *E. coli* is divided into persistent signalling (~ 0.5 mM for an extended period) and pulse signalling (~ 5 mM for a short period during the transition from exponential to stationary phase). We tracked the cytoplasmic pH of *E. coli* growing in LB vs M9 by introducing the cytoplasmic pH indicator “pHlourin” into a wild-type strain. The cytoplasmic pH was conserved throughout the growth at ~ 7.2 in LB and ~ 7.8 in M9. When M9 was supplemented with tryptophan, the cytoplasmic pH became ~ 7.2 . “pHlourin” was then introduced into a mutant that lost the ability to produce indole. The cytoplasmic pH of the mutant growing in LB was ~ 7.8 , and remained unaffected by indole supplementation ($30 \mu\text{M}$ to 0.5 mM). However, an indole-pulse mimic (20 min treatment with 5 mM) restored the cytoplasmic pH to ~ 7.2 . Considering that indole is a proton ionophore, the treatment was repeated with other ionophores (CCCP and DNP) and the cytoplasmic pH of the mutant was ~ 7.2 . Our work suggests that indole pulse signalling is regulating the cytoplasmic pH of *E. coli* by an ionophore-based mechanism.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P479

Mutation to amoxicillin-clavulanic acid resistance during evolution experiments on *Escherichia coli* is affected by growth media

Alasdair Hubbard¹, Nicholas Feasey^{1,2}, Adam Roberts¹

¹Liverpool School of Tropical Medicine, Liverpool, United Kingdom. ²University of Malawi, Blantyre, Malawi

Abstract

Background: Mutations leading to antibiotic resistance are routinely generated using sub-inhibitory concentrations of the antibiotics in a range of media. We wanted to determine whether different media affect the emergence of mutations leading to amoxicillin-clavulanic acid resistance.

Methods: Using a susceptible clinical isolate of *E. coli*, mutations were selected for via 24-hour exposure to sub-minimum inhibitory concentrations in defined (M9), semi-defined (iso-sensitest broth) and undefined (LB broth) media. Resistant colonies were isolated using LB agar containing 8 (MIC) or 16 µg/ml (2xMIC) amoxicillin-clavulanic acid. Fitness costs of two mutant lineages from each media-type were assessed using a competitive fitness assay and mutations in the genome identified through PCR or whole genome sequencing.

Results: No resistant colonies were recovered with pre-incubation in M9, whereas Iso-sensitest and LB broth incubation resulted in mutations that allowed growth at 8 and 16 µg/ml of amoxicillin-clavulanic acid, respectively. There was within media-type variability in the fitness costs associated with the mutations that conferred resistance, with no obvious link between higher levels of resistance and fitness cost. Mutations identified varied between and within media-type, for example within the -35-promoter region of *ampC*.

Conclusions: In this study, we found that amoxicillin-clavulanic acid resistant isolates could not be isolated from M9. There was also between and within media-type variation in the mutations that conferred resistance and subsequent fitness costs in isolates recovered from LB and iso-sensitest broth. This suggests that media-type is an important consideration when generating *in vivo* mutations and interpreting evolutionary studies.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P480

Role of indole in antibiotic persister formation

Isabel Nuñez Santiago, Stephen Trigg, Dr. David Summers

University of Cambridge, Cambridge, United Kingdom

Abstract

Persister cells are a physiologically distinct subpopulation (<1%) of genetically sensitive bacteria with the ability to tolerate high concentrations of antimicrobial drugs. The mechanism of persistence is unclear, but it appears that the inter-kingdom signalling molecule, indole, may sometimes be involved in the process. Indole is produced from tryptophan by the enzyme tryptophanase (TnaA). To test whether indole is involved in persister generation, *E. coli* wild-type and tryptophanase-deficient ($\Delta tnaA$) strains were challenged with high (100x MIC) concentrations of quinolone antibiotics and the number of surviving cells was followed over 5 hours. Wild-type showed 80-fold higher survivals than $\Delta tnaA$, when treated with ciprofloxacin, 100 times MIC.

To confirm the role of indole in persister formation, the indole-deficient mutant was supplemented with 5 mM indole prior to antibiotic challenge. The presence of indole increased survival after exposure to ciprofloxacin 18-fold compared to the untreated indole-deficient strain, and 7-fold compared to the indole-producing wild-type, which suggests that indole has a protective effect against antimicrobial drugs. Quinolones target the essential bacterial enzyme DNA gyrase, interrupting its DNA cleavage-religation activity by stabilising the DNA-enzyme cleavage intermediate. This leads to an accumulation of DNA double-stranded breaks (DSB), resulting in cell death. *In vitro* gyrase cleavage assays indicate that indole reduces the activity of DNA gyrase, with 5 mM indole reducing the presence of ciprofloxacin and moxifloxacin induced DNA DSB by 15% and 45%, respectively. We propose that this mechanism is responsible for at least part of the persister subpopulations that survive quinolone treatment.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P481

Dissecting the regulation of the three tandem promoters of the *Escherichia coli* *kps* gene cluster at chromosomal level.

Esraa Aldawood, Ian Roberts

University of Manchester, Manchester, United Kingdom

Abstract

There are three tandem promoters (PR1-1, PR1-2 and PR1-3) within the PR1 regulatory region of *Escherichia coli* *kps* capsule gene cluster. In this study, we dissect out the regulation of the PR1 promoters in the chromosome. Several constructs were made in UTI89 and UTIP1*lacZ* strains with mutations in the -10 of the selected promoter using gene doctoring followed by K1 phage sensitivity assay in UTI89 strain and measuring the transcriptional response using chromosomal *lacZ* reporter fusion assay in UIP1*lacZ* strain. The mutation of PR1-2 UTIP1*lacZ* reduced b-galactosidase activity by 50% indicating that PR1-2 is a functional promoter. In contrast mutation of PR1-1 reduced b-galactosidase activity by 90% abolishing transcription from both PR1-1 and PR1-2 indicating that PR1-2 is a functional promoter whose activity is dependent in PR1-1. These data were mirrored by K1 capsule production as detected by sensitivity to K1-specific bacteriophage. Mutation of PR1-2 reduced the plaque size by a half whereas in the PR1-1 and PR1-2 double mutant both the plaque size and bacteriophage titre was dramatically reduced suggesting a substantial reduction of capsule expression compared to the wild type. Overall, these results demonstrate the importance of PR1-1 promoter in the PR1 regulatory region and the complex interplay between multiple promoters. Going forward I intend to study the regulation of these different promoters by global regulators known to regulate transcription of the *kps* gene cluster.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P482

Adherence to human intestinal epithelium is associated with disease potential of Enteroaggregative *E. coli*

Samuel Ellis^{1,2}, Marie Chattaway³, Lisa Crossman², Bernard Brett⁴, John Wain², Stephanie Schüller^{2,1}

¹Quadram Institute Bioscience, Norwich, United Kingdom. ²University of East Anglia, Norwich, United Kingdom. ³Public Health England, London, United Kingdom. ⁴Norfolk and Norwich University Hospital, Norwich, United Kingdom

Abstract

Enteroaggregative *E. coli* (EAEC) is a heterogeneous pathotype associated with diarrhoeal illness in children and travellers in developing countries, as well as foodborne outbreaks worldwide. Previous work with clinical EAEC isolates identified sequence types ST31 and ST40 as significantly associated with asymptomatic carriage and disease, respectively.

The aim of this study was to use experimental models to investigate ST31 and ST40 EAEC isolates from cases and controls for differences in virulence-associated phenotypes, supported by comparative analysis of virulence gene profiles from sequence data.

Adherence and colonisation phenotypes were investigated by *in-vitro* assays using the human colon carcinoma cell line T84, and *ex-vivo* experiments using colonic biopsies obtained from consenting patients undergoing routine endoscopy. Assays were also designed to evaluate early-stage biofilm formation, mucus degradation, and host inflammatory response.

Complex ST40 demonstrated significantly greater colonisation of both T84 cells and colonic biopsies compared to ST31. Biofilm formation was also significantly higher for ST40 isolates than ST31. Within a complex, case isolates did not demonstrate greater virulence phenotypes than controls. Sequence analysis identified virulence gene variations between complexes, including adhesin genes which may contribute to the observed differences in adherence ability.

These results demonstrate that ST40 isolates have an enhanced ability to adhere to human colonic epithelium and form biofilms, which are both important features of EAEC pathogenesis. These findings complement the previous clinical associations of ST40 with disease and ST31 with carriage. The lack of phenotypic differences between case and carriage isolates suggests that disease is also dependant on host susceptibility.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P483

When replication forks collide – containing fork fusion-mediated pathologies in *Escherichia coli*

Christian Rudolph, Juachi Dimude

Division of Biosciences, Brunel University London, Uxbridge, United Kingdom

Abstract

The bacterium *Escherichia coli* has been used for decades to study increasingly intricate details of its biology. Nevertheless, many cell biology aspects still remain poorly understood. We have demonstrated that the fusion of two replication forks as DNA replication terminates can have surprisingly severe consequences. In *E. coli* two replication forks are recruited at the single *oriC* and proceed in opposite directions until they fuse opposite *oriC* in a specialised region that acts as a fork trap, allowing replisomes to enter but not to leave. Our recent work in *E. coli* has highlighted that an unexpected number of proteins are involved in the processing of fork fusion intermediates, including RecG, 3' exonucleases and polymerase I. In the absence of the correct processing steps fork fusion intermediates persist for longer, allowing replication to restart in an already fully replicated chromosomal area. This over-replication leads to the formation of double-stranded DNA ends, triggering RecBCD-dependent recombination which, in turn, results in recombination-dependent replication that further exacerbates over-replication. Excessive amounts of over-replication are lethal for cells, highlighting that maintaining accuracy as replication forks fuse is a key event in genome duplication. The severe consequences of fork collisions illustrate the advantages of the bacterial chromosome arrangement: use of a single origin intrinsically limits the number of fork encounters to one per cell cycle. In addition, the defined termination zone provides an area for the safe processing of fork fusion intermediates. Changes to this arrangement cause problems to cell cycle control, chromosome dynamics and cell viability.

Escherichia Coli: The Model Microbe

Zone D

Presentations: Tuesday and Wednesday Evening

P484

Host niche environments play a critical role in dictating the motility phenotype of *Escherichia coli*.

Aaron Ming Zhi Tan^{1,2}, Senan Almashta^{1,2}, David Picton^{1,2}, Lauren Drage^{1,2}, Romy Tuin³, Gary Forster-Wilkins³, Simon Gould³, Tim Potter⁴, Mark Fielder³, Robert Pickard⁵, Judith Hall², Phillip Aldridge^{1,2}

¹Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom. ²Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom. ³Kingston University, London, United Kingdom. ⁴Wespoint Farm Vets, Horsham, United Kingdom. ⁵Institute of Cellular Medicine, Newcastle upon Tyne, United Kingdom

Abstract

Escherichia coli is a fascinating and diverse species with respect to how it interacts with us. We appreciate that *E. coli* is a member of our commensal gastrointestinal flora but also can be pathogenic. When pathogenic *E. coli* it is associated with gastrointestinal, urinary tract, and blood stream infections. Genome analysis shows that *E. coli* can be divided into 6 clades (A, B1, B2, D, E and F).

Our perception of motility in *E. coli* is biased towards a strong literature foundation exploring domesticated clade A strains such as MG1655, MC4100 and W3110. However, discuss *E. coli* motility in a clinical setting and an alternative picture appears where many isolates are deemed non-motile.

We have taken advantage of two *E. coli* strain collections sourced from either recurrent UTI patients or cow herds across the south of England. We have assessed motility, its regulation and the isolates clade distribution to define motility for *E. coli* the species rather than a specific clade. We will present data based on this species wide assessment to argue the case for a new regulatory architecture of the *flhDC* chromosomal loci. Our data identifies a further level of regulation for *flhDC* transcription and explains the impact of IS element insertion in the *flhDC* loci.

We conclude that *E. coli* is extremely pleiotropic with respect to motility. Our data suggests that the observed diversity is dependent upon the environmental niche within a host that *E. coli* is colonising.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P485

Comparison of the epidemiology of *E. coli* from patients from Salford Royal Hospital in the United Kingdom and Fort Portal Hospital in Uganda

Yuanyuan Ren

University of Salford, Salford, United Kingdom

Abstract

E. coli is a well-studied bacterium that has increasingly become a public health concern in all parts of the world. This is due to its abundance, the ability to cause diseases, and its profound antimicrobial resistance (AMR) profile. So far, 30 isolates from sepsis/bacteraemia patients and 30 isolates from urinary tract infection patients have been collected from Salford Royal Hospital. Clinical phenotypes have been tested using disk diffusion assays, and they have been typed phylogenetically using Polymerase chain reaction (PCR) method. Ten of the samples were sequenced using Illumina whole genome sequencing.

Results show that, the incidence of AMR against ciprofloxacin (40%) is higher than that of the national average (between 9% and 23.7%), and more than a third of the strains were multi-drug resistant. Moreover, in the samples collected, 70% of them belongs to the phylo-group B2, which is commonly found in pathogenic *E. coli*.

This study has helped gain interesting insight into the local AMR profile of clinical *E. coli* strains from Salford Royal Hospital. Further work is required to identify more samples from both study sites. The genetic basis of these pathogenic strains will be depicted using the whole genome sequencing data in future studies.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P486

Assessing the copper-response pathway in *E. coli* as a model of metal homeostasis in bacteria.

Andrea Giachino¹, Jon Marles-Wright², Kevin Waldron¹

¹Newcastle University - Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom. ²Newcastle University - School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom

Abstract

The copper (Cu) response pathway of *Escherichia coli*, which relies on the Cu-sensing activator, CueR, and the Cu efflux pump, CopA, provides a minimal model for cellular homeostasis of metal ions. In this study, we quantified the activation of the CueR-dependent promoter in a GFP-reporter construct to measure cellular response to increasing Cu concentrations.

We show that WT *E. coli* (BW25113) featured no response to Cu concentrations <10mM in complex medium, whereas higher concentrations (up to 1mM) induced proportional reporter production without affecting growth. At Cu concentrations >1mM, both growth and reporter production were impaired, up to complete lethality at 10mM. However, we report that 5mM Cu was often lethal, but sometimes (frequency ~15%) resulted in a long lag phase, after which the culture grew to high OD without significant promoter activation. The duration of such lag phase varied across experiments, suggesting it arose from random mutations.

Deletion of the *copA* gene resulted in high basal reporter production in complex medium but not in Cu-depleted minimal medium, indicating that CueR features a strong response to even trace ions. The *DcopA* strain showed a similar growth pattern to WT but increased intracellular Cu concentration and >10-fold increase in reporter production, even in growth-impairing conditions. Also, no adaptation to high Cu concentrations was observed.

Our results suggest that CopA effectively caps cytosolic Cu concentration in *E. coli*, but this regulation is not required for growth. High Cu concentrations are mutagenic and can induce Cu-resistance, perhaps increasing the efficiency of CopA.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P487

Development of a phage therapy treatment option for multidrug resistant *E. coli* infections in dogs.

Alison Low, David Gally

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

Abstract

The rise of antibiotic resistant bacterial infections is a threat to the treatment of infectious diseases in both humans and animals, with companion animals acting as a potentially important reservoir in the transmission of resistant bacteria to and from humans. Our on-going research has been studying and sequencing multi-drug resistant (MDR) *E. coli* associated with urinary tract infections in dogs. Conventional treatments for these MDR infections are limited and we are joining the resurgent group of researchers interested in using lytic phages for treatment. Phage therapy has been used clinically in the former Soviet Union and Eastern Europe for decades and in the current era of antibiotic resistance there is renewed interest in using wild type and modified phages as therapeutic agents to treat MDR infections. One limitation with phage therapy is the variability in bacterium-phage interactions and this is especially true when applied to Gram-negative bacteria such as *Escherichia coli*. We aim to develop 'predictive phage therapy' based on measuring *E. coli*-phage interactions with high throughput methods. With this data, bioinformatics approaches and machine-learning, predict specific phage combinations based on the whole genome sequence of an isolate. We have been using a reduction assay in 96 well plates to measure phage activity against sequenced canine MDR *E. coli* isolates and our initial data is generated from interactions with 40 phages. Following safety trials, the ambition is to produce bespoke phage cocktails that can be trialled to treat unresponsive UTIs in dogs.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P488

Protein Interactions Facilitated by CsgE are Vital to Amyloid Fiber Assembly in *E. coli*

Roger D. Klein¹, Qin Shu², Zachary Cusumano, Jerry Pinkner, Carl Frieden, Matthew R. Chapman³, Scott Hultgren

¹Department of Molecular Biology, Washington University School of Medicine, St Louis, USA.

²Department of Biochemistry and Molecular Biophysics, University School of Medicine, St Louis, USA.

³Department of Molecular, Cellular, and Developmental Biology, University of Michigan

Abstract

Curli amyloid fibers are produced by *E. coli* and other enteric bacteria as part of the extracellular biofilm matrix. The major structural subunit of the curli fiber, CsgA, is secreted through an outer membrane secretion complex comprised of a nonameric CsgG pore and the accessory protein, CsgE. In this study, we utilized NMR chemical shift perturbation studies to identify two solvent-exposed, charged patches on CsgE involved in the CsgE-CsgA interaction. Residues within these patches were mutated and further interrogated using *in vivo* measurements of curli production and *in vitro* amyloid polymerization assays. We found that the R47 residue at the “head” of CsgE is required for stabilization of CsgA and consequently for curli fiber formation on the surface of *E. coli* cells. Introduction of positive charge at the E31 and E85 residues decreased curli fiber formation despite increased stabilization of CsgA. We also interrogated residues hypothesized to mediate CsgE-CsgG complex formation and found that no single point mutants in the positively-charged ‘head’ of the molecule nor the negatively-charged tails abrogate this interaction. In aggregate, these findings suggest that CsgE binds to unfolded CsgA using surfaces comprised of R47, E31, and E85 to mediate a series of finely-tuned specific and nonspecific charge-charge interactions with CsgA. CsgE then forms a high-affinity complex with CsgG, which facilitates the secretion of CsgA and assembly of curli fibers. This mechanistic insight into the directed assembly of functional amyloids in extracellular biofilms could provide novel insights to possible treatments for biofilm-associated bacterial infections.

Escherichia Coli: The Model Microbe
Zone D
Presentations: Tuesday and Wednesday Evening

P489

Functional Characterization of Bacterial and Fungal Ammonium Transporters

Gordon Williamson, Arnaud Javelle, Gaetan Dias-Mirandela

University of Strathclyde, Glasgow, United Kingdom

Abstract

Ammonium is a vital source of nitrogen for bacteria, fungi, and plants and is a toxic metabolic waste for animals. Hence, ammonium transport across biological membranes is a process of fundamental importance in all living organisms. The first genes encoding ammonium transporters were identified 20 years ago in the Baker's yeast (named Mep gene) and plants (named Amt gene). Later it was shown that the rhesus protein (Rh) is an Amt/Mep ortholog in vertebrates and since then, members of the Amt/Mep/Rh protein family have been identified in almost all sequenced organisms. The physiological importance of this family of proteins is underlined by the role of Mep proteins in yeast filamentation, a dimorphic transition often related to the virulence of pathogenic yeast. A plethora of functional studies aimed at elucidating the mechanism of ammonium transport by the Amt/Mep/Rh proteins has led to considerable controversy and the exact mechanism of ammonium transport by these proteins remains largely elusive and controversial. We have recently used an original and powerful methodological approach, based on an *in vitro* assay associated with molecular dynamic simulation to elucidate the molecular detail of Amt protein function. Using our approach, we are now able to answer the intriguing mechanistic questions concerning this fascinating and ubiquitous family of membrane transporters.

Escherichia Coli: The Model Microbe

Zone D

Presentations: Tuesday and Wednesday Evening

P490

Stick to the salad: mechanisms of VTEC adherence to fresh leafy produce

Ashleigh Holmes¹, Yannick Rossez², Leighton Pritchard¹, Ian Toth¹, Nicola Holden¹

¹The James Hutton Institute, Dundee, United Kingdom. ²Université de technologie de Compiègne, Compiègne, France

Abstract

The consumption of fresh produce, such as spinach and lettuce, is increasingly being linked to foodborne outbreaks of verocytotoxigenic *E. coli* (VTEC). There is growing evidence that STEC use plants as alternative hosts rather than as a vector for transmission. Understanding how the bacterium interacts with the plant host can inform on strategies for intervention and control.

Attachment to the host is the first stage of colonisation. Transcriptome analyses, Bacterial Artificial Chromosome (BAC) library and glycan array screening approaches were used to identify factors that are involved in VTEC adherence to plant tissues. The expression of the *E. coli* Common Pilus (ECP) operon was upregulated *in planta*, and functional characterisation determined that ECP recognise arabinosyl residues present on plant cell walls. Screening purified H7 flagella on glycan arrays showed interactions with a positively charged polysaccharide. After further investigation, we show that H7 flagella interact with ionic lipids present in the plant plasma membrane. An *E. coli* O157:H7 strain Sakai BAC library screened for attachment to spinach roots enriched for the *etp* Type II Secretion (T2S) system. In attachment assays, *E. coli* O157:H7 Sakai is recovered from spinach tissues at significantly greater numbers than its isogenic T2S-deficient mutants. Whether this interaction is via a secreted adhesin is yet to be elucidated.

VTEC attachment to the plant host is a multifactorial process with specific and general interactions via fimbrial and afimbrial adhesins; many of which have also been characterised for adherence to *in vitro* and *in vivo* models of infection.



Charles Darwin House,
12 Roger Street, London,
WC1N 2JU, UK
www.microbiologysociety.org

The Microbiology Society is a membership charity for scientists interested in microbes, their effects and their practical uses. It is one of the largest microbiology societies in Europe with a worldwide membership based in universities, industry, hospitals, research institutes and schools.

Our members have a unique depth and breadth of knowledge about the discipline. The Society's role is to help unlock and harness the potential of that knowledge.