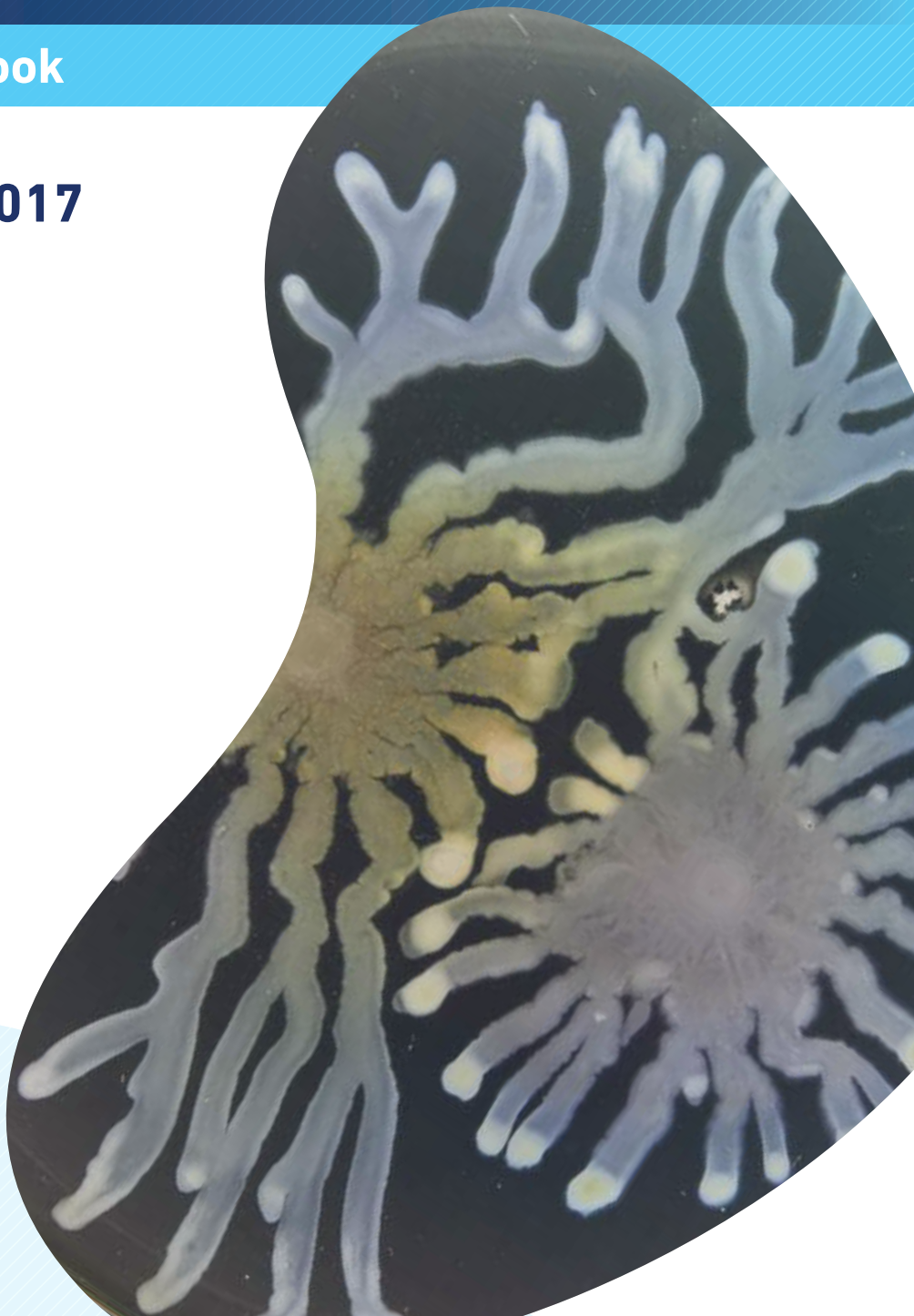
MICROBIOLOGY SOCIETY

FOCUSED MEETING
2017

16th International Conference on Pseudomonas

Poster Abstract Book

5–9 September 2017
St. George's Hall,
Liverpool, UK



@MicrobioSoc
#Pseudomonas17

http://microb.io/pseudomonas17

Contents

Poster sessions

Poster presenters are welcome to leave their poster up until Friday 8 September at 14:00

Odd numbered posters will present on Wednesday 6 September between 16:00– 18:00.

Even numbered posters will present on Thursday 7 September between 17:00–19:00.

Antibiotics & Biofilms

P1

The basis for adaptive changes in multidrug antibiotic resistance and virulence associated with swarming motility in *Pseudomonas aeruginosa*

Shannon R. Coleman, Erin E. Gill, Reza Falsafi, Robert E. W. Hancock
University of British Columbia, Vancouver, British Columbia, Canada

Swarming motility in *Pseudomonas aeruginosa* is a social behavior that facilitates the rapid colonization of semisolid surfaces under nitrogen-limiting conditions and retains many of the features associated with acute infections. Previous studies have shown that *P. aeruginosa* exhibits adaptive antibiotic resistance while swarming. To elucidate the mechanism behind this adaptive resistance, and to discover more about swarming motility in general, the transcriptome of swarming cells was compared to that of swimming cells by the highly accurate next-generation sequencing method RNA-Seq. Briefly, RNA was extracted from swarm and swim plates and cDNA libraries were prepared for Illumina platform and sequenced. Differential gene expression analysis revealed 561 upregulated and 530 downregulated genes. Eleven known and putative drug efflux pumps were upregulated, as well as 62 resistance-determining genes mined from the resistome literature. Mutants in these genes are being tested for their role in swarming-mediated antibiotic resistance, notably revealing pyocin genes influencing trimethoprim susceptibility. Interestingly, swarming cells also overexpressed numerous virulence factors, and genes in several secretion systems and iron-scavenging pathways were upregulated. Intriguingly, two putative universal stress protein homologs were upregulated while 23 ribosomal proteins were modestly downregulated. This indicates that *P. aeruginosa* swarm cells may be in a stressed state, potentially leading to a resilient phenotype. Taken together, these results indicate that *P. aeruginosa* swarm cells have the potential for robust multifactorial virulence and drug resistance *in vivo*. Since conditions in the human lung are similar to those that allow swarming, this study has relevance for the treatment of lung infections.

Antibiotics & Biofilms

P2

Mechanisms influencing *Pseudomonas aeruginosa* susceptibility to antimicrobials in host-mimicking conditions

Corrie Belanger, Amy Lee, Robert Hancock
University of British Columbia, Vancouver, British Columbia, Canada

Antibiotic resistance is becoming increasingly critical, causing drug choices for treatment of many bacterial infections, such as those caused by *Pseudomonas aeruginosa*, to become limited or non-existent. Conventional antibiotic assays do not reflect the *in vivo* environment of infected hosts, and thus are not predictive of antibacterial activity. Our research assessed the ability and mechanism of antibiotics and antimicrobial peptides to treat *P. aeruginosa* in host-mimicking media compared to standard synthetic conditions. Testing susceptibility in media that mimicked the lungs of cystic fibrosis patients or the blood of sepsis patients, macrolides, vancomycin and trimethoprim showed differential inhibitory and antibiotic:peptide synergistic effects against *Pseudomonas* when compared to susceptibility in standard laboratory media. To unravel potential causes for this altered susceptibility, RNA-Seq was used to explore changes in gene expression of *P. aeruginosa* grown or treated with azithromycin in host-mimicking conditions compared to standard media. More than 60 known *P. aeruginosa* resistome genes were dysregulated in host conditions with and without azithromycin treatment, including downregulated surface modification genes such as *oprH* and those in the related PhoP/Q and Arn systems. Dysregulation of these genes may disrupt Lipopolysaccharide modification and prevent *Pseudomonas* from blocking antibiotic uptake in host conditions. Further research will be instrumental for the development of new treatments for *P. aeruginosa* and for the discovery of novel antimicrobial activities of peptides and antibiotics. Understanding the mechanisms that underlie bacterial susceptibility to antimicrobials in varying conditions could help change standard approaches to drug testing and lead to more predictive drug discovery routes.

Antibiotics & Biofilms

P3

SURVIVING AND THRIVING IN THE AIRWAY: HOW THE ARGININE SPECIFIC AUTOTRANSPORTER AMINOPEPTIDASE COULD PROMOTE THE SUCCESS OF *PSEUDOMONAS AERUGINOSA* IN THE CYSTIC FIBROSIS LUNG

Daniella Spencer[1], Rupa Rai[1], Monir Mohammad[1], Nick Tucker[3], Alan Smyth[2], Miguel Camára[1], Kim Hardie[1]
[1]University of Nottingham, Nottingham, UK, [2]Queens Medical Centre, Nottingham, UK, [3]Strathclyde Institute, Glasgow, UK

Pseudomonas aeruginosa is a prominent pathogen within the Cystic Fibrosis (CF) community, surviving challenges from the immune system and antibiotic treatment through biofilm formation. Furthermore, it can thrive under hypoxic conditions. *P. aeruginosa* possesses an Arginine Specific Autotransporter Aminopeptidase (AaaA) which releases arginine from protein N-terminals, enabling its use as a carbon and nitrogen source. This should confer a fitness advantage in oxygen limited environments, including areas of the CF lung. To further understand the relevance AaaA plays in chronic *P. aeruginosa* CF airway infections, we investigated whether *aaaA*: (i) is controlled by regulators of relevance to the CF environment; (ii) is conserved in CF isolates and (iii) plays a role in biofilm formation. We found that RpoN, ArgR, NarX/NarL and AaaA itself control *aaaA* expression and that binding sites for these regulatory proteins are located in the *aaaA* promoter region. Correspondingly, exogenous NO_3 reduced *aaaA* expression. A panel of 18 CF isolates contained a highly conserved *aaaA* gene with some SNPs outside the active site. The levels of AaaA activity in these strains will be presented. Comparison of *aaaA* promoter sequences from GenBank revealed a 200bp promoter deletion in a LES strain with changes to the ArgR and NarL binding sites but with an intact RpoN binding domain. Finally, the ability of a *ΔaaaA* PAO1 mutant to form biofilms was impaired when compared to its WT counterpart using a 24 hour microchamber and 15 hour flow system. These data suggest AaaA plays an important role in the CF lung.

Antibiotics & Biofilms

P4

Evaluation of Pseudomonas aeruginosa Strain Specific Antimicrobial Activity of Gallium-Doped Phosphate Based Glasses

Wenqing Hu, Jo Fothergill, Sabeel P Valappil
University of Liverpool, Liverpool, UK

Evaluation of *Pseudomonas aeruginosa* Strain Specific Antimicrobial Activity of Gallium-Doped Phosphate Based Glasses

Wenqing Hu[1], Jo Fothergill[2], Sabeel P Valappil[3]

1 School of Life Sciences, University of Liverpool, UK
2 Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, UK
2 Department of Health Services Research and School of Dentistry, University of Liverpool, UK

Pseudomonas aeruginosa infections are increasingly complex to treat in hospitalized patients due to increasing antibiotic resistance. Hence development of alternative strategies such as the use of gallium doped phosphate-based glasses (Ga-PBGs) is of considerable interest. In this study, the antibacterial effect of Ga-PBGs on 64 well-characterised isolates of *P. aeruginosa* from an international panel. In addition, the synergistic effect of Ga-PBGs and tobramycin on a subset of strains was evaluated. A modified disc diffusion assay was used to examine the antibacterial effect of Ga-PBGs with gallium free PBGs as controls. Subinhibitory tobrmaycin was used in combination with Ga-PBG to determine the synergistic effect.

Ga-PBGs showed strain specific P.aeruginosa growth inhibition, with the largest zone of inhibition > 20 mm (STDEV= 0.816) and smallest < 10 mm (STDEV = 0.767).There was no significant difference in the activity of Ga-PBG against antibiotic susceptible isolates and multidrug resistant isolates (resistant to at least 8 different antibiotics). The inhibitory effects of Ga-PBGs were enhanced in the presence of tobramycin for PAO1 and PA14. Ga-PBGs displayed activity against all 64 *P. aeruginosa* isolates and were effective against highly antibiotic resistant isolates thus highlighting its potential use as a novel therapeutic agent.

Antibiotics & Biofilms

P5

Combination antibiotic therapy for *P. aeruginosa* blood stream infection in febrile neutropenic patients? The question still remains.

Kate McCarthy, David Cherian, Minyon Avent, David Paterson
University of Queensland, Brisbane, Queensland, Australia

Aim

The benefit of combination antibiotic therapy (CT) is not yet clear in the setting of *Pseudomonas aeruginosa* BSI. This study aimed to look at the effect of CT in either the empirical or definitive phase of therapy in the febrile neutropenic host.

Methods

The study was conducted at a large tertiary care institution with a haematology, oncology and a bone marrow transplant service. Positive blood cultures for *P. aeruginosa* in adult febrile neutropenic patients were identified over a 7 year and 3 month time period from the 1st of January 2006 to the 1st of April 2014. Extensive clinical, laboratory and outcome data was obtained.

Results

Fifty seven patients were included in the epidemiology analysis. The majority of infections were hospital acquired, in a patient with leukaemia or in a patient who had received chemotherapy in the preceding 30 days. The mean length of neutropenia in the cohort was 6 days. Antibiotic resistance was limited. CT consisted mainly of a beta-lactam with an aminoglycoside and the length of dual therapy was relatively short. Twenty seven patients were studied in the outcome analysis. No significant benefit in the modified SIRS score at 3 and 7 days was seen. A low rate of mortality was also seen in the cohort studied and thus the effect of combination antibiotic therapy could not be studied.

Conclusion

A randomised controlled trial is required to determine if CT does provide an outcome benefit in the setting of *P. aeruginosa* BSI in the febrile neutropenic patient.

Antibiotics & Biofilms

P6

eDNA in *Pseudomonas* biofilms: from chromosome to higher-order extracellular structure

Thomas Seviour, Wong Lan Li, Staffan Kjelleberg
Nanyang Technological University, Singapore, Singapore

While apparently similar to and providing full coverage of whole-genome DNA, key questions regarding differences between the organization and behavior of DNA in the extracellular milieu (i.e. extracellular DNA, or eDNA) and inside the cell are unanswered. Despite the likely implications various secondary DNA structures might have for biofilm structure and function, eDNA structure and its contribution to biofilm structure has so far only been described in vague terms. In this study we exploit the solubility of *Pseudomonas* biofilms in designer solvent 1-ethyl-3-methyl imidazolium acetate (EMIM-Ac) to isolate eDNA and reconstitute it as representative gels. eDNA was the only *Pseudomonas* exopolymer able to mimic this property of native biofilms. eDNA additionally dominated the elastic response of biofilms, further qualifying it as a foundation biofilm polymer. An explanation was sought for this unusual tendency of eDNA to form gels and elastic networks by performing comparative biophysical assays on eDNA isolates and unprocessed biofilms. The stability and orientation of the DNA secondary structure strongly depended on the availability of specific cations. Evidence of specific nucleobase proton-proton interactions was obtained from nuclear magnetic resonance spectra, and the secondary structure of *Pseudomonas* biofilms could then be assigned. Thus, the secondary structure of eDNA was preserved during isolation and is fundamental to its ability to support exopolymer networking, as well as the various emergent biofilm properties that result from this, such as enhanced virulence and multi-drug resistance.

Antibiotics & Biofilms

P7

Structure-dependent efficacy of biofilm-dispersing agents

Tae-Hyeon Kim, Soo-Kyoung Kim, Joon-Hee Lee
Pusan National University, Busan, Republic of Korea

Pseudomonas aeruginosa is an opportunistic human pathogen causing various infections, such as cystic fibrosis, microbial keratitis, and burn wound infections. Most chronic infections by *P. aeruginosa* are closely related to biofilm formation. In the highly structured architectures of biofilms, bacterial cells are protected in extracellular polymeric matrix from various environmental stresses, antimicrobial treatment, and host immunity. Many strategies have been attempted to control the biofilm for *P. aeruginosa* and the induction of biofilm dispersion is one of the most promising methods. In this study, clinical isolates of *P. aeruginosa* from hospitalized patients were investigated for their biofilm structures and tested for the biofilm dispersion. Clinical isolates formed structurally distinct biofilms that could be classified into three different groups; 1) prototypic mushroom structure, 2) thin flat, and 3) thick flat structures. We attempted to induce the dispersion of these differently structured biofilms by using anthranilate and SNP (sodium nitroprusside), the known biofilm-dispersing agents. While both SNP and anthranilate were able to disperse all type of biofilms, thick flat biofilms were less efficiently dispersed, compared with the biofilms of other structures. This result suggests that biofilm-dispersing agents have better efficacy on the biofilms of porous structure than on densely packed biofilms.

Antibiotics & Biofilms

P8

Differential evolution landscapes towards resistance of *Pseudomonas aeruginosa* under selective pressure with ribosome-targeting antibiotics

Fernando Sanz García, Sara Hernando Amado, José Luis Martínez
National Centre for Biotechnology, Madrid, Spain

Pseudomonas aeruginosa is an opportunistic pathogen that presents a characteristic low susceptibility to several drugs. In addition, *P.* aeruginosa can acquire increased levels of resistance, being mutation the main cause of resistance during chronic infections as those of cystic fibrosis patients. Given these premises, tools for predicting the emergence of resistance may help in understanding the pathways of evolution towards resistance of this microorganism. For this purpose, we compared the evolutionary trajectories towards resistance of *P. aeruginosa* PA14, to two ribosome-targeting antibiotics: tobramycin and tigecycline. The first one is regularly used in therapy, whereas this bacterium is considered to be intrinsically resistant to the second one. Deciphering whether or not "intrinsically-resistant" bacteria can acquire further resistance is a proof of concept required to predict whether anti-resistance approaches might allow the resuscitation of drugs for which organisms are currently resistant.

In order to elucidate the different evolutionary pathways towards resistance to these antimicrobials we evolved four biological replicates in parallel under selective pressure, doubling the concentration of each antibiotic every five days, from MIC to 32MIC. Subsequently, the genomes of each of the populations at the last day of the experiment were sequenced. The order of fixation of mutations during the evolution and the susceptibility of evolved populations to a large range of antibiotics, in order to determine whether the acquisition of resistance was specific to the chosen antibiotic, were determined.

All this information may allow developing new strategies to manage and predict resistance in this relevant nosocomial pathogen.

Antibiotics & Biofilms

P9

Quorum Sensing inhibition modifies evolution landscapes of antibiotic resistance in *Pseudomonas aeruginosa*.

Sara Hernando Amado, Fernando Sanz García, Jose Luís Martínez
National Center for Biotechnology, Madrid, Spain

The bacterial Quorum Sensing (QS) response is a critical element for intraspecific communication, as well for interspecies communication. In *Pseudomonas aeruginosa*, the QS response governs the expression of a large set of elements involved in bacterial-host interactions, including motility, expression of virulence factors and biofilm formation, all of them involved in the success of infection. However, it is intriguing to find that clinical strains of *P. aeruginosa*, isolated from sputum of chronically infected cystic fibrosis patients are frequently defective in QS, being this defect frequently due to mutations in *lasR*, which encodes the QS regulator LasR. These mutants have been described as cheaters because they can make use of the public goods produced by the cooperative part of the population. In addition, *lasR* mutants may outcompete wild type cooperators leading to reduced virulence, reason why social interactions have been proposed as a target for medical applications. However, the effect of *lasR* inhibition in the evolution of antibiotic resistance, which may allow us to designe more efficient treatments against these bacteria, has not been analyzed to date. Here, we have used *in vitro* experimental evolution and whole genome sequencing to compare evolutionary landscapes of ribosome-targeting antibiotics in a *lasR* and a wt population of PA14 *P. aeruginosa*. In addition we have reconstructed, in both backgrounds, some of the mutations experimentaly selected with the purpose of shed light into the complex relationship between virulence and antibiotic resistance.

Antibiotics & Biofilms

P10

Identification of antagonists of the quorum sensing regulator PqsR that inhibit virulence traits in *Pseudomonas aeruginosa* clinical isolates

Eduard Vico Oton, Fadi Soukarieh, Michael Stocks, Paul Williams, Stephan Heeb, Miguel Cámara
University of Nottingham, Nottingham, Nottinghamshire, UK

The use of antibiotics to treat *Pseudomonas aeruginosa* infections has resulted in a rapid increase in resistance as they inhibit cell viability. To minimize this problem, the targeting of virulence through the inhibition of quorum sensing signalling systems (QS) has been proposed as a promising alternative. One of these QS systems is driven by 2-alkyl-quinolone (AQ) signal molecules. The major *P. aeruginosa* AQs are 2-heptyl-4-quinoline (HHQ) and 2-heptyl-3-hydroxy-4-quinolone (PQS) which through binding to the LysR-type regulator PqsR (MvfR) control virulence factor production, biofilm formation and the sensitivity of biofilms to antibiotics.

We have been searching for inhibitors of the interaction between PQS/HHQ and PqsR. Using structure-based drug discovery and virtual screening approaches we have identified a number of PqsR inhibitors with potencies of ≤ 10 μM as determined *in-vitro*. These compound series have been further optimised using medicinal chemistry methodology to improve potency and physicochemical properties. These compounds inhibit *pqsA* expression and AQ biosynthesis and attenuate virulence factor production in *P. aeruginosa* clinical isolates. We are currently investigating their impact on sensitizing biofilms to antibiotics.

Antibiotics & Biofilms

P11

Alginate oligomers as novel therapies to treat life-threatening pseudomonal multi-drug resistant bacterial infections

Manon Pritchard[1], Lydia Powell[1], Juliette Oakley[1], Philip Rye[2], Katja Hill[1], David Thomas[1]
[1]Cardiff University, Cardiff, UK, [2]AlgiPharma AS, Sandvika, Norway

Background: Chronic antibiotic exposure of pseudomonal biofilm in the cystic fibrosis (CF) lung is associated with the emergence of multi-drug resistant (MDR) bacteria, resistance to treatment and patient mortality. OligoG CF-5/20, a low molecular weight alginate derived from seaweed, has previously been shown to alter surface charge and motility of *Pseudomonas aeruginosa*, and potentiate antibiotic efficacy.
Methods: Confocal laser scanning microscopy was employed to visualise biofilm disruption of mucoid *P. aeruginosa* (NH57388A), and quantified using COMSTAT image analysis. Rate of acquisition of antibiotic resistance to azithromycin in *P. aeruginosa* PAO1 over 45 days (± OligoG), and antibiotic cross-resistance (n=10) was determined using a "bead-biofilm" model (Poltak and Cooper 2010: doi:10.1038/ismej.2010.136).
Results: Imaging demonstrated that OligoG treatment (>0.5%) inhibited biofilm formation resulting in bio-volume reduction ($P<0.05$), induced disruption of 24 h established biofilms, with corresponding marked decreases in biofilm height (control vs. 2% OligoG; $P<0.05$). Furthermore, OligoG treatment (≥2%) induced significant alteration in the extracellular polymeric substance of 24 h established biofilms as shown by altered distribution of sugar residues ($P<0.05$), and decreased extracellular DNA. Whilst OligoG failed to affect the rate of acquisition of azithromycin resistance, mutants formed in the presence of OligoG (+azithromycin) demonstrated >3-7 fold reduction in resistance to antibiotics such as aztreonam, oxy-tetracycline and meropenem.
Conclusion: These studies highlight the potential of novel polymer therapies, such as OligoG, to disrupt MDR pseudomonal biofilms and potentiate conventional antibiotic treatment. OligoG is currently in human trials for the treatment of life-threatening, MDR lung infections.

Antibiotics & Biofilms

P12

Molecular epidemiology of MBL-producing Pseudomonas aeruginosa isolates in Poland, 2005-2015: clonality and MBL genetic determinants.

Pawel Urbanowicz[1], Radoslaw Izdebski[1], Anna Baraniak[1], Dorota Zabicka[2,3], Monika Machulska[1], Katarzyna Bojarska[2,3], Elzbieta Literacka[2,3], Małgorzata Herda[2,3], Waleria Hryniewicz[2], Marek Gniadkowski[1]
[1]National Medicines Institute, Department of Molecular Microbiology, Warsaw, Poland, [2]National Medicines Institute, Department of Epidemiology and Clinical Microbiology, Warsaw, Poland, [3]National Medicines Institute, National Reference Centre for Susceptibility Testing (NRCST), Warsaw, Poland

Background: Pseudomonas aeruginosa is one of the most common and clinically-relevant nosocomial pathogens, with notoriously broad antimicrobial resistance accumulating in few pandemic clones. Metallo-β-lactamases (MBLs) are the major mechanism of acquired resistance in P. aeruginosa.

Material/methods: 1314 carbapenem-resistant, putative MBL-producing Pseudomonas spp. isolates were submitted to the Polish National Reference Centre for Susceptibility Testing between 2005-2015. Species were reidentified using Vitek 2 (bioMérieux). MBLs were detected originally by EDTA-DDST and, recently, the Carba NP test. 450 confirmed non-duplicate MBL-producing P. aeruginosa (MPPA) isolates were selected for molecular analysis, including PCR for MBL genes, PFGE, MLST and PCR-sequencing of MBL gene-carrying integrons.

Results: Of the 450 isolates, 440 (~98%) produced VIM-like MBLs, mostly of the VIM-2 type. The isolates comprised 136 pulsotypes, of which seven pulsotypes grouped 246 isolates (~55%) from multiple regions. The MPPA subpopulation was dominated by four STs: ST235, ST111, ST273 and ST654. ST235 has spread all over the country, while ST111 and ST654 have been frequent mainly in western and southern provinces, respectively. ST273, reported rarely in other countries, has disseminated all over Poland since 2006. MBL genes were located in class 1 integrons of large diversity in variable regions. The most prevalent integron contained cassettes aadB-blaVIM-2-aadA6/aadA10 and was present in multiple clones in the entire country.

Conclusions: This study in progress has shown a dramatic increase and proliferation of MPPA in Poland in 2005-2015. This has been due to few epidemic clones, including specific contribution of ST273. One integron type has broadly spread by horizontal transfer.

Antibiotics & Biofilms

P13

Molecular characterization of MBL-producing Pseudomonas putida from Poland, 2003-2016: clonal relatedness, occurrence and MBLs genetic determinants.

Pawel Urbanowicz[1], Radoslaw Izebski[1], Anna Baraniak[1], Dorota Zabicka[2,3], Waleria Hryniewicz[2], Marek Gniadkowski[1]
[1]Department of Molecular Microbiology, National Medicines Institute, Warsaw, Poland, [2]Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Warsaw, Poland, [3]National Reference Centre for Susceptibility Testing (NRCST), National Medicines Institute, Warsaw, Poland

Background: Pseudomonas putida has been repeatedly reported as a host of acquired antimicrobial resistance (AMR) determinants, including metallo-β-lactamases (MBLs). This study comprised 61 non-duplicate MBL-producing P. putida (MPPP) strains isolated in Polish medical institutions in 2003-2016. The aim was to reveal clonal relationships between the isolates, identify MBL genes and characterise their genetic context.
Material and methods: Carbapenem-resistant, putative MPPP isolates have been collected during surveillance of MBL producers run by the National Reference Centre for Susceptibility Testing. Species were identified using Vitek 2 (bioMérieux). MBLs were detected by phenotypic assays (CarbaNP, EDTA-DDST). The molecular analysis included PFGE, PCR for MBL genes and PCR-mapping of MBL gene-carrying integrons.
Results: The PFGE analysis revealed 61 PFGE patterns clustered into 32 pulsotypes, with the majority of isolates (n=36) being grouped in 7 pulsotypes. All isolates produced VIM-like MBLs, mostly of the VIM-2 type. MBL genes were located in class 1 integrons of which the most prevalent one contained the gene cassette array aadB-blaVIM-2-aadA6/aadA10. This integron was observed first in Poland in P. aeruginosa and P. putida in 2003. Since then it has widely spread in the two species all over the country.
Conclusions: This first study focused on MPPP in Poland shows high genetic diversity of the organism. However, several genotypes have been observed in distant locations, suggesting increased transmission potential. The high prevalence of one integron type, which occurs also in P. aeruginosa, seems to confirm the hypothesis on P. putida being a significant reservoir of AMR genes.

Antibiotics & Biofilms

P14

Biosynthesis of the Pel polysaccharide in *Pseudomonas aeruginosa* requires the formation of a multipurpose membrane complex

Gregory B. Whitfield[1,2], Lindsey S. Marmont[1,2], Jacquelyn Rich[3], Joe J. Harrison[3], Matthew R. Parsek[4], P. Lynne Howell[1,2]
[1]Program in Molecular Medicine, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada, [2]Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada, [3]Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada, [4]Department of Microbiology, University of Washington, Seattle, Washington, USA

Pseudomonas aeruginosa has adapted to life in nearly every niche on Earth due to its ability to maximize fitness through dynamic responses to changing environmental conditions. One survival strategy *P. aeruginosa* employs is to form a biofilm, which requires secretion of specialized macromolecules such as the Pel polysaccharide. Pel is integral to the community response to redox stress and shields cells within the biofilm from aminoglycoside antibiotics. Despite the importance of the Pel polysaccharide as an adaptive tool of *P. aeruginosa*, there is little data describing the molecular mechanisms of its biosynthesis. To this end, we examined the proteins thought to be involved in the initial stages of Pel biosynthesis to map their connectivity to each other to define the minimum regulatory/biosynthetic complex. Using a combination of genetic dissection, co-immunoprecipitation, bacterial two-hybrid analyses, analytical size exclusion, and X-ray crystallography, we have identified a complex of PelDEFG that we believe represents the unit responsible for biosynthetic regulation, polymerization, and transport of Pel across the inner membrane. By pairing co-immunoprecipitation with mass spectrometry, we discovered a number of potential regulatory partners, most notably the cyclic diguanylate phosphodiesterase BifA. The proposed architecture of this complex suggests that the mechanism for Pel biosynthesis may be unique amongst those identified in gram-negative bacteria, and indicates a direct link between Pel biosynthesis and major cellular signaling networks.

Antibiotics & Biofilms

P15

Oligomeric lipoprotein PelC guides polysaccharide export across the outer membrane of *Pseudomonas aeruginosa*

Lindsey Marmont[1,2], Jacquelyn Rich[3], John Whitney[4], Gregory Whitfield[1,2], Howard Robinson[5], Matthew Parsek[6], Joe Harrison[3], Lynne Howell[1,2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]The Hospital for Sick Children, Toronto, Ontario, Canada, [3]University of Calgary, Calgary, Alberta, Canada, [4]McMaster University, Hamilton, Ontario, Canada, [5]Brookhaven National Laboratory, Upton, New York, USA, [6]University of Washington, Seattle, Washington, USA

Exopolysaccharides are critical structural and functional components of the *Pseudomonas aeruginosa* biofilm matrix. Pel is a cationic polysaccharide that protects *P. aeruginosa* from aminoglycoside antibiotics and contributes to biofilm architecture through ionic interactions with extracellular DNA. Our bioinformatics analysis of genome databases suggests that *pel* gene clusters are present in >125 bacterial species, yet little is known about how this biofilm exopolysaccharide is synthesized and exported from the cell. The role of the outer membrane lipoprotein PelC is unclear, therefore to gain insight into its function we determined the crystal structures of PelC from *Geobacter metallireducens* and *Paraburkholderia phytofirmans*. These data coupled with disulfide crosslinking studies suggest that PelC assembles into a twelve-subunit ring-shaped oligomer. In this arrangement, an aromatic belt in proximity to the lipidation site positions a highly electronegative surface towards the periplasm. The absence of membrane-spanning segments in PelC and its structural similarity to the periplasmic domain of the *Escherichia coli* amyloid secretion channel CsgG, which utilizes a β-barrel domain for export, suggests that another protein is required for Pel translocation across the outer membrane. We show that the multi-domain protein PelB localizes to the outer membrane and exhibits properties suggestive of a β-barrel fold, and propose that PelC functions as a molecular funnel guiding the positively charged Pel polysaccharide towards an exit channel formed by PelB. Together, our findings provide insight into the unique molecular architecture and export mechanism of the Pel apparatus, a widespread exopolysaccharide secretion system found in environmental and pathogenic bacteria.

Antibiotics & Biofilms

P16

A genome-wide epidemiological study of Metallo-β-lactamases-producing *Pseudomonas aeruginosa* isolates from an Italian hospital

Irene Bianconi[1], Clotilde Bettua[1], Elisabetta Giacobazzi[1], Lucia Collini[2], Paolo Lanzafame[2], Olivier Jousson[1]
[1]Centre for Integrative Biology (CIBIO) - University of Trento, Trento, Italy, [2]Microbiology and Virology Unit - Santa Chiara Hospital, Trento, Italy

The spread of antibiotic resistance represents a severe risk to human health. The WHO has recently updated the list of bacterial species causing community-acquired infections for which the development of new antimicrobials should be prioritized and has listed carbapenem-resistant *Pseudomonas aeruginosa* as a top priority. These strains carry various types of Metallo-β-lactamases (MBL) located on specific mobile and transferable genetic elements within the genome.
Currently, no effective clinical anti β-lactamase inhibitors are available, and since very few innovative and effective antibiotics will reach the market in the near future, it is of primary importance to understand the epidemiology and molecular characteristics of MBL-producing *P. aeruginosa* to prevent and control infections caused by these strains. Although recent studies on MBL-producing *P. aeruginosa* have been carried out in several countries, few updated epidemiological data are available for Italy. We thus performed a retrospective molecular epidemiological study to provide a description of the clinical, epidemiological, and molecular characteristics of infections caused by MBL-producing *P. aeruginosa* isolated from patients hospitalised at the Santa Chiara Hospital in Trento, Italy.
A total of 416 MBL-producing strains has been collected from 2010 to 2017. Antimicrobial susceptibility testing was performed and MBL phenotype was assessed. Whole-genome sequencing and analysis will lead to a better understanding of the epidemiology of MBL-producing *P. aeruginosa*, thus assisting in the design of targeted guidelines for infection control.

Antibiotics & Biofilms

P17

Effect of efflux pumps inhibition on *Pseudomonas aeruginosa* transciptome and virulence

Giordano Rampioni[1], Cejoice Ramachandran Pillai[2], Francesca Longo[1], Roslen Bondí[1], Valerio Baldelli[1], Marco Messina[1], Francesco Imperi[3], Paolo Visca[1], Livia Leoni[1]
[1]University Roma Tre, Dept. of Science, Rome, Italy, [2]Kannur University, Inter University Centre for Bioscience, Palayad, Kerala, India, [3]Sapienza University of Rome, Department of Biology and Biotechnology "Charles Darwin", Rome, Italy

Efflux pumps of the resistance-nodulation-cell-division (RND) family increase antibiotic resistance in many bacterial pathogens and are considered candidate targets for the development of antibiotic adjuvants. RND pumps have also been proposed to contribute to bacterial infection in animals and plants, implying that efflux pump inhibitors (EPIs) could also act as anti-virulence drugs. Nevertheless, EPIs are usually investigated for their properties as antibiotic adjuvants, while their potential anti-virulence activity is seldom taken into account.

Here we show that RND efflux pumps contribute to *Pseudomonas aeruginosa* PAO1 pathogenicity in an insect model of infection and that the well-characterized EPI Phe-Arg-β-naphthylamide (PAβN) is able to reduce the virulence of this pathogen both *in vitro* and *in vivo*. The protection exerted by PAβN from *P. aeruginosa* PAO1 infection *in vivo* correlates with the down-regulation of key virulence genes, as revealed by transcriptomic and/or phenotypic analyses. However, the PAβN-mediated repression of virulence-related traits is strain dependent, as assessed in a collection of *P. aeruginosa* clinical isolates.

Given that efflux pump inhibition has an impact on *P. aeruginosa* virulence, anti-virulence properties of EPIs are worthy to be explored, taking into account the strain-specificity of their activity.

Antibiotics & Biofilms

P18

Assessing the suitability of quorum sensing inhibition in cystic fibrosis therapy

Giulia Giallonardi[1], Valeria Lancia[1], Ersilia Fiscarelli[2], Francesca Ungaro[3], Francesco Imperi[4], Paolo Visca[1], Giordano Rampioni[1], Livia Leoni[1]
[1]University Roma Tre, Rome, Italy, [2]Bambino Gesù Hospital, Rome, Italy, [3]University Federico II, Naples, Italy, [4]University Sapienza, Rome, Italy

The quorum sensing (QS) system of *Pseudomonas aeruginosa* is considered a good target for the development of anti-virulence drugs, as testified by the high number of new molecules targeting *P. aeruginosa* QS identified every year. However, the suitability of anti-QS therapy in the treatment of cystic fibrosis (CF) patients is under debate. Indeed, *P. aeruginosa* isolates from CF lung infections disclose high genetic variability, and isolates producing low levels of virulence factors or QS-defective mutants are frequently isolated from CF patients.

Here, a collection of 100 *P. aeruginosa* isolates from CF patients infected for different years have been tested to evaluate the suitability of anti-QS therapy in CF lung infection. In particular these strains have been characterized for: *i)* antibiotic susceptibility; *ii)* QS signal molecules production; *iii)* susceptibility to niclosamide (NCL), a strong QS and virulence inhibitor targeting the *N*-3-oxododecanoyl-homoserine lactone ($3OC_{12}$-HSL)-dependent QS system in the model strain PA14. Results have shown that the majority of the strains (69%) produce $3OC_{12}$-HSL and could be in principle susceptible to NCL. Nevertheless, NCL-mediated inhibition of $3OC_{12}$-HSL and virulence factor production is overall low and highly variable. Statistical analysis has shown no significant correlation between drug resistance, $3OC_{12}$-HSL production, NCL susceptibility and duration of the infection.

Overall, this study highlights that the effect of anti-QS drugs could be strongly strain-dependent, indicating that each new molecule should be tested on a large collection of clinical strains before further studies leading to translation to CF therapy.

Antibiotics & Biofilms

P19

Ajoene, a novel therapeutic that shows enhanced activity in *in vivo* and *in vitro* models of *Pseudomonas aeruginosa* infection.

Laura Bricio-Moreno[1], Elaine Waters[1], Dan Neill[1], Gareth Evans[2], Michael Graz[2], Jo Fothergill[1], Aras Kadioglu[0]
[1]University of Liverpool, Liverpool, UK, [2]Neem Biotech, Abertillery, UK

Pseudomonas aeruginosa is an opportunistic pathogen that causes a variety of infections in humans and is one of the ESKAPE pathogens described as clinically relevant and highly multidrug resistant. In this study we tested the antimicrobial properties of Ajoene against the epidemic *P. aeruginosa* strain LESB65 *in vivo* and *in vitro*. Artificial Sputum Media was used to study antimicrobial properties of Ajoene against developing and mature *P. aeruginosa* biofilms to mimic both early and chronic infections. In the early infection *in vitro* model, Ajoene significantly inhibited the growth of LESB65 during the first 48h of infection when administered in combination with Tobramycin, and it induced a reduction in pyocyanin production during the 7 days of the experiment. The Ajoene/Tobramycin combination was more effective against pre-established biofilms. Changes in gene expression of virulence-related genes were also studied. In the *in vivo* model of infection, we observed a reduction in the number of bacteria colonising the upper respiratory tract of mice treated with Tobramycin alone, however, this effect was stronger when Tobramycin was administered in combination with Ajoene. By day 5, 70% of mice treated with Ajoene/Tobramycin treatment had cleared *P. aeruginosa* from the upper respiratory tract; in contrast, only 30% of mice treated with Tobramycin alone had cleared the infection. Ajoene alone had, however, no effect on the bacterial density in the upper respiratory tract when compared to the untreated control group. These results suggest that Ajoene in combination with Tobramycin could be used to treat infections with *P. aeruginosa*.

Antibiotics & Biofilms

P20

Identification of FDA-approved anti-virulence drugs targeting PqsE

Valerio Baldelli, Francesca D'Angelo, Viola Pavoncello, Paolo Visca, Livia Leoni, Giordano Rampioni
University Roma Tre, Dept. of Science, Rome, Italy

In *Pseudomonas aeruginosa* the quorum sensing effector protein PqsE is required for the expression of a large array of virulence factors, and *pqsE* mutation results in decreased virulence in plant and animal infection models. Therefore, PqsE is a candidate target for the development of *P. aeruginosa* anti-virulence drugs.

In the attempt to identify PqsE inhibitors, a library of FDA-approved drugs has been screened by using a *P. aeruginosa* biosensor strain carrying a transcriptional fusion between the promoter of the *pqsABCDE* operon (P*pqsA*) and the *luxCDABE* reporter genes for bioluminescence emission, and in which PqsE expression is IPTG-inducible. Since PqsE negatively affects P*pqsA* activity, light emission in the biosensor is low in the presence of IPTG, and is expected to increase in the presence of PqsE inhibitors.

The screening campaign led to the identification of three drugs increasing bioluminescence in the biosensor and decreasing the expression of PqsE-dependent virulence traits in *P. aeruginosa* wild type (*i.e.* pyocyanin production and swarming motility).

Preliminary analyses showed that the most promising PqsE inhibitor (named PqsE-I2) does not affect P*pqsA* activity in a *P. aeruginosa pqsE* mutant strain, supporting target specificity. Moreover, Real Time PCR analysis confirmed that PqsE-I2 increases the mRNA levels of the *pqsABCDE* operon in *P. aeruginosa* wild type, thus suggesting interference with PqsE at the post-transcriptional level.

Analyses are ongoing to elucidate the mechanism of action of PqsE-I2, and to assess its effect on *P. aeruginosa* pathogenic potential both *in vitro* and *in vivo*.

Antibiotics & Biofilms

P21

The antimicrobial efficacy of NO-releasing polymeric substrates

George Fleming, Jenny Aveyard, Jo Fothergill, Raechelle D'Sa
University of Liverpool, Liverpool, UK

Given the global epidemic of antibiotic resistance, coupled with 1 million new cases of healthcare-related infections reported each year in England, developing new antimicrobial strategies is an urgent clinical need. *Pseudomonas aeruginosa* is responsible for 10% of all hospital-acquired infections and a leading cause of nosocomial infections. Approximately 60-70% of nosocomial infections are related to implanted medical devices. Polyethylene terephthalate (PET) and silicone elastomer (SE) are commonly employed as biomaterials for use as shunts, catheters and grafts, and are susceptible to microbial infection and biofilm formation.

Nitric oxide (NO) is a small messenger molecule produced endogenously that has showed antimicrobial properties. In this work we aim to modify PET and SE to release NO at the surface, through the use of silane chemistry, as a protective mechanism in combatting infection upon implantation into the body. We have shown an environmentally-friendly, cost-effective method of generating polymeric NO donors, with varying NO release properties. X-ray photoelectron spectroscopy confirmed the presence of the NO donor group and electrochemical detection allowed the quantification of NO released from the polymers. The NO-releasing PET surfaces released 3500 nM of NO and 5000 nM for SE grafted surfaces. Antibiofilm efficacy was monitored with colony forming unit assays using *P. aeruginosa*, PA01 and PA14. Reductions of up to 1-log have been observed in the modified polymers after 24 hour biofilm formation. Further modification will be undertaken in order to maximise the antimicrobial activity. Ultimately, these surfaces could be used in widespread medical applications against antibiotic resistant *P. aeruginosa*.

Antibiotics & Biofilms

P22

Identification of novel virulence targets in *Pseudomonas aeruginosa* for antimicrobial development

Ioanna Chatzigianni, Jean-Frederic Dubern, Simon Caulton, Ingrid Dreveny, Jonas Emsley, Paul Williams, Stephan Heeb, Miguel Cámara
University of Nottingham, Nottingham, UK

Pseudomonas aeruginosa is an important human opportunistic pathogen in immunocompromised patients and is intrinsically resistant to antibiotics. Using a genome wide approach involving the screening of a large Tn*5* P. aeruginosa PAO1-L mutant library, we identified a number of mutants severely attenuated in virulence but presenting normal growth, and which could potentially be exploited as novel targets for anti-virulence agents. One of the mutated genes codes for a putative S-adenosyl-L-methionine-dependent methyltransferase (SamA). A samA deletion mutant (Δ*samA*) exhibits impaired twitching and swarming motilities and reduced biofilm formation. Since some of these traits are controlled by quorum sensing (QS) we investigated the relationships between SamA and the QS systems in *P. aeruginosa* and found that mutations in the QS genes *rhlI*, *lasI* and *pqsA* have an impact on *samA* expression but also that a Δ*samA* mutation has an impact on the expression of these QS genes. To gain further insights into the function of SamA we are currently purifying this protein for enzymatic characterization. This work will set the grounds for the future development of SamA inhibitors which can be used to attenuate the virulence of *P. aeruginosa* as an alternative therapeutic approach.

Antibiotics & Biofilms

P23

Identification of the mode of action of peptide antibiotics in *Pseudomonas aeruginosa*

Alessandra Vitale[1], Gabriella Pessi[1], Steven Higgins[1], Katja Zerbe[2], John Robinson[2], Leo Eberl[1]
[1]Plant and Microbial Biology, Zurich, Switzerland, [2]Department of Chemistry, Zurich, Switzerland

One of the biggest challenge of our century is the increasing antibiotic resistance in pathogenic bacteria such as the opportunistic human pathogen *Pseudomonas aeruginosa*. New antibiotic treatments are urgently needed and this challenge could be overcome with new peptidomimetic antimicrobial compounds, which have been shown to be especially active against many Gram-negative bacteria.
In this study, we investigated the mechanisms of action of those new antibiotics by using the transposon sequencing method. A saturated library of transposon mutants (approx. 1 million) was constructed in *P. aeruginosa* PA14 and challenged with a sub-inhibitory concentration of the well-known cyclic peptide antibiotics polymyxins B and E. We performed a fitness based data analysis and established a list of candidate genes, which are needed for autonomous growth or provide a fitness advantage in the presence of the antimicrobial compounds. Among them, three genes involved in outer-membrane biosynthesis and one coding for a multidrug resistance efflux pump were found to be essential in the presence of both polymyxins (B and E). In addition, we are currently challenging individual mutants with polymyxins in order to confirm the importance of specific proteins, as for example the multidrug resistance efflux pump.
In conclusion, we have constructed a highly saturated library of mutants in *P. aeruginosa* PA14 and identified new potential mechanisms of polymyxin resistance. In the next step, we will apply this approach to characterize the mode of action of novel peptidomimetic antibiotics.

Antibiotics & Biofilms

P24

Alginate oligomers potentiate antibiotics by disrupting biofilm

Anne Tøndervik[1], Randi Aune[1], Philip Rye[2], Are Kristiansen[2], Håvard Sletta[1]
[1]SINTEF, Dept Biotechnology and Nanomedicine, Trondheim, Norway, [2]AlgiPharma AS, Sandvika, Norway

Objectives: Low molecular weight alginate (OligoG CF-5/20) has previously been shown to reduce the MIC of antibiotics (up to 512-fold reduction) for a wide range of bacteria and fungi. However, the mechanism of action is unclear. Since alginate oligomers have also been shown to disrupt biofilm, we hypothesized the potentiating effect may be due to disruption of a defensive biofilm response at sub-lethal MIC's.

Methods: Biofilms of *P. aeruginosa* (PAO1) and *C. difficile* were grown in defined growth medium, and incubated at 37°C for 16 hours before detection with BacTiterGlo. Planktonic growth in the supernatant was also detected by BacTiterGlo. Concentration gradients of the antibiotics used were aztreonam (0.02-16 µg/ml), ciprofloxacin (0.004-0.079 µg/ml), azithromycin (0.125-128 µg/ml), and vancomycin (0.01-16 µg/ml), with and without the presence of OligoG CF-5/20 (4% w/v) in the growth medium.

Results: With increasing antibiotic concentration there is a gradual decrease in the planktonic growth. However, biofilm formation increases, reaching a maximum level preceding the MIC value, indicating a protective biofilm response at sub-lethal MIC's. In the presence of OligoG CF-5/20, this biofilm response is absent, with a concomitant 2-fold reduction in MIC (ciprofloxacin 0.125 to 0.0625 µg/ml; aztreonam 4 to 2 µg/ml.)

Conclusions: At sub-lethal concentrations of antibiotic, some bacteria appear to initiate a stress response by an increased production of biofilm. The anti-biofilm effects of OligoG appear to disrupt this response, thereby reducing the MIC. This mechanism of action may explain the potentiating effect of OligoG across a wide range of antibiotic classes.

Antibiotics & Biofilms

P25

A lytic bacteriophage cocktail improves the killing effects of standard antimicrobials in pre-established Pseudomonas aeruginosa biofilms from cystic fibrosis airway cultures

Isaac Martin[1,2], Sandra Morales[3], Eric Alton[1,2], Jane Davies[1,2]
[1]Imperial College London, London, UK, [2]Royal Brompton Hospital, London, UK, [3]Ampliphi Biosciences Corporation, Sydney, Australia

Objectives: In the context of cystic fibrosis (CF), Pseudomonas aeruginosa (Pa) persists in the lungs due to a biofilm mode of growth, which provides a barrier to antimicrobials and host defences. We explored the effects of a cocktail of 4 phages when added to standard antibiotic therapy in pre-formed Pa biofilms.

Methods: 16 biofilm forming Pa strains were selected from CF patients at the Royal Brompton Hospital based on laboratory sensitivity to ceftazidime and tobramycin as well as to phage in plaque assay. We incubated 96 well plates with Pa for 24 hours, allowing adherent biofilm formation. Ceftazidime and tobramycin (both at 2 × MIC) were then added, with and without bacteriophage for a further 24 hours. Cell viability and biomass were measured using fluorescent resazurin and crystal violet assays, respectively.

Results: Values were expressed as percent reduction in cell viability and biomass compared to negative control. Addition of bacteriophage resulted in a 1.3-fold reduction in cell viability ($p<0.001$) and 1.7-fold reduction in biomass ($p<0.001$) when compared with antibiotics alone. In the 4 biofilms most resistant to antibiotic treatment, the addition of phage conferred a 50 ± 15% reduction in cell viability and 60 ± 12% reduction in biomass (95% CI).

Conclusion: We show significant reduction in pre-formed Pa biofilms when bacteriophage was added to standard antibiotic therapy. The most striking benefits were seen in the 4 clinical strains with the least response to antibiotics. Bacteriophage is a promising treatment adjunct in strains that have little response to conventional antimicrobials.

Antibiotics & Biofilms

P26

Influence of biosurfactants on *Pseudomonas putida* membrane vesicles

Ratchara Kalawong, Masanori Toyofuku, Leo Eberl
Department of Plant and Microbial Biology, Zurich, Switzerland

An increasing number of Gram-negative bacteria have been demonstrated to release membrane vesicle (MVs) which play important roles in biological processes including virulence, stress response, quorum sensing, gene and protein transfer and biofilm formation. Despite their importance for various functions the biogenesis of MVs is only poorly understood.
Pseudomonas putida IsoF is a plant growth promoting strain which was isolated from the rhizosphere of a tomato plant. This bacterium produces the signal molecule 3-oxo-C10HSL, which in turn positively regulates the production of the biosurfactant putisolvin. Biosurfactants are amphiphilic compounds that reduce the interfacial tension of two phases, and are known to dissolve membranous materials such as vesicles. In *P. putida* IsoF, putisolvin is primary associated with the cell, presumably adsorbed to the cellular membrane. It has been shown that putisolvin is required for swarming motility, but given its association with the producing cell, it is not a common good and thus is not shared among swarm cells. In this study we examined the ability of *P. putida* to produce MVs under different conditions, including DNA damaging stress conditions that was previously reported to induce explosive cell lysis in *P. aeruginosa*. Our result suggest that *P. putida* IsoF produces MVs, albeit stress conditions did not significantly enhance MV production, much in contrast to many other bacteria. However, we observed that the production of putisolvin affects MV formation, possibly because of its affinity for the outer membrane**.** The role of biosurfactants on of MV biogenesis will be discussed.

Antibiotics & Biofilms

P27

Exploiting Bacterial Lifestyles: A Strategy for the Treatment of *Pseudomonas aeruginosa* Biofilms

Giulia Ballerin[1,2], Rosalia Cavaliere[1], Paul Young[2], Daniela Traini[2], Cynthia Whitchurch[1]
[1]The ithree institute, Sydney, Australia, [2]Woolcock Institute of Medical Research, Sydney, Australia

Background: We have demonstrated that *P. aeruginosa* planktonic cells survive exposure to β-lactam antibiotics by transitioning to a spherical morphotype and unlike normal bacillary cells, these cells were killed by antimicrobial peptides (AMPs) [1]. The aim of this study was to investigate whether this morphological transition also occurs in biofilms and if so, whether this could be exploited to provide a strategy to eradicate *P. aeruginosa* biofilms.

Methods: *P. aeruginosa* biofilms were grown in flow cells for 3 days in M9 minimal medium, treated with the β-lactam antibiotic meropenem up to 24 h and stained with a fluorescent membrane dye for morphological analysis. Biofilms were also treated with a combination of meropenem and the AMP nisin for 24 h, stained with a membrane/dead cell dye mixture for quantification of biomass and to assess the degree of cell death. Samples were visualised with deconvolution microscopy and quantified using COMSTAT.

Results: Numerous spherical cells could be detected in meropenem-treated biofilms after 16 h and 24 h of treatment. Treatment with a meropenem/nisin combination resulted in a significant decrease in total biomass and a significant increase in dead biomass after 24 h of treatment compared to untreated and single treatment samples. Moreover, the dead biomass remained trapped within the biofilm matrix after the combination treatment.

Conclusion: We showed that cells in *P. aeruginosa* biofilms convert to spherical cells when exposed to a β-lactam antibiotic and that a combination of β-lactam antibiotic with AMPs could provide a novel strategy for treating *P. aeruginosa* infections.

Antibiotics & Biofilms

P28

Towards the function of βαββββ-module containing proteins in *Pseudomonas aeruginosa*

Wulf Blankenfeldt, Allegra Vit, Monika Popp
Helmholtz Centre for Infection Research, Braunschweig, Germany

Gaining detailed insights into resistance mechanisms might deliver new approaches in the battle against emerging multidrug-resistant *P. aeruginosa* isolates. Many members of the βαββββ-module containing protein family are involved in resistance mechanisms. Some of these proteins catalyze metal-dependent degradation of antibiotic compounds while others have chaperone-like functions by binding toxic compounds through π-stacking. According to conserved amino acid residues, the 22 βαββββ-module-containing proteins found in *P. aeruginosa* are classified as metal-dependent (class I) and aromatic compound binding proteins (class II). Proteins that cannot be assigned to both of these groups define class III. Our interest lies in the functional characterization of these class III proteins, because we assume their participation in unidentified resistance mechanisms. To corroborate this we compared the phenotypes of the wild type and transposon insertion strains in genes that code for class III βαββββ-proteins under several growth conditions with Biolog Phenotype MicroArrays™. These assays revealed that the disruption of the genes of interest makes *Pseudomonas* more susceptible towards several toxic compounds. Subsequent experiments, such as binding affinity measurements and protein x-ray crystallography, pointed out that one βαββββ-module protein directly interacts with one of those compounds. If and how this interaction is connected to the molecular function of the investigated protein remains to be verified. Nevertheless, these preliminary results indicate that at least one class III βαββββ-module protein is involved in the mediation of detoxifying mechanisms.

Antibiotics & Biofilms

P29

Transcriptional regulation during NO-induced biofilm dispersal of *Pseudomonas aeruginosa* biofilms

Katrin Gerbracht, Susanne Zehner, Nicole Frankenberg-Dinkel
TU Kaiserslautern, Kaiserslautern, Rheinland-Pfalz, Germany

Biofilms are surface-associated microbial communities with great medical relevance. As an opportunistic human pathogen *Pseudomonas aeruginosa* causes a broad range of acute and chronic infections often involving the formation of biofilms. Therefore, understanding of the mechanisms leading to biofilm dispersion may help to combat biofilms in the future. Several environmental cues have been identified to trigger biofilm dispersal. Many (if not all) have been linked to cause low intracellular c-di-GMP (bis-(3'-5')-cyclic dimeric guanosine monophosphate) concentrations generated by the c-di-GMP degrading phosphodiesterases (PDEs). Non-lethal concentrations of nitric oxide (NO) trigger PDE activity in *P. aeruginosa* and lead to detachment of motile cells from the biofilm. The membrane-anchored protein NbdA (NO-induced biofilm dispersion locus A) with the domain organisation MHYT-AGDEF-EAL was shown to be a major player in the NO-induced biofilm dispersal response of *P. aeruginosa* [1 Li *et.al.*, 2013]. While the main hypothesis postulates direct NO sensing via the MHYT-domain resulting in increased PDE activity, initial qPCR experiments also suggested regulation at the transcriptional level [1]. Within this study we further examined the transcriptional regulation of *nbdA* using transcriptional promoter *lacZ*-fusions. In order to investigate whether endogenous NO has an impact on *nbdA* transcription a markerless *nirS* mutant strain, deficient in dissimilatory nitrite reductase, has been generated. Additional experiments aimed at elucidation of a predicted RpoS consensus sequence in the promoter region of *nbdA*. The obtained data will be presented and discussed with respect to the current model of NO-induced biofilm dispersal in *P. aeruginosa.*

Antibiotics & Biofilms

P30

Anti-biofilm effects of novel alginate oligosaccharides are cation-independent.

Stephen McKenna[1], Lydia Powell[1], Philip Rye[2], Katja Hill[1], David Thomas[1]
[1]Advanced Therapies Group, Cardiff University School of Dentistry, Cardiff, South Glamorgan, UK, [2]AlgiPharma AS, Sandvika, Norway

Background: We have previously demonstrated that the alginate oligomer, OligoG, is able to inhibit/disrupt biofilm formation and potentiate antibiotic activity. The effect of divalent cations, found in bodily fluids, can enhance biofilm development as well as causing gelation of alginates. Here, we hypothesised that OligoG is able to chelate divalent cations, thus limiting bacterial growth and biofilm formation.

Methods: Studies on the effect of divalent cation concentration (calcium; 5-102 mg/ml and magnesium; 6-30 mg/ml) on bacterial growth and biofilm development of non-mucoid wound (PAO1) and mucoid cystic fibrosis (NH57388A) P. aeruginosa isolates ± OligoG (0.5-6%) were conducted. Minimum inhibitory concentration (MIC) assays in Mueller-Hinton broth (MHB), growth curves (24-48h), and confocal laser scanning microscopy (CLSM) imaging of 24h biofilms with LIVE/DEAD® staining were performed.

Results: MIC and growth curve assays demonstrated that variations in cation concentration did not affect growth rate or antibiotic potentiation. However, CLSM imaging demonstrated distinct differences in biofilm formation between both strains. For non-mucoid PAO1, biofilms grown in low cation medium were more diffuse; however, as cation concentration was increased, aggregated structures were apparent. These differences were not observed in the mucoid biofilms. Furthermore, alterations in divalent cation concentration did not affect the antimicrobial properties of OligoG with reduced bacterial growth, reduced MIC and inhibition of biofilm formation all unaffected.

Conclusion: This study suggests that the mode of action of OligoG is cation-independent. These results may inform future research and development of new oligosaccharide therapies for clinical use in the treatment of biofilm-associated diseases.

Antibiotics & Biofilms

P31

Expression of *Pseudomonas aeruginosa* antibiotic resistance genes during infection in cystic fibrosis

Lois Martin[1], Annabelle Watts[1], Cynthia Robson[1], Andrew Gray[1], Joyce Cheney[3], Claire Wainwright[3], Scott Bell[2], Kay Ramsay[2], Jan Cowan[1], David Reid[2], Ben Brockway[1], Iain Lamont[1]
[1]University of Otago, Dunedin, New Zealand, [2]Queensland Berghofer Institute for Medical Research, Brisbane, Australia, [3]Royal Children's Hospital, Brisbane, Australia

The lungs of patients with cystic fibrosis (CF) become chronically infected by *Pseudomonas aeruginosa.* We hypothesized that the variable effectiveness of antibiotics in treating infections may be due to variations in expression of antibiotic resistance genes. The aims of this research were to measure expression of two key antibiotic resistance genes, *ampC* that encodes a b-lactamase and *mexX* that encodes part of an efflux pump, both during infection and in laboratory culture. To do so, RNA was extracted from bacteria grown on agar or, to measure gene expression during infection, from sputum samples expectorated by patients. Expression of *ampC* and *mexX* was measured by RT-qPCR. Isolates of *P. aeruginosa* from 36 CF patients had over 2000-fold variation in expression of both *ampC* and *mexX*. Expression of both genes was increased by the presence of sub-inhibitory concentrations of antibiotic. Expression of *ampC* correlated with resistance to meropenem, a b-lactam antibiotic, but expression of *mexX* did not correlate with resistance to the substrate aminoglycoside antibiotic tobramycin. During infection, expression of *ampC* varied over 4000-fold and that of *mexX* varied over 100-fold in sputum samples from 31 patients. Expression of *ampC* was significantly lower during infection than in laboratory-grown bacteria whereas expression of *mexX* was significantly higher during infection. These data demonstrate that expression of antibiotic resistance genes varies greatly during infection in CF and also between *P. aeruginosa* isolated from patients. These findings may explain the variable effectiveness of anti-pseudomonal antibiotics in treating infection.

Antibiotics & Biofilms

P32

Interactions of microbes with *P. aeruginosa* during chronic lung infection in cystic fibrosis patients.

Laura Wright[1], Sian Pottenger[1], Nick Loman[2], Craig Winstanley[1], Jo Fothergill[1]
[1]University of Liverpool, Liverpool, UK, [2]University of Birmingham, Birmingham, UK

The Cystic Fibrosis (CF) lung facilitates the cohabitation of microbes and we are just beginning to understand the extent of this diversity. The bacteria in chronic lung infections can form complex, interacting communities, which adapt and diversify due to interactions with both the environment, including host responses, and each other. We aimed to study the interspecies interactions components using an *in vitro* model.

We have developed co-culture biofilm models in which microbial interactions can be investigated. Using an artificial sputum medium (ASM) model, which mimics CF sputum, *Pseudomonas aeruginosa* was co-cultured with pathogens, including *Staphylococcus aureus* and *Burkholderia cenocepacia,* and common CF lung commensals, including *Rothia sp.* and *Streptococcus sp.,* to determine whether interspecies interactions facilitate changes in the virulence and diversification of *P. aeruginosa*. Virulence testing using the *Galleria mellonella* model showed significant changes in *P. aeruginosa* virulence after co-culture, and phenotypic differences between isolates were also apparent, including changes in antimicrobial susceptibility leading to the development of clinically resistant isolates. Whole genome sequencing revealed mutations in quorum sensing (QS) associated genes and two-component regulators. In addition, alterations in the gene expression of virulence-associated genes were detected including genes linked to QS, exopolysaccharide production and iron acquisition.

Interactions with other species can change the behaviour of *P. aeruginosa*, impacting on virulence and resistance. Understanding complex interactions between different bacterial species and in combination with immune components may uncover novel therapeutic targets and ultimately lead to altered CF patient management.

Antibiotics & Biofilms

P33

Uncovering the full toxin repertoire of the Type VI secretion system of Pseudomonas aeruginosa using a global genomic approach

Laura Nolan[1], Amy Cain[2], David Albesa-jové[3], Gordan Dougan[2], Julian Parkhill[2], Alain Filloux[1]
[1]Imperial College London, London, UK, [2]Wellcome Trust Sanger Institute, Cambridge, UK, [3]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

The Type-VI secretion system (T6SS) is a bacterial weapon which delivers toxic effectors into target cells, and plays an important role in interbacterial competition within polymicrobial niches. Within this context bacteria have evolved T6SS-dependent toxins to efficiently and selectively target particular bacterial components, and thus the targets of these toxins represent already validated antibacterial targets. To date a number of T6SS toxins have been characterised with the major targets of these being the bacterial cell wall, the inner and outer membrane, and nucleic acids however, it is expected that the full repertoire of toxin targets is more extensive. To expand the range of known T6SS toxin targets we used a global genomic approach called transposon directed insertion site sequencing (TraDIS) to identify novel toxins associated with the first (H1-T6SS) of 3 T6SSs in Pseudomonas aeruginosa. This approach allowed the identification of all previously described H1-associated toxin-immunity pairs and revealed a whole series of putative novel toxins which may direct us to new targets for antimicrobial development.

Antibiotics & Biofilms

P34

Anti-pseudomonal activity of multifunctional nanoapatites co-doped with lanthanide and silver ions

Agata Dorotkiewicz-Jach[1], Katarzyna Zawisza[2], Paulina Sobierajska[2], Rafael J. Wiglusz[2], Zuzanna Drulis-Kawa[1]
[1]Institute of Genetics and Microbiology, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland, [2]Institute of Low Temperature and Structure Research, Polish Academy of Sciences, Okolna 2, 50-422 Wroclaw, Poland

In a response to emerging problem of Pseudomonas aeruginosa biofilm and multidrug resistance new approaches have to be considered. One of the idea is the design of antibacterial biomaterials . It has been shown that the microbiological investigation of hydroxyl- (HAp) , chlor-(ClAp) and fluorapatites (FAp) undoped and co-doped with lanthanide and silver ions as a promising antimicrobials and as well as luminescence materials for bio-imaging.
The antimicrobial activity of nanoapatites were performed on P. aeruginosa PAO1 and ATCC27853 with the colony forming units (cfu/ml) determination using microdilution method as recommended for antibacterial testing. Furthermore, the structural and morphological properties of the obtained samples were determined by using XRD (X-ray powder diffraction) and TEM (Transmission electron microscopy) technique. The spectroscopic properties of the lanthanide ions doped nanoapatites depending on dopant concentration and sintering temperature were recorded.
The lowest concentration exhibiting the bacterial count reduction was established as 100μg/ml. In the case of the FAp co-doped with Eu3+ and Ag+ ions 2-log reduction in cfu/ml was observed. The best anti-pseudomonal effect has been shown for the ClAp co-doped with Eu3+ and Ag+ ions as 4-log reduction. The biomaterials composing Ag+ or Eu3+ ions separately exhibited minor or non-antibacterial activity, respectively. It turned out that the undoped nanoapatites in some cases have stimulated the growth of bacterial cultures.
As non-cytotoxic effect of the tested biomaterials has also been found and the potential application of the co-dopant nanomaterials for biomedicine using the biocompatible implants or dressing was demonstrated.

Antibiotics & Biofilms

P35

Unraveling the genome of a *Pseudomonas aeruginosa* isolate belonging to the high-risk clone ST235 exposes a putative integrative conjugative element housing a *bla*$_{GES-6}$ carbapenemase

João Botelho, Filipa Grosso, Luísa Peixe
UCIBIO/REQUIMTE, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Porto, Portugal

Background: GES-6 carbapenemase among *Pseudomonas aeruginosa* was previously associated with an In1076 class I integron. Here, we conducted a genome-based analysis and explored the genetic backbone associated with the mobilization of this integron.

Methods: *bla*$_{GES-6}$-harboring *P. aeruginosa* isolate (FFUP_PS_690) was collected in February 2015 from the urine of a 90-year-old outpatient. Antimicrobial susceptibility testing was conducted according to EUCAST guidelines and clonal analysis by MLST. WGS was achieved with Illumina HiSeq, *de novo* assembly by SPAdes and subsequent bioinformatic analysis concerning antibiotic resistance (ResFinder and CARD databases), virulence (VRprofile and Virulence Factor databases) and mobile genetic elements (IslandViewer 3 and plasmidSPAdes tools).

Results: The FFUP_PS_690 isolate exhibited an extensive-drug resistant (XDR) profile and belonged to the ST235 high-risk clone. The FFUP_PS_690 genome was highly related to a set of ST235 genomes. Main differences were observed among phage-associated proteins and other horizontally acquired regions. *bla*$_{GES-6}$-harboring In1076 was here associated with a novel putative integrative conjugative element (ICE), hereby named ICE*Pae*690. This *clc*-like ICE is 86,203-bp long, exhibits 63,1% GC content and comprises a bacteriophage P4 integrase, conjugative elements and genes encoding for maintenance functions. The cytotoxic type III secretion system effector ExoU was here associated with an island A variant.

Conclusion: The presence of a 'hitch-hiking' *bla*$_{GES-6}$-harboring In1076 integron in a putative ICE highlight the potential spread of this element through conjugation and/or clonal expansion of the ST235 lineage. The presence of an *exoU*-carrying genomic island helps to confirm the most likely association of the ST235 lineage with the *exoS*-/*exoU*+ genotype.

Antibiotics & Biofilms

P36

Quantification of diffusion in single microcolonies of Pseudomonas aeruginosa using Single Plane Illumination Microscopy-Fluorescence Correlation Spectroscopy (SPIM-FCS)

Jagadish Sankaran[1,2], Kumaravel Kandaswamy[4,5], Yehuda Cohen[3,4], Scott Rice[3,4], Thorsten Wohland[1,2]
[1]Centre for BioImaging Sciences, National University of Singapore, Singapore, Singapore, [2]Department of Biological Sciences, National University of Singapore, Singapore, Singapore, [3]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, [4]Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore, [5]Interdisciplinary Graduate School, Nanyang Technological University, Singapore, Singapore

Pseudomonas aeruginosa exists in two different life styles, the unicellular planktonic form, and the multicellular, matrix encased form called the biofilm. The mobility of molecules in and around a biofilm microcolony are altered by the extracellular polymeric substances (EPS) surrounding the microcolony. The axial sectioning provided by Single Plane Illumination Microscopy (SPIM) facilitates imaging at different depths of the microcolony. Imaging in SPIM is carried out using water immersion objectives and hence P. aeruginosa is grown using flow-chamber in tubes made of a specialized polymer called fluorinated ethylene polymer (FEP) whose refractive index matches that of water. Fluorescence Correlation Spectroscopy (FCS) is a technique to quantify diffusion coefficient of molecules. Combining SPIM with FCS allows one to quantitate the mobility of molecules at micrometer resolution enabling one to visualize the diffusion profile of an entire microcolony in 3D. Using SPIM-FCS, we have probed the roles of size of microcolony and charge of the molecule in determining the diffusion properties of the P. aeruginosa biofilms. Our results suggest that the diffusion coefficient of fluorescent probes decreased with increasing size of microcolony. Positively charged molecules localized into the biofilm and negatively charged molecules formed a ring around microcolony. Taken together, our results can help to delineate the roles of various physicochemical factors in altering the mobility of molecules in and around a biofilm microcolony, which ultimately is important in the selection of compounds such as antibiotics based on charge and size that can penetrate the biofilm matrix to kill the underlying cells.

Antibiotics & Biofilms

P37

Analysis of biofilm disruption by laser interferometry

Michal Arabski[1], Slawomir Wasik[2], Zuzanna Drulis-Kawa[3]
[1]Deparment of Microbiology, Jan Kochanowski University, Kielce, 25-406, Poland,
[2]Department of Molecular Physics, Jan Kochanowski University, Kielce, 25-406, Poland,
[3]Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wroclaw, Wroclaw, 51-148, Poland

Laser interferometry technique enables a quantitative substance diffusion by the measurement of the difference between light refractive indexes - changes of the solution's refractive index corresponding to changes in substance concentration. The unique interferometric set-up, constructed at the Department of Molecular Physics of Jan Kochanowski University in Kielce, Poland, was previously used for physical and pharmaceutical researches. This technique has a potential for new applications in microbiology, especially for biofilm degradation assay. The changes in the amount of transported medium trough biofilm structure, biofilm permeability and diffusion coefficient, might reflect as biofilm matrix degradation. The biofilm is usually formed on membrane surface (PET or Nephrophane) serving as a scaffold for biofilm structure formation. In our studies we used this technique for *Pseudomonas aeruginosa* PAO1 biofilm eradication by different lytic phages, including KTN6 and KT28 (PB1-like) phages; KTN4 and 5oct giant myoviruses, as well as *Pseudomonas* LKA1 phage and LKA1-gp49 recombinant depolymerase. We were able to observe increased diffusion of TSB medium through treated biofilm in time-depended manner, what corresponded to matrix degradation. Laser interferometry allows for very precise evaluation of the diffusion rate of particular amount of transferred compound enabling quantitative measurement of effective concentration of antibacterial/antibiofilm compound.

In conclusion, laser interferometry technique is a useful tool in real-time analysis of biofilm disruption in unmixed system, mimicking natural conditions.

Antibiotics & Biofilms

P38

Determining the molecular effects of 5-fluorocytosine on the pathogenicity of *Pseudomonas aeruginosa*

Jessica Thomson, Iain Lamont
University of Otago, Dunedin, New Zealand

Like many pathogenic bacteria, *Pseudomonas aeruginosa* presents a challenge to conventional antibiotic therapeutics because of the evolution of resistance. One approach to facilitate antibiotic discovery is to screen clinically used drugs for anti-pseudomonal activity. This approach was previously used to identify the antimycotic drug 5-fluorocytosine as reducing pathogenicity of *P. aeruginosa* in a murine infection model. The aim of the presented research is to determine how 5-fluorocytosine reduces pathogenicity of *P. aeruginosa*.
RNAseq was used to identify genes whose expression is affected by 5-fluorocytosine. After 4 h of treatment, more than 300 genes involved in a variety of different functions and pathways were found to be differentially expressed. More than 30 percent of these genes were pathogenicity-related, including genes required for type IV secretion systems and for production of hydrogen cyanide, and pyocyanin, which is implicated in facilitating redox balance in *P. aeruginosa*. A proteomics approach confirmed the proteins involved in pyocyanin synthesis were upregulated in 5-fluorocytosine treated bacteria.
Our data show that 5-fluorocytosine treatment has a broad effect on *P. aeruginosa* gene expression. 5-fluorocytosine altered the production of a wide range of *P. aeruginosa* virulence factors, explaining the observed reduction in pathogenicity of treated bacteria.

Antibiotics & Biofilms

P39

Development of high throughput assays to identify compounds that exploit cell wall deficient (CWD) *Pseudomonas aeruginosa*

David.O Max, James.J Lazenby, Cynthia.B Whitchurch
University of Technology Sydney, Sydney, NSW, Australia

Background: *Pseudomonas aeruginosa* employs several strategies to tolerate the effects of a broad range of antibiotics including possession of a highly impermeable outer-membrane (OM) and multi-drug efflux pumps. We have recently shown that *P. aeruginosa* tolerates β-lactam antibiotics by undergoing a rapid *en masse* conversion from bacilli to cell wall deficient (CWD) forms that lack the OM [1]. We hypothesized that CWD *P. aeruginosa* may be susceptible to antibacterial compounds that are normally inhibited by the Gram-negative cell wall.

Methods: We found that β-lactam-induced CWD *P. aeruginosa* is rapidly killed by antimicrobial peptides (AMPs), that are normally ineffective against bacillary *P. aeruginosa* [1]. These observations suggest that β-lactam antibiotics in combination with compounds that exploit the susceptibility of CWD may be novel therapeutic options. However, the increasing prevalence of β-lactam resistance in clinical settings has created a need to develop novel drugs that induce the transition to the CWD form for this strategy to be effective. The redox indicator resazurin was used to track cell growth using a fluorescence plate reader. Screens were performed in a 96 well plate format to allow for high throughput screening and validated using meropenem and nisin to induce and kill CWD *P. aeruginosa*.

Results: We have developed simple, cheap high throughput screening methods that will enable the development of novel therapeutic approaches that exploit the susceptibility of CWD *P. aeruginosa*.

Conclusion: These high throughput screening methods can be used as drug discovery tools to develop novel therapeutic options against *P. aeruginosa*.

Antibiotics & Biofilms

P40

Pseudomonads contact-dependent competition in mixed biofilms

Gabriela Purtschert, Gerardo Cárcamo-Oyarce, Claudio Aguilar, Leo Eberl
University of Zurich, CH-8008 Zurich, Switzerland

Bacterial biofilms represent the most relevant form of bacterial growth in the environment. Since multiple bacterial species coexist within most natural biofilms, the competition for resources and space has triggered the evolution of competitive strategies to survive and persist in such multispecies communities.
In this study, we evaluate competition ability of the plant-associated bacterium Pseudomonas putida strain IsoF in mixed species biofilms. Using different experimental approaches, we show how IsoF is able to outcompete various soil and plant associated Pseudomonas species in a contact-dependent manner. Moreover, IsoF antagonism is shown to be particularly in mixed-species biofilms. Using flow-through chambers we demonstrate that IsoF is capable of invading and displacing a pre-established biofilm of other Pseudomonas strains. By screening of a mini-Tn5 transposon insertion library we identified a gene cluster that is responsible for contact-dependent killing by the strain. Bioinformatic analyses revealed that the locus encodes a putative type IV secretion system (T4SS). Inactivation of genes encoded by this cluster resulted in mutants that were no longer able to kill other bacteria nor were they able to invade existing biofilms, in contrast to the wild-type strain. In conclusion, we provide evidence that the competition strategy of P. putida is based on a T4SS and show that this system is not only used for self-defense but also as an offensive weapon for the invasion of biofilms.

Antibiotics & Biofilms

P41

Pseudomonas colicin M-type bacteriocins target different TonB-dependent outer-membrane proteins

Maarten Ghequire, René De Mot
KU Leuven, Heverlee, Belgium

Pseudomonads are equipped with an armamentarium of secreted antibacterial proteins to harness themselves in competitive environments. A major subset of these bacteriocins are synthesized as modular proteins and include a receptor-binding domain, a membrane translocating motif and a toxin-immunity module at the C-terminus. Bacteriocins equipped with a colicin M (ColM) toxin domain kill target cells via degradation of lipid II, hereby impeding peptidoglycan biosynthesis.
We performed a comprehensive *in silico* analysis for the presence of ColM modules in proteobacterial genomes. In addition to identifying two ColM-type bacteriocin organizations with a dual toxin architecture, our search revealed that ColM modules fall apart in two ancestral clusters. M colicins in *Escherichia coli* and other *Enterobacteriaceae* are consistently equipped with a *cmi*-type immunity gene, whereas immunity to ColM-type bacteriocins from other genera (including *Pseudomonas*) is displayed by a distinct set of small proteins with diversified transmembrane helix topologies. The complete lack of sequence similarity of the predicted receptor-binding domains of ColM-type bacteriocins from different genera suggests that these bacteriocins have recruited different outer-membrane targets to gain access to target cells. Using recombinantly-produced proteins and a transposon mutagenesis approach of susceptible cells, we show that ColM bacteriocins in *Pseudomonas* target at least two different TonB-dependent outer-membrane proteins, both involved in the uptake of iron via siderophores.
Together, our results demonstrate that colicin M-type bacteriocins are truthful polymorphic toxins that have been subject of extensive recombination events. The piracy of receptors involved in iron uptake highlights the critical role of this compound in niche colonization.

Antibiotics & Biofilms

P42

Nudix hydrolase PA5176 is linked to rhamnolipids production in Pseudomonas aeruginosa.

Mateusz Ziecina, Elzbieta Kraszewska
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Pseudomonas aeruginosa is an ubiquitous bacterium found in different environments and in association with various organisms. In humans, it causes a broad spectrum of opportunistic infections that are difficult to eradicate. Thus, there is an urgent need to search for novel virulence - associated factors, which might serve as targets for antibacterial therapies.

The PA5176 hydrolase belongs to a family of Nudix pyrophosphatases which are widely distributed among all classes of organisms. These enzymes catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives and have been shown to contribute to virulence of other pathogenic bacteria species. Recently, we have shown that the mutant strain lacking *PA5176* gene exhibits virulence - associated phenotype. It displayed impaired swarming motility and its virulence toward *Caenorhabditis elegans* was significantly decreased.

The aim of this work was to study function of PA5176 protein in swarming motility. We have established that the mutated strain produced clearly less swarming motility - associated rhamnolipids than the wild type. To address the question of the PA5176 substrate preference, the protein was expressed in *Escherichia coli* and purified. Its ability to hydrolyze substrates was tested with a number of nucleoside diphosphate derivatives. The preliminary results indicate that PA5176 is a multi-substrates enzyme that hydrolyzes ADP-ribose, FAD, CoA and NADH, but not nucleotides nor dinucleotide polyphosphates. Therefore, its ability to act on dTDP-glucose or dTDP-rhamnose, nucleotide sugars involved in rhamnolipids biosynthesis, is not excluded.

This work was supported by the grant no: UMO-2014/15/B/NZ6/02562 from the National Center of Science.

Antibiotics & Biofilms

P43

Two decades of *bla*$_{VIM-2}$-producing *Pseudomonas aeruginosa* dissemination: the decisive role of mobile genetic elements and successful clones

João Botelho, Filipa Grosso, Luísa Peixe
UCIBIO/REQUIMTE, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Porto, Portugal

Background: Information on clonal dynamics and genetic surroundings of carbapenemase-producing *Pseudomonas aeruginosa* (CPPA) strains in Portuguese hospitals is scarce. Here, we unveil the drivers contributing for the occurrence of CPPA in Portuguese hospitals for two decades.

Methods: A collection of carbapenem-resistant *P. aeruginosa* clinical isolates (n=263, 1995-2014) was screened for CPPA by Blue-Carba and PCR. Antimicrobial susceptibility testing was performed according to EUCAST and clonal analysis by MLST. Eight isolates representing different integrons, STs and time periods were selected for WGS, followed by *de novo* assembly (SPAdes), mapping (Bandage), automatic annotation (RAST), plasmid (plasmidSPAdes), genomic island (GI, IslandViewer 3), antibiotic-resistance (ResFinder and CARD) and virulence (VRprofile) analysis.

Results: Twenty-seven CPPA were detected, twenty-two defined as XDR and five as MDR. All isolates carried *bla*$_{VIM-2}$, located on 7 different class I integrons, of which In58 was the most prevalent. On 3 isolates from ST175, ST179 and ST282, *bla*$_{VIM-2}$ was inserted on related integrons (In58 and In103) associated with different 30-kb plasmids. *bla*$_{VIM-2}$-harboring Tn*3*-like transposons were linked to megaplasmid backbones (350-460kb) on 2 isolates from ST253 and ST244. *bla*$_{VIM-2}$-carrying GIs (80-120kb) were associated with different integrons (In58 and In100) (n=3 isolates, ST111 and ST235). An intact prophage region was identified next to the tRNA^{Gly} gene on the ST235 lineage.

Conclusion: Our results link the dissemination of *bla*$_{VIM-2}$ with several integron structures, most of them related to the In58 integron. These 'hitch-hiking' elements are associated with different mobile genetic elements, highlighting the genetic plasticity and wide dispersion of this carbapenemase gene.

Antibiotics & Biofilms

P44

Poly (ε-lysine) dendrons as modulators of virulence and biofilm formation in *Pseudomonas aeruginosa*

Rahaf Issa, Steve Meikle, Stuart L. James, Ian R. Cooper
University of Brighton, Brighton, UK

Natural and synthetic molecules capable of modulating virulence in *P. aeruginosa* have been previously described. Little is however known regarding hyperbranched peptide-based macromolecules such as poly (ε-lysine) dendrons. This study aims to determine their suitability as novel modulators of virulence, and their efficiency in an *in vitro* co-culture infection model. Dendrons (consisting of an arginine root and branching points of lysine) were synthesised using N-Fmoc-solid-phase peptide synthesis. Dendrons were tested for their effects on biofilm formation using the *P. aeruginosa* strain PAO1. Treatment at 400μM, a non-toxic concentration, inhibited swarming/swimming motility, reduced surface coverage and microcolony formation, and attenuated extracellular polymeric substance (EPS) matrix production. All of which reduced the bacterium's biofilm forming capacity and increased susceptibility to ciprofloxacin by 50%. Microscopic analysis using fluorescently labelled dendrons revealed their intracellular accumulation at the cell poles, an area where virulence is controlled. Using transcriptional and translational *lacZ* reporters demonstrated that these hyperbranched peptides reduce the expression of two quorum sensing autoinducer synthases, *lasI* and *rhlI*, independent of changes to growth kinetics. Subsequently, the expression of genes that encode for elastase (*lasB*) and rhamnolipid (*rhlA*) production, and PQS (*pqsA*) signalling was downregulated. The efficiency of poly (ε-lysine) dendrons was finally evaluated in a co-culture model of human bronchial epithelial cells and PAO1. Treatment reduced PAO1-induced cytotoxicity, bacterial adherence, and delayed the onset of irreversible airway epithelial barrier damage. Our findings demonstrate the anti-virulence effects of hyperbranched peptide-based macromolecules and provide an alternative approach to challenge the increasing resistance of bacteria to antibiotics.

Antibiotics & Biofilms

P45

The role of the phosphodiesterase NbdA in NO-induced biofilm dispersal of *Pseudomonas aeruginosa*

Martina Rüger, Susanne Zehner, Nicole Frankenberg-Dinkel
Technical University Kaiserslautern, Kaiserslautern, Rhineland-Palatinate, Germany

Dispersal is the last step in the life cycle of a *Pseudomonas aeruginosa* biofilm, a process used by bacteria to switch between the sessile and the planktonic lifestyle. Changes in c-di-GMP (bis-(3'-5')-cyclic dimeric guanosine monophosphate) levels have been linked to biofilm dispersal in a number of different bacteria and were shown to be induced by a variety of signals. The signalling molecule nitric oxide (NO) induces biofilm detachment through stimulation of c-di-GMP degrading phosphodiesterase (PDE) activity. In *P. aeruginosa*, the membrane-anchored proteins MucR and NbdA (NO-induced biofilm dispersion locus A) are known to be involved in NO-induced biofilm dispersal. Both proteins share a similar organisation consisting of MHYT-GGDEF-EAL domains. Biochemical analyses of recombinant protein variants lacking the transmembrane MHYT-domain revealed NbdA being an active PDE. In contrast, MucR showed diguanylate cyclase and PDE activity in vitro [1]. Functional complementation of a swimming deficient *E. coli* phosphodiesterase mutant strain with different NbdA variants was performed. Obtained results will be presented and discussed with respect to protein function and in comparison to previously gathered biochemical data.

Reference

[1] Li Y, Heine S, Entian M, Sauer K, Frankenberg-Dinkel N. (2013) NO-induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by an MHYT domain-coupled phosphodiesterase. *J Bacteriol*. **195**: 3531-3542.

Antibiotics & Biofilms

P46

Identification of anti-virulence FDA-approved drugs targeting the *pqs* quorum sensing system of *Pseudomonas aeruginosa*

Francesca D'Angelo[1], Valerio Baldelli[1], Nigel Halliday[2], Fabio Polticelli[1], Paul Williams[2], Paolo Visca[1], Livia Leoni[1], Giordano Rampioni[1]
[1]University Roma Tre, Department of Science, Rome, Italy, [2]University of Nottingham, Centre for Biomolecular Sciences, Nottingham, UK

Targeting bacterial virulence processes rather than growth is considered a promising strategy for the treatment of drug-resistant infections. The *pqs* quorum sensing (QS) system of *Pseudomonas aeruginosa* relies on 2-alkyl-4-quinolones (AQs) as signal molecules. Since this QS system positively controls the expression of multiple virulence determinants, it is considered a potential target for the development of anti-virulence drugs.

In this study a library of drugs has been screened by using a co-culture reporter system composed of *P. aeruginosa* PAO1 wild type and an AQs-biosensor, in which light emission depends on AQs produced by the wild type strain. This reporter system, in principle, allows identification of molecules targeting the *pqs* signaling cascade at multiple levels, including signal synthesis, import/export or reception.

Three hits specifically inhibiting the *pqs* QS signaling system have been identified. *In silico* molecular docking and phenotypic characterization of *ad hoc* engineered strains indicate that all the newly identified inhibitors hamper the *pqs* system by targeting the transcriptional regulator PqsR. These molecules inhibited the expression of *pqs*-controlled virulence traits in *P. aeruginosa*, such as pyocyanin production and swarming motility. Further phenotypic analyses have shown the ability of the most promising *pqs*-inhibitor to reduce biofilm formation in *P. aeruginosa,* to protect *Galleria mellonella* larvae from *P. aeruginosa* infection and to hamper the *pqs* QS system in several clinical isolates from cystic fibrosis patients.

Antibiotics & Biofilms

P47

Deletion of periplasmic chaperones and Bam complex components leads to a decreased outer membrane integrity and increased antibiotic sensitivity in Pseudomonas aeruginosa

Kristina Klein, Michael Sonnabend, Lisa Frank, Paul Schmitt, Ingo B. Autenrieth, Monika Schütz, Erwin Bohn
University Hospital Tuebingen, Tuebingen, Germany

The outer membrane (OM) of Gram-negative bacteria is an essential organelle acting as a physical barrier and protecting Gram-negative species from a harmful environment. Penetration across the outer and inner membrane appears to be a limiting factor for drug efficacy against Gram-negatives. To achieve insertion of outer membrane proteins (OMPs) into the outer membrane, unfolded proteins are first transported through the inner membrane and guided by chaperones (SurA, HlpA) to the Beta barrel assembly (Bam) machinery which mediates insertion of OMPs. In this study we ask the question how deletion of chaperones and non-essential components of the Bam complex (BamB, BamC) may affect membrane integrity, membrane composition and virulence of P. aeruginosa.
Therefore, the ability of deletion mutants of SurA, HlpA (Skp homologue), BamB and BamC to withstand physiological detergents and antibiotics was investigated. In addition, we tested the OM integrity with the fluorescence based NPN assay.
The increased influx of the dye NPN into a SurA deletion mutant indicates an important role for SurA in maintaining OM integrity. Consistently, the ΔsurA as well as the ΔbamB mutant show a significantly reduced growth in a physiological concentration of bile salts as well as susceptibility to various antibiotics to which the wildtype strain is otherwise resistant.
Our results demonstrate that Bam complex components and linked periplasmic chaperones, are important factors for Pseudomonas aeruginosa OM integrity and could serve as attractive targets to treat multi-resistant Pseudomonas strains. Studies to determine alteration of the composition of OMPs and of virulence are in progress.

Antibiotics & Biofilms

P48

Pseudomonas Biofilm Exopolymer Processing and Structural Characterisation

Lan Li Wong[1,2], Thomas William Seviour[1,2], Scott Rice[1,2]
[1]*Singapore Centre on Environmental Life Sciences Engineering, Singapore, Singapore,*
[2]*Nanyang Technological University, Singapore, Singapore*

Despite extensive research into the composition and conformation of *Pseudomonas* biofilm matrix exopolymers, characterisations of structural molecules have been limited to those that are water-soluble. In this study, extraction efficacies of representative *Pseudomonas* biofilm exopolymers, including various polysaccharides, proteins and DNA, in designer solvent and ionic liquid 1-ethyl-3-methylimidazolium acetate (EMIM-Ac), were assessed. Specific *Pseudomonas* exopolymers were then targeted by fractional precipitation using different anti-solvents. Chemically distinct network-forming constituents from various *Pseudomonas* biofilms (*P. aeruginosa, P. protegens and P. putida*) were isolated. The solubility and behaviour of their exopolymers in the ionic liquid, as well as their responses to anti-solvents, informed the identity and secondary structure of dominant exopolymers. The high MW exopolymer isolate from all strains self-assembled into a highly networked, gel-like material upon transfer back into water from ionic liquid. Its behaviour in isolation thus resembled that in native biofilm and it displayed the same physical responses to environment conditions (pH, temperature and salt gradient) as observed for the native biofilm, indicating that its secondary structure was preserved during ionic liquid processing. Furthermore, the viscoelastic character of the biofilm effectively transferred to the solvent in each case, as indicated by normal force elicited by the dissolved biofilms under conditions of high shear rate. The results gathered highlight the potential for ionic liquids in the analysis and isolation of hitherto inaccessible but nonetheless critical exopolymers from *Pseudomonas* biofilms.

Antibiotics & Biofilms

P49

TonB-dependent receptors involved in the uptake of siderophore-drug conjugates in *Pseudomonas aeruginosa*

Alexandre Luscher[1], Lucile Moynié[2], James H. Naismith[2], Thilo Köhler[1]
[1]Department of Medical Specialties, Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [2]Biomedical Sciences Research Complex, University of St. Andrews, St. Andrews, UK

Background: To overcome the slow permeation of antibiotics in *P. aeruginosa and A. baumannii*, drugs can be conjugated to siderophores, which are essential for iron acquisition in bacteria, and are transported via specific TonB-dependent receptors (TBDR) across the outer membrane. *P. aeruginosa* and *A. baumannii* encode 35 and 22 TBDRs, respectively, 23 of which are predicted to be involved in siderophore transport in *P. aeruginosa*.

Methods: TBDR genes from *P. aeruginosa* and *A. baumannii* have been cloned and were expressed from a constitutive promoter in PAO1. Minimal inhibitory concentrations (MIC) were determined by 2-fold dilution series in MH-medium. Gene expression was measured by qPCR.

Results: Within the "Translocation" project of the ND4BB program, we and others previously identified PiuA and PirA as the main TBDRs, involved in siderophore-beta-lactam uptake. Here we identified a constitutively expressed orthologue of PiuA, termed PiuD, for which we obtained the crystal structure. The structure reveals a typical 22-beta stranded sheet structure with a flexible loop extending from the plug domain towards the extracellular medium. We further cloned the majority of siderophore specific TBDR genes and identified novel TBDRs, which when overexpressed, increase the susceptibility to three different siderophore-beta-lactam conjugates (BAL30072, MC-1, cefiderocol) currently considered for clinical trials.

Antibiotics & Biofilms

P50

Rapid adaptation of clinical *Pseudomonas aeruginosa* populations to antibiotic therapy

Leif Tüffers[1], Ingrid Bobis[2], Sabine Schubert[2], Femke-Anouska Heinsen[1], Anette Friedrichs[2], Helmut Fickenscher[2], Burkhard Bewig[2], Stefan Schreiber[2], Hinrich Schulenburg[1]
[1]Kiel University, Kiel, Schleswig-Holstein, Germany, [2]University Hospital Schleswig-Holstein, Kiel, Schleswig-Holstein, Germany

Pseudomonas aeruginosa is the major airway pathogen in adult cystic fibrosis. Antibiotics are used extensively to maintain lung function in these patients, but empirical support for therapy success is scarce. While the long-term adaptation of *Pseudomonas* to the cystic fibrosis lung was shown to lead to diversification and antibiotic resistance, there is little data on fast adaptation to newly applied antibiotic pressures. Meanwhile, designing therapies along evolutionary principles to minimize resistance evolution is a promising concept. Using sputum metagenomics and deep sampling of *Pseudomonas* isolates from a cohort of cystic fibrosis patients taking a 14-day course of intravenous antibiotics, we could show that this therapy has a limited effect on bacterial load, but can lead to rapid population-wide shifts towards resistance. In order to test alternative antibiotic regimens, which have been shown to reduce adaptation of laboratory *Pseudomonas* strains, we performed experimental evolution of these clinical isolates. Our results show that individual therapy courses can contribute to the overall antibiotic resistance within cystic fibrosis patients. At the same time, modeling future therapy regimens to exploit bacterial evolutionary constraints could improve the management of chronic *Pseudomonas* lung infections, resulting in higher rates of successful clearance while reducing antibiotic resistance.

Antibiotics & Biofilms

P51

Identification of a small molecule inhibitor of quorum sensing that acts in part via Vfr in *Pseudomonas aeruginosa*

Leon Tan[1,2], Ziyan Hong[2], Joey Yam[2], Bau Yi Woo[1], Liang Yang[2], Michael Givskov[2,3], Shu Sin Chng[1,2]
[1]National University of Singapore, Singapore, Singapore, [2]Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore, [3]Costerton Biofilm Center, Copenhagen, Denmark

Pseudomonas aeruginosa is widely attributed as the leading cause of hospital-acquired infections[1]. Due to intrinsic antibiotic resistance mechanisms, as well as the ability to form biofilms[2], *P. aeruginosa* infections are notoriously difficult to treat. An attractive strategy to combat *P. aeruginosa* infections is the use of anti-virulence compounds. Key to the regulation of *P. aeruginosa* virulence is the quorum sensing (QS) network, which coordinates the expression of virulence genes in response to population density. Here, we report the identification of a small molecule, termed compound A, that inhibits QS in *P. aeruginosa*. Using fluorescence reporter assays, we show that compound A exhibits sub-micromolar inhibition of *las*, *rhl* and *pqs* systems. Interestingly, compound A also inhibits Type III secretion (T3SS) and Type IV pili (TFP) formation, indicating that it may target Vfr, the global virulence regulator that positively controls QS, T3SS, and TFP. Indeed, we demonstrate that compound A strongly inhibits the ability of Vfr to bind target promoter sequences in vitro. Consistent with potent inhibition of QS and TFP, we further show that compound A attenuates biofilm maturation, and displays strong synergy in eradicating *P. aeruginosa* biofilms when used in combination with colistin. Compound A may have immediate applications as an anti-virulence drug against *P. aeruginosa* infections.

Antibiotics & Biofilms

P52

Differential susceptibility of *Pseudomonas aeruginosa* biofilm to topical and systemic concentrations of gentamicin investigated using a soft tissue model.

Bianca Price, Krishanu Baruah, Curtis Dobson
University of Manchester, Manchester, UK

We have developed a soft tissue model of diabetic foot infection and compared the activity of gentamicin against *P. aeruginosa* biofilm when the antibiotic was applied at its $C_{max,}$ or released from topically applied calcium sulfate beads.

Collagen matrices, including hyaluronic acid, extracellular matrix proteins, fibroblasts and keratinocytes, were prepared in 6-well tissue culture inserts. PA01 was added to the surface and grown for 72 hours; biofilm was verified using SEM. Gentamicin was introduced either in the media or in calcium sulfate beads. After 72 hours incubation the model was sectioned, colonies were enumerated and concentrations of antibiotics in the matrix were measured.

The MIC, MBEC and MBC in the collagen model was 8µg/ml 31µg/ml and 532µg/ml respectively. The C_{max} of gentamicin after oral administration has previously been reported to be 20µg/ml. When this concentration of gentamicin was added to the model, an increase in colony counts of 1.2 logs was recorded and corresponding concentration of gentamicin in the matrix was found to be 0.6µg/ml. When calcium sulfate beads loaded with gentamicin were added to the model, a 3.2 log reduction in colony counts was recorded, corresponding to a concentration of 22µg/ml in the matrix.

We conclude that high local concentrations of gentamicin released in close proximity to biofilm effectively reduce the *P. aeruginosa* bioburden. We confirm that PAO1 can tolerate gentamicin at concentrations above MIC when growing in a soft tissue environment. Our data suggest topically applied antibiotics may be more effective than systemic antibiotics for treating such infections.

Antibiotics & Biofilms

P53

SiaABCD regulates cellular aggregation of *Pseudomonas aeruginosa* PAO1 in response to growth permitting conditions

Brendan Colley[2], Wee-Han Poh[3], Nicolai Müller[4], Staffan Kjelleberg[2,3], Scott A. Rice[2,3], Janosch Klebensberger[1]
[1]University of Stuttgart, Institute of Technical Biochemistry, Stuttgart, Germany, [2]University of New South Wales, Centre for Marine Bio-Innovation, Sydnes, Australia, [3]Nanyang Technological University, Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore, [4]University of Konstanz, Department of Biology, Konstanz, Germany

Background: We recently identified the c-di-GMP dependent signaling module SiaA/D to regulate suspended macroscopic cell aggregation of *P. aeruginosa* in response to surfactant stress. The present study was conducted to investigate whether SiaA/D has a more general role in cellular aggregation.

Methods: To visualize and quantify suspended cell aggregates, we used scanning electron microscopy (SEM) and laser diffraction analysis (LDA). For studying the dynamics of biofilm formation and dispersal under various growth conditions, we used a combined approach consisting of a crystal violet staining method, optical density measurements, and the determination of substrate concentrations in 24-well plates over time.

Results: We show that all genes encoded by the *siaABCD* operon are involved in the regulation of biofilm formation, either existing as suspended aggregates or attached to an inorganic surface. We demonstrate that the SiaABCD pathway impacts the ratio between single cells *versus* cells residing in biofilms as a response towards growth permitting conditions. Our data provide evidence that SiaABCD-mediated biofilm formation is in principle independent of the presence of toxic chemicals such as surfactants, but can be adapted in responsive to harmful environmental conditions.

Conclusion: From these and previous data, we conclude that SiaABCD balances cellular aggregation in response to growth permitting conditions and harmful environmental stimuli, which might play an important role as an adaptive strategy of the organisms in particular in medical relevant situations.

Antibiotics & Biofilms

P54

Novel two component systems promote swarming in *Pseudomonas aeruginosa*

Varsha Singh, Ayushi Mishra, Shubham Joge, Mohammad Ameen
Indian Institute of Science, Bangalore, Karnataka, India, India

Swarming in *P. aeruginosa* is a quorum dependent process which results in rapid growth and expansion of bacterial population over semisolid surfaces. Two major requirements of swarming include flagella dependent motility and surfactant namely rhamnolipid. We have found that the extent and pattern of swarming depends upon nutrient environment, using three defined minimal media-M8, M9 and BM2 media- and three undefined media- BHI, LB and peptone growth media. Suppression of swarming by rich media indicated that the ability to sense external environment is central to Pseudomonas' decision to swarm. We formulated the hypothesis that 2 component Histidine kinase sensors of Pseudomonas assist in sensing of nutrients. Using a collection of mutants affecting 116 two component system genes, we have found 12 genes that regulate swarming on undefined media. We call these swarming promoting factors (SPF). Of these, five are known genes, while rest of the seven genes are novel genes. Of the novel SPF genes, three regulate swarming without affecting flagella dependent swimming and type IV pili dependent twitching. Regulation of surfactant production by 2CS class of swarming promoting factors provides new insights into complex regulation of swarming. We have also studied regulation of virulence by these three SPFs using *Caenorhabditis elegans* as host.

Antibiotics & Biofilms

P55

Long-term effects of the CFTR potentiator ivacaftor on *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis.

Laura Smith[1,2], Mary McCullagh[1,2], Freddy Frost[1,2], Martin Ledson[1]
[1]*Liverpool Heart & Chest Hospital, Liverpool, UK,* [2]*University of Liverpool, Liverpool, UK*

Background:

Ivacaftor is a CFTR potentiator used in cystic fibrosis patients who have a G551D mutation. It significantly improves lung function and reduces exacerbations however its effect on respiratory microbiology remains controversial. This study aims to assess whether ivacaftor use is associated with discernible impact on *Pseudomonas aeruginosa (Pa)* colonisation.

Methods:

Retrospective analysis of case records was undertaken for all patients receiving ivacaftor at a large regional adult CF centre. Baseline characteristics were recorded and sputum results spanning over 7 years were included. The rates of *Pa* positive sputum samples before and after commencing ivacaftor were assessed temporally. Leeds' Criteria was utilised to determine whether ivacaftor reduced the frequency of *Pa* positivity in individuals.

Results:

We reviewed 22 patients (16 male, average age 27.7yrs and FEV1 83.1% predicted) and 564 sputum samples for the time period 2010-2017. 12 patients were *Pa* positive prior to starting ivacaftor (8 Liverpool epidemic strain [LES] positive). Overall *Pa* positivity remained static (59.8% vs. 59.1% of samples respectively, odds ratio 0.97 [95% confidence intervals 0.69-1.37]). Applying the Leeds criteria, 10 patients remained in the same group and 2 patients (16.6%) moved down a classification following ivacaftor initiation (one patient [LES positive] "chronic" to "intermittent", one patient [1 LES positive] "intermittent" to "free of *Pa"*.)

Conclusion:

Our results indicate ivacaftor does not result in overall reductions in *Pa* positive sputum cultures and only a minority of patients were downgraded when the Leeds criteria were applied. Our findings do not support an anti-pseudomonal effect for ivacaftor.

P56

Application of the ECOFF values for the interpretation of antibiotic susceptibility of *Pseudomonas aeruginosa* environmental isolates

Olga Pappa[1,2], George Vantarakis[5], Apostolos Beloukas[6,1], Apostolos Vantarakis[3], Alexis Galanis[4], Athena Mavridou[1]
[1]Department of Medical Laboratories, Athens University of Applied Science, Athens, Greece, [2]Central Public Health Laboratory, Hellenic Centre for Disease Control and Prevention, Athens, Greece, [3]Environmental Microbiology Unit, Department of Public Health, School of Medicine, University of Patras, Patra, Greece, [4]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupoli, Greece, [5]Region of Western Greece, Patra, Greece, [6]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

Background: Antibiotic-resistant *Pseudomonas aeruginosa* isolates are continuously discharged into natural water basins. However, the characterisation of antibiotic resistance in environmental reservoirs warrants further investigation.

Methods: A total of 245 isolates from various aquatic sites in Greece were analyzed and interpreted according to the Epidemiological cut-off value (ECOFF) proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Isolates with various resistance patterns such as cefotaxime–ceftazidime–cefepime–aztreonam–meropenem were screened phenotypically for the presence of extended spectrum β-lactamases (ESBLs), while a subset of 77 isolates were additionally screened for the presence of class 1 integrase gene.

Results: The isolates were divided to Wild (155/245; 63.3%) and Resistant (90/245; 36.7%) and thereafter into four sub-groups representing the main intrinsic resistance mechanisms of *P. aeruginosa*: R1 (AmpC, partially/fully derepressed with resistance to aztreonam; 38/90; 42.2%), R2 (increased efflux; 6/90; 6.6%), R3 (loss of OprD with resistance to imipenem; 5/90; 5.5%) and R4 (resistance to aminoglycosides; 15/90; 16.6%). Finally, twenty-three isolates (23/90; 25.5%) were confirmed phenotypically as ESBL producers and in 13/77 isolates class 1 integron was detected.

Conclusions: The ECOFF values were able to distinguish between organisms without and with phenotypically expressed resistance mechanisms, where aztreonam-resistant isolates and ESBL producers were the main phenotypes across habitats tested. Greek water sites might serve as a potential reservoir of resistant *P. aeruginosa* isolates while the ECOFF values were the most fitting criteria for non-clinical environmental studies.

P57

Insights into the Mechanisms of Promysalin, a Secondary Metabolite with Genus-Specific Antibacterial Activity Against *Pseudomonas*

Krista Giglio[1], Paul Stodghill[1], Colleen Keohane[2], Andrew Steele[2], William Wuest[2], Melanie Filiatrault[1]
[1]USDA-ARS, Ithaca, NY, USA, [2]Emory University, Atlanta, GA, USA

Promysalin, a secondary metabolite produced by *Pseudomonas putida* RW10S1, has antibacterial activity against a wide variety of *Pseudomonas sp*., including both human and plant pathogens. Promysalin induces swarming and biofilm formation in the producing species, and inhibits growth of susceptible species. *P. putida* KT2440 is only mildly susceptible yet displays increased swarming in the presence of exogenous promysalin. In order to uncover mechanisms of promysalin action, we performed RNA-sequencing on *P. putida* KT2440 cells grown in the presence or absence of promysalin and analyzed changes in gene expression. RNA-sequencing results showed 455 differentially regulated genes in response to promysalin; 431 genes were downregulated and 22 were upregulated (FDR < 0.05). Promysalin decreased expression of genes involved in flagella-mediated motility, and genes involved with iron storage and regulation, while expression of genes that function in transport either as multi-drug resistance efflux transporters or in the uptake of nutrients was increased in the presence of the compound. Our data provide insight into the mechanism of action of promysalin and indicate that 1) the increased swarming seen in promysalin-treated cells is not due to flagella-dependent motility; 2) promysalin-treated cells may be experiencing an iron-deficient environment; and 3) efflux transport may play a role in resistance to promysalin in species that are less susceptible. Additionally, we tested the effect of promysalin on the plant pathogen P*seudomonas syringae* pv *tomato* DC3000 and found the IC_{50} value to be around 500 nM, indicating that promysalin may be an effective agent against this pathogen.

Antibiotics & Biofilms

P58

Mining 'Microbe-Speak' for Biofilm Blockers

F. Jerry Reen[1], John Phelan[1], David Woods[1], Gerard McGlacken[1], Fergal O'Gara[1,2]
[1]University College Cork, Cork, Ireland, [2]Curtin University, Perth, Australia

The ability of pathogens to form structured surface-associated populations contributes significantly to their ability to develop clinical infections. The inherently resistant characteristics of these biofilms has limited the effectiveness of conventional antimicrobial therapies in the treatment of infections. This presents a major challenge as the rapid decline in the development of new antimicrobial therapeutics has coincided with the emergence of new and more aggressive multidrug resistant pathogens. Adding to the complexity of the challenge is the fact that many sites of infection are colonised by mixed communities of fungi, bacteria, and viruses. These multiple interactomes constitute what is effectively a chorus of communication, exquisitely coordinated and highly evolved. Deciphering the underlying intercellular signalling networks offers an opportunity to develop a new generation of drugs that effectively silence pathogenesis and the formation of persistent biofilms, while avoiding dysbiosis by retaining the diverse structure of the microbial community.

We discovered that alkylquinolone signal molecules produced by *P. aeruginosa* could suppress biofilm formation in co-occurring bacterial and fungal pathogens, perhaps the microbial equivalent of esperanto. Synthetic decoration of the interkingdom alkylquinolone framework enhanced the therapeutic properties of these small molecules and provided a suite of compounds for further development. Importantly, while biofilm cells were significantly reduced, planktonic (non-biofilm) cells were unaffected. This may be significant, as microbial life tends to 'finds a way' to counter the biocidal activity of existing antibiotics. The biofilm-blocking potential and chemical tractability of these compounds makes them a suitable platform for the development of potent virulence suppressing anti-infectives.

Antibiotics & Biofilms

P59

SENBIOTAR: Sensiting Pseudomonas aeruginosa biofilms to antibiotics and reducing virulence through novel target inhibition

Fadi Soukarieh[1], Tomás Sou[2], Mirko Iubatti[3], Irena Kukavica-Ibrulj[4], Eduard Vico[1], Nigel Halliday[1], Christel A.S. Bergström[2], Michael J. Stocks[1], Peter E. Nielsen[3], Paul Williams[1], Roger C. Levesque[4], Miguel Cámara[1]
[1]School of Life Sciences, Centre for Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK, [2]Department of Pharmacy, Uppsala University, Uppsala, Sweden, [3]Department of Cellular and Molecular Medicine, Panum Institute, University of Copenhagen, Copenhagen, Denmark, [4]Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Québec City, Canada

The traditional therapeutic approaches targeting viability often induce strong selective pressures resulting in the rapid emergence of antimicrobial resistance. An alternate approach is to inhibit virulence rather than bacterial viability. In the opportunistic human pathogen Pseudomonas aeruginosa, virulence is co-ordinately controlled through quorum sensing (QS), a global cell-to-cell communication system employing diffusible signal molecules.
The aim of the SENBIOTAR project is to optimise hit compounds and peptide nucleic acids (PNAs) which target Pseudomonas Quinolone Signal (PQS) biosynthesis and/or PQS signal transduction. These hits have been shown to not only render P. aeruginosa avirulent but also to sensitise biofilms to the action of antibiotics. One of the limitations of using inhibitors of virulence is the fact that immunocompromised patients may not be able to clear the targeted pathogen efficiently. However, if the pathogen can, at the same time, be sensitised to antibiotics \ there is great scope for dual therapy using PQS inhibitors and antibiotics.
The SENBIOTAR project brings together world experts to optimise the activity of hit compounds and PNAs which strongly inhibit QS, attenuate virulence and sensitise biofilms to conventional antibiotics. These studies use a combination of in vitro virulence and biofilm bioassays alongside experimental animal chronic lung infection models.
The lead compounds developed by SENBIOTAR could also have significant potential for the treatment of wound, bloodstream and medical-device associated infections caused by P. aeruginosa.

Antibiotics & Biofilms

P60

The Pseudomonas aeruginosa pan-genome: was sequencing 1000 isolates worth it?

Luca Freschi[1], Anthony T. Vincent[1,2], Julie Jeukens[1], Jean-Guillaume Emond-Rheault[1], Irena Kukavica-Ibrulj[1], Marie-Josée Dupont[1], Steve Charette[1,3], Brian Boyle[1], Roger C. Levesque[1]
[1]Institut de biologie intégrative et des systèmes (IBIS), Université Laval, Quebec City, Canada, [2]Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ), Quebec City, Canada, [3]Département de biochimie, de microbiologie et de bio-informatique, Université Laval, Quebec City, Canada

Background - An emerging paradigm of bacterial genetics is the analysis and the comparison of pan-genomes, i.e. the full set of genes of a given bacterial species or clade. With the revolutions of next-generation sequencing technologies and high-performance computing, it is now possible to sequence thousands of isolates of a given bacterial species, and compare their genomes quickly. But is sequencing a lot of genomes and updating existing pan-genomes worth it?
Methods - Here, we combined P. aeruginosa genomes from IPCD (https://ipcd.ibis.ulaval.ca) and NCBI to obtain a high-quality dataset of 1,311 assemblies. We then calculated the pan-genome using a new bioinformatic tool called SaturnV and performed phylogenetic analysis. Plasmid, antibiotic resistance and virulence gene content was also identified.
Results - We found that 54,272 genes constitute the P. aeruginosa pan-genome. The 665 core genes identified were used to construct what is arguably the most detailed P. aeruginosa tree to date, which partitions the species into five distinct groups. Interestingly, antibiotic resistance and virulence gene content was linked to this population structure. We also found that P. aeruginosa is expected to have undergone the most horizontal gene transfer events with genera Sinorhizobium, Ralstonia, Klebsiella and Escherichia. Hence, both environmental and pathogenic bacteria likely contributed to the evolution of pathogenicity in this organism.
Conclusion - Our study shows that the current efforts to sequence thousands of bacterial isolates for different bacterial species are justified and will allow us to explore new dimensions of bacterial genomics and evolution.

P61

Antibiotics and carbapenem-resistant *P. aeruginosa* isolation in intensive care unit: case-case-control study nested in a multicenter cohort.

MAIDER COPPRY[1,2], CAMILLE JEANNE-LEROYER[1], MARION SALY[1,2], PERNELLE NOIZE[1,2], CATHERINE DUMARTIN[3,2], ROMUALD RIEM[4], ALEXANDRE BOYER[1], VERONIQUE DUBOIS[1,2], ANNE-MARIE ROGUES[1,2]
[1]CHU de Bordeaux, Bordeaux, France, [2]Université de Bordeaux, Bordeaux, France, [3]CCLIN Sud-Ouest, Bordeaux, France, [4]CHU de Nantes, Nantes, France

Background
Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), is associated with an increased mortality in intensive care unit (ICU). Studies showed association between antibiotics and CRPA isolation with several limitations, in fact, we aimed to identify other antibiotics than carbapenem associated with CRPA isolation among adults in ICU, using a nested case-case-control study design.

Methods
We conducted a retrospective case-case-control study nested in DYNAPYO prospective multicenter cohort: 1808 patients hospitalized more than 24 hours in 10 French ICU, weekly screened for *P. aeruginosa* from admission to discharge. Study compared resistant-cases (inpatient with CRPA isolation) and susceptible-cases (inpatient with carbapenem-susceptible *P. aeruginosa* (CSPA) isolation) to the same controls (inpatient without *P. aeruginosa* isolation) matched (1-1) according to ICU, length of stay and hospitalization period. CRPA and CSPA isolation were outcomes in two logistic regression models assessing effect of antibiotics grouped by family and anti-pseudomonal activity, adjusted for carbapenem exposure, non-antibiotic exposures (colonization pressure, invasive devices or procedures) and inpatient characteristics.

Results
Were included 59 resistant-cases, 83 susceptible-cases and 142 controls, without major differences in baseline characteristics. After adjusting for carbapenem use, piperacillin(-tazobactam) and ticarcillin(-clavulanic acid) exposure remained a protective factor for CSPA isolation (OR .0,868 ; p=0.018) and exposure to group b-lactam inactive on *P. aeruginosa* remained an independent risk factors for CRPA isolation (OR 1.101; p=0.029).

Conclusion

Using unusual confounding factors, we fortunately identify that exposure to b-lactam inactive on *P. aeruginosa* is a risk factor for CRPA isolation. Clinicians should take into account our results to define probabilistic treatment protocol in ICU.

P62

Direct microfabrication of 3D hydrogel architectures for bacterial culturing

Irene Henning, Paul Williams, Mischa Zelzer, Morgan Alexander, Richard Hague
University of Nottingham, Nottingham, UK

Bacteria naturally grow in communities in which they build a protective three-dimensional architecture that accommodates a variety of different species. Cell-to-cell communication enables them to organise spatially and enhances the survival of the entire population. Although, it is clinically important to understand the mechanisms that make them successful as a complex community, the vast majority of studies focusses on in vitro studies with lacking control over population size and spatial distribution of microbes.
Recent advances in 3D printing help to overcome this challenge by making it possible to produce complex three-dimensional structures of arbitrary shape. Herewith, we explore the high resolution 3D printing technique, called two-photon lithography, to culture *Pseudomonas aeruginosa* in defined spatial hydrogel architectures. For this purpose, we compared the suitability of gelatin methacrylate (GelMA) and bovine serum albumine (BSA) to form hydrogels by photoactivated cross-linking and optimised the printing parameters to enable the rapid microfabrication of structures. Different geometries were tested to investigate both, the most robust designs that endure the culturing conditions overnight and to determine a potential influence on bacterial growth. We show that micro-architectures with volumes between 100-200 pL were efficiently fabricated within 20 to 60 seconds. Scanning electron microscopy (SEM) was used to verify the structural integrity of the micro-architectures. The growth of bacterial cultures within the structures could be monitored with a confocal microscope over time. In this work, we present the great capability of producing tailored 3D in vitro models to culture bacteria in a customised and controlled environment.

Antibiotics & Biofilms

P63

Adaptation to octenidine leads to decreased sensitivity to octenidine formulations and other biocides in Pseudomonas aeruginosa

Matthew J. Shepherd, Matthew E. Wand, J. Mark Sutton, Lucy. J Bock
Public Health England, National Infection Service, Salisbury, UK

Background: Multi-drug resistance puts increasing pressure on biocides such as octenidine for infection control. There have been no reports of reduced susceptibility to octenidine.

Aim: To investigate the susceptibility of P. aeruginosa to octenidine and its formulations, and to identify if P. aeruginosa is capable of adapting to octenidine. Adapted strains were phenotyped to identify any cross-resistance.

Methods: Seven clinical isolates of P. aeruginosa were adapted to increasing concentrations of octenidine. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of a variety of antimicrobials were measured at 1 minute, 5 minutes and 24 hours exposure for the parental and adapted P. aeruginosa strains.

Results: Octenidine formulations were efficacious against clinical P. aeruginosa isolates at 50% working concentration at the recommended exposure time. However, P. aeruginosa stably adapted to octenidine when continuously exposed to the biocide. Adaptation increased tolerance to octenidine formulations and other cationic biocides (chlorhexidine, alexidine, and didecyldimethylammonium bromide). Adaptation to octenidine in one strain increased the MIC of gentamicin from 8 to 32 μg/mL, of colistin from 1 to 4 μg/mL and conferred increased MICs to additional aminoglycoside antibiotics.

Conclusion: Infection Prevention and Control measures should take into account that multidrug resistant P. aeruginosa strains can increase their tolerance of octenidine in response to continuous exposure and that this can lead to cross-resistance to antibiotics. The rotational use of different classes of chemicals for disinfection and antisepsis may decrease exposure and consequent adaptation of clinical strains.

Ecology, Evolution & Environment

P64

Biosorption of hexavalent chromium by *Pseudomonas aeruginosa* strain S164S: equilibria isothermic, kinetic and thermodynamic studies

Kingsley Ojiagu, Nnenna Ojiagu, Kingsley Agu
Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Pseudomonas aeruginosa strain S164S, a non-genetically engineered bacterial strain isolated from soil was used to study and evaluate its biosorption potentials for hexavalent chromium (Cr[VI]) from aqueous solution. Living, heat-killed, and permeabilised cells were all used and found to be capable of reducing and sorbing Cr(VI). The influences of initial Cr(VI) ion concentration (50 to 150 µg/mL), contact time (2 h), pH (2 to 7), temperature (30 to 60°C) and biosorbent mass (1 to 5 g) were reported. Adsorption of Cr(VI) is highly pH- and temperature-dependent, and the results indicate that the optimum pH and temperature for the removal was found to be 2 and 60 °C respectively. The hexavalent chromium biosorption equilibrium could be better described by the Langmuir isotherm than it could by Freundlich isotherm. A comparison of kinetic models applied to the adsorption of Cr(VI) ions onto the biosorbents was evaluated for the pseudo first-order, the pseudo second-order, and intra-particle diffusion kinetic models. Results show that the pseudo second-order kinetic model was evidenced to correlate better the experimental data. The rate of hexavalent chromium adsorption increased following permeabilisation of the outer and/or cytoplasmic membrane by surfactants such as triton X100, tween 80, toluene, sodium deoxycholate and sodium dodecyl sulphate. The adsorption process has been found endothermic in nature, and thermodynamic parameters of Gibb's free energy ($\Delta G°$), change in enthalpy ($\Delta H°$) and change in entropy ($\Delta S°$) have been calculated. *Pseudomonas aeruginosa* strain S164S evidenced an effective biosorbent for the removal of hexavalent chromium in aqueous form.

Ecology, Evolution & Environment

P65

Temperate phage accelerates the loss of siderophore cooperation in *Psuedomonas aeruginosa*

Siobhan O'Brien[1,2], David Guymer[2], Craig Winstanley[4], Steve Paterson[4], Michael Brockhurst[3]
[1]ETH Zurich, Zurich, Switzerland, [2]University of York, York, UK, [3]University of Sheffield, Sheffield, UK, [4]University of Liverpool, Liverpool, UK

Bacteriophages (phages) are viruses of bacteria that infect and replicate within their hosts, outnumbering eukaryotic viruses both in abundance and diversity. While lytic phages follow a strictly parasitic life cycle, temperate phages may either complete the lytic cycle or integrate into the bacterial chromosome as a prophage. Temperate phage integration can shape bacterial genomes, not only by disrupting and deactivating host genes, but also by introducing new autonomous genes. Furthermore, transposable temperate phage that insert randomly throughout the genome can enhance bacterial genomic diversity, facilitating rapid adaptation to a novel environment. Despite the potential influence of temperate phages on pathogen genomes, there have been few experimental tests of the contribution of temperate phages to bacterial adaptive evolution. Here, we investigate whether random mutations and enhanced diversity generated by transposable phage integration can shape adaptation to the social environment, in this case the cooperative production of iron-scavenging siderophores in *Pseudomonas aeruginosa.* We show that, under iron-limited conditions, temperate phage accelerates the production of non-siderophore producing 'cheats' by integrating into global regulator genes within their host cell. This suggests that transposable phage can profoundly and predictably impact the dynamics of social evolution in microbial populations.

Ecology, Evolution & Environment

P66

The Role of Environmental Drivers in Creating and Constraining Opportunities for Novel Gene Regulator Recruitment

Tanya Arseneault[1,2], Louise Johnson[2], Robert Jackson[2], Tiffany Taylor[3,1]
[1]Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu Research and Development Centre, Quebec, Canada, [2]University of Reading, School of Biological Sciences, Reading, UK, [3]University of Bath, Milner Centre for Evolution and Dept of Biology and Biochemistry, Bath, UK

The environment plays a key role in shaping gene regulatory network (GRN) arrangement, with natural selection able to make adjustments to specific interactions. However, we do not yet understand the evolutionary processes that create and constrain opportunities for GRN adaptation and expansion. Our model organism is a genetically modified *Pseudomonas fluorescens* SBW25 mutant, which is non-motile and non-flagellate by deletion of the master regulator of flagellar synthesis (*fleQ*) and a transposon-insertion disrupting viscosin production. We have shown that under ecological conditions that imposed strong selection for dispersal, these immotile bacteria re-evolved flagellar motility within 96 hours via a repeatable, two-step evolutionary pathway. A switch-of-function mutation allowed NtrC, a distant homologue of FleQ, to commandeer control of the *fleQ* regulon, which putatively increased affinity for FleQ target genes but decreased affinity for NtrC target genes. The evolved NtrC combines its pre-existing nutritional regulatory function with a novel function as a flagellar regulator, but there is a trade-off between these two roles. This trade-off shapes the opportunity for rewiring between networks and constrains evolutionary routes in different nitrogen environments. Our results demonstrate the role of environmental drivers in creating opportunities for recruitment of novel gene regulators – a central process in GRN expansion and adaptation.

Ecology, Evolution & Environment

P67

Enhanced catabolism of adenosine by gene duplication-amplification in *Pseudomonas aeruginosa*

Jean-Paul Toussaint, Anna Farrell-Sherman, Tamar Feldman, Nicole Smalley, Amy Schaefer, E. Peter Greenberg, Ajai Dandekar
University of Washington, Seattle, Washington, USA

The laboratory strain of *Pseudomonas aeruginosa*, PAO1, regulates the catabolism of adenosine and other nucleosides through the quorum sensing transcription factor LasR. We previously reported that PAO1 grows poorly on adenosine as a sole carbon and energy source, with doubling times >40 hours. When PAO1 is grown on adenosine and casein, variants emerge that grow more rapidly on adenosine as the sole carbon source. We investigated the mechanism that confers rapid growth on adenosine. To do so, we selected five individual variants of PAO1 with doubling times ranging from 5-12 hours on adenosine, and performed whole genome sequencing. A gene duplication-amplification (GDA) event was the only consistent mutation across all the variants. The common amplified region to all variants involved several genes in the nucleoside degradation pathway including the quorum-regulated nucleoside hydrolase, *nuh*; an adenine deaminase, PA0148; and other genes possibly involved in nucleoside catabolism. We show that expression of nine genes in the GDA region on a plasmid conferred rapid adenosine growth to PAO1 while no gene individually conferred the phenotype. Furthermore, we show that the regulation of *nuh* remains quorum-sensing dependent in the setting of amplification. Therefore, GDA in *P. aeruginosa* confers an enhanced nucleoside catabolic ability in *P. aeruginosa* while maintaining quorum sensing regulation of nucleoside catabolism.

Ecology, Evolution & Environment

P68

Pseudomonas aeruginosa from healthy animals: antimicrobial resistance, virulence and molecular typing.

Lidia Ruiz-Roldán[1], Beatriz Rojo-Bezares[1], Carmen Lozano[1], Carmen Torres[2], Yolanda Sáenz[1]
[1]Área de Microbiología Molecular. Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain, [2]Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain

BACKGROUND: *Pseudomonas aeruginosa* (PA) is a ubiquitous pathogen that shows high antimicrobial resistance and virulence.

Our aim was to characterise PA isolates recovered from faecal samples of healthy animals.

METHODS: 608 faecal samples from healthy animals (pets, farm animals, ducks, wild boars, ticks, micromammals, deers and rabbits) were processed. The following analyses were performed: molecular typing by *SpeI*-Pulsed-Field-Gel-Electrophoresis (PFGE), multilocus-sequence-typing and serotyping; susceptibility to 13 antibiotics by disc-diffusion method; OprD mutations by PCR and sequencing; presence of thirteen virulence markers by PCR; pigments and elastase production by biochemical methods, and biofilm production by crystal violet staining (biomass) and fluorescein diacetate assay (metabolic activity).

RESULTS: PA was detected only in 6 animals (one sheep and five wild boars) and 33 isolates were obtained (1-9 strains/sample). These isolates, grouped in 7 different PFGE patterns, were susceptible to antibiotics, except two piperacillin/tazobactam-ticarcillin-meropenem-aztreonam resistant isolates. Sequence types ST1648, ST1711, ST1732, ST2096, ST2194 and ST2252 were detected among 9 selected isolates; six of them were serotype 3 and three isolates serotype 6. Four OprD mutational patterns and two virulotypes were detected. Type 3 Secretion System and *exoA* genes were absent in 6 strains. All strains produced more biofilm and elastase than control strain, and 66.6% of them produced more pyocyanin.

CONCLUSION: Low occurrence of PA was detected (1%). Animal PA isolates showed low levels of antimicrobial resistance and virulence, but diversity of genetic lineages and elevated biofilm, elastase and pyocyanin production. These data underline the variable adaptation capacity of PA.

Ecology, Evolution & Environment

P69

Evolution of repetitive sequences in bacterial genomes

Frederic Bertels, Chaitanya Gokhale, Arne Traulsen
Max Planck Institute for Evolutionary Biology, Plön, Germany

Short repetitive extragenic palindromic sequences are a ubiquitous feature of bacterial genomes, in particular *Pseudomonas* genomes. REP sequences are replicated as part of a REP doublet called a REPIN. We show that REPINs within a genome are evolving populations and as such show features typical of population growth, decline and interaction with other REPIN populations as well as other genetic elements. Studying a whole population can be achieved by sequencing a single genome. In our study we model REPIN populations from a very diverse set of bacterial strains as quasispecies. From this model we infer duplication rates that suggest that hundreds of years can pass between two successive duplication events. If duplication rates were any lower then it would be impossible to maintain sequence identity over long periods of time. Because these rates are very similar between the populations of the studied bacteria (MRCA existed about 600mya) we hypothesize that REPIN duplication rates are optimal. REPIN populations can only persist if duplication rates are low, because higher rates are likely to negatively affect the host bacterium and lower rates would lead to the extinction of the REPIN population.

Ecology, Evolution & Environment

P70

Intraclonal competitive fitness of sequential P. aeruginosa strains from CF-patients differs by the clinical severity of infection

Nina Cramer, Sebastian Fischer, Silke Hedtfeld, Marie Dorda, Jens Klockgether, Lutz Wiehlmann, Burkhard Tümmler
Medical School, Hannover, Germany

The common Gram-negative bacterium P.aeruginosa is an important causative agent in nosocomial infections and the pathogen which contributes most to the shortened life expectancy of CF-patients. In this work we wanted to analyze if there is a fitness advantage or disadvantage due to pathoadaptive mutations in the CF-lung.
Therefore, serial clonal P. aeruginosa isolates taken from 12 CF-patients (six patients with a mild and six with a severe course of infection) were competitively grown together in different media to mimic environmental and CF-lung conditions. Samples were taken at 0 h, 48 h (with continuous culturing every 12 h) and 120 h (without continuous culturing), DNA was prepared, multiplex PCR was performed and resulting amplicons were sequenced. The percentage of each isolate within a sample was calculated based on isolate specific SNPs.
In nearly all 12 courses one or two strains could outcompete all others. For the severe courses we observed in all cases that the first isolates outcompeted the later isolates, whereas in the mild courses no tendency could be detected. For some of these winner strains we identified SNPs, stop-mutations, deletions or extra genes which could be directly linked to a fitness advantage. The late isolates of the severe courses acquired more drastic genomic changes which caused a growth disadvantage compared to the non-adapted first isolates.

Ecology, Evolution & Environment

P71

The adaptation-process of Pseudomonas aeruginosa in CF-lungs: Correlation between phenotype and genotype

Nina Cramer, Jens Klockgether, Sebastian Fischer, Burkhard Tümmler
Medical School, Hannover, Germany

Chronic airway infections with P. aeruginosa typically determine the clinical course of patients suffering from CF. During chronic infection the bacteria undergo microevolution which supposedly reflects the adaptation to the CF lung habitat and to the antimicrobial treatments administered over the years.
Here, the adaptation process of P. aeruginosa was observed in sequential isolates of the initially colonizing clone from twelve CF-patients, six with a mild and six with a severe clinical course. Therefore, the isolates were analyzed pheno- and genotypically. More than 250 sequential isolates were characterized in mutation rates and phenotypic traits such as morphology, motility and virulence effector secretion. Of this pool of strains isolates were chosen in one year intervals and sequenced by next-generation sequencing. Genetic peculiarities such as nucleotide variations, small indels and larger deletions compared to the initial isolate were extracted and used for clade reconstruction. Hotspots of mutations were determined.
Contrary to what is written in the literature box plots of phenotypes displayed no clear loss of motility or siderophore secretion over the course of infection. For all analyzed phenotypic traits, except for swarming, a broad range of characteristics could be verified. Phenotypes were not linked with clades. Overall, genes associated with antibiotic resistance and adaptation were overrepresented among hot spots of mutations. To see if those mutations are functional, various bioassays are planned for the future.
For detail on the genomic analyses please refer to the poster of Jens Klockgether.

Ecology, Evolution & Environment

P72

Delineation of *Pseudomonas* species based on genomic sequences

Christopher Huptas, Michaela Kreitmeier, Mareike Wenning, Siegfried Scherer
Chair of Microbial Ecology, ZIEL - Institute for Food and Health, Technische Universität München, Freising, Bavaria, Germany

Background

The genus *Pseudomonas* currently consists of 164 species according to the *LPSN* (April 2017). Since the number of species described is increasing constantly, accurate species and strain identification is crucial. Commonly two different methods are used to delineate *Pseudomonas* species: multilocus sequence analysis (MLSA) and whole genome relatedness like average nucleotide identity (ANI).

Methods

Strict ANIb and ANIm thresholds were determined by binary logistic regression based on DNA–DNA hybridization data of 127 prokaryotic strain pairs. In case of gANI the recommended species cut-off ≥ 96.5% was adopted. ANI cut-offs were applied to a set of 350 *Pseudomonas* strains comprising 63 type strains. Furthermore, the MLSA scheme of Mulet *et al.* (2010) was used to predict the phylogeny of the 350 *Pseudomonas* strains considering 96 additional type strains, for which MLSA sequence data was available at NCBI.

Results

Goodness of fit of all logit models (ANIb, ANIm) was confirmed by the pseudo-R^2 statistics of Cox&Snell (~0.59), McFadden (~0.73) and Nagelkerke (~0.83). Additionally, ROC-Curves yielded AUCs around 0.97 indicating high classification ratings. Hence, reliable strict species thresholds were defined for ANIb and ANIm. Finally, ANI-derived species clusters and MLSA-phylogeny were combined and visualized with the iTOL software to highlight the species membership of each of the 350 *Pseudomonas* strains.

Conclusion

In contrast to identifications based on single indices only, polyphasic comparison based on phylogenetic and whole-genome distance metrics is more sensitive for ambiguous species assignments and avoids premature and misleading classification.

Ecology, Evolution & Environment

P73

Hot spots of side-by-side sequence variation and RGP variants in *Pseudomonas aeruginosa* strains.

Sebastian Fischer, Leonie Greipel, Katarzyna Pienkowska, Nina Cramer, Jens Klockgether, Patricia Moran Losada, Lutz Wiehlmann, Burkhard Tümmler
Hannover Medical School, Hannover, Germany

Early antimicrobial treatment has been established as an efficacious measure to eradicate *P. aeruginosa* from CF airways when it is detected for the first time by culture-dependent diagnostics.
Due to the recent developments in next generation sequencing technologies we got an in-depth insight into the intraclonal diversity of *P. aeruginosa* in CF lungs during the onset of colonization that is not accessible by any established genotyping method such as genome fingerprinting, oligonucleotide microarray or Sanger-based multilocus sequence typing. With the accurate SOLiD technology we were able to detect subclonal variants within one strain whereby the two possible nucleotides of a SNP were commonly present in different proportions in pre- and post-treatment samples indicating a variable composition of SNPs variants in *P. aeruginosa* colonies. Most subclonal SNPs do not change the coding sequence and may be classified as neutral substitutions that do not confer much or any advantage or disadvantage to the bacteria. Exceptions were missense and stop mutations in *lasR*. The co-existence of LasR isoforms may reflect diversifying selection to improve the fitness of *P. aeruginosa*.

Similar variation was also recognized during the analysis of the accessory genome of the clone C reference genome. Resequencing of the respective isolate NN2 using SMRT technology revealed the absence of accessory DNA at some loci for a minor proportion of genome copies indicating that also larger blocks of DNA can vary within an isolate.

Ecology, Evolution & Environment

P74

Spectrum of mutations in pathoadaptive loci of *P. aeruginosa*.

Sebastian Fischer, Nina Cramer, Jens Klockgether, Burkhard Tümmler
Hannover Medical School, Hannover, Germany

Pathoadaptive loci are defined as genes that acquire a high number of mutations during persistence in a chronically infected patient habitat like the CF lung. Twenty genes including *lasR*, *nuoL*, *algG*, *pelA* and *pelF* had been identified as pathoadaptive loci in a set of 12 CF patient courses (6 mildly and 6 severly infected patients). The aim of this project was to determine the abundance of mutations in the investigated loci in isolates from environmental habitats, acute infection and cross-sectional chronic infection isolates. Using amplicon sequencing on an Illumina NextSeq we were able to sequence the pathoadaptive loci of more than 500 isolates (66 acute infections, 98 environmental, 375 chronic infection isolates) in a fast and cost effective manner. After filtering for decent coverage and read distribution we were able to detect a various number of mutations in the strain panel. The range of mutations in a loci varied between a dozen and more than 100 candidate positions whereby some rare positions seem to be specific for one habitat. Both synonymous and non-synonymous mutations seem to be present in the same amount of affected positions within the genes with the limitation that neutral mutations tend to be present in more isolates. An example for a rare mutation is an early stop mutation in *algG* only found in two chronic infection isolates.

Ecology, Evolution & Environment

P75

Pseudomonas aeruginosa transcriptional responses to potable water and identification of pathways important in the potable water environment

Erika English, Graham Willsey, Matthew Wargo
University of Vermont, Burlington, VT, USA

Many *P. aeruginosa* infections are caused directly by environmental isolates, primarily derived from potable water (PW) sources and related fixtures. We propose that the PW pre-infection niche is an important component of the natural history of many *P. aeruginosa* infections. To better understand *P. aeruginosa* response to PW, we analyzed the PA14 transcriptome in response to half-strength LB, half-strength R2B (1/2R2B), and PW. Surprisingly, very few changes were seen between LB and R2B, even given the large difference in nutrient concentrations between these two media. Using 1/2R2B as the comparator condition, 485 transcripts were significantly changed more than 3-fold in PW. One of the most notable and obvious differences between 1/2R2B and PW is the concentration of divalent metals, particularly copper (induction of 22 transcripts involved in transition metal resistance and efflux, and decrease in 11 transcripts involved in metal acquisition). In addition to the heavy metals, transcripts related to lipids, polyamines, biofilm formation, stress, and nucleotide utilization were substantially altered, and induction of the genes encoding enzymes in the glyoxylate shunt suggests altered central metabolism. We selected a few target genes and investigated their role in growth and competition in PW. One operon, *betBA*, showed a competitive defect against wt in PW and our reporter assays suggest a small but measureable level of choline in PW sources. Overall, these results shed light on *P. aeruginosa* biology in one of its fundamental environmental niches.

Ecology, Evolution & Environment

P76

Collective action at multiple scales in the *Pseudomonas fluorescens* biofilm

Neal Jahren, Jessica Dewey, Sarah M Hong, Michael Travisano
University of Minnesota, Saint Paul, MN, USA

Many of the "major transitions" in evolution involve independent units joining into a higher-level collective. We present results from a new system for investigating the emergence and maintenance of collective action in *P. fluorescens* SBW25. Replicate populations of *P fluorescens* were maintained in shaking culture with daily serial transfers and settling selection for 58 transfers. Settling selection was accomplished by inoculating a subsequent culture with the bottom 5% of a sample from the previous culture after it had rested undisturbed for five minutes. All populations evolve towards a long-term coexistence of strains with distinct adaptations. Some strains respond to settling selection by producing a costly extra-cellular matrix (ECM) that promotes clustering, while other strains evolve the capability to survive the settling selection by free riding. Previous research into the collective action of *P. fluorescens* has focused on the pellicle that forms on the surface of a static culture. A subset of ECM-producing strains isolated from our experiments also demonstrate adaptation to static culture by outcompeting a pellicle-forming strain. Evidently, the collective action that emerged at a small scale to survive settling selection also provides an effective strategy for large scale co-operation in a pellicle. These results provide evidence for the existence of a new mode of collective action in bacterial biofilms.

Ecology, Evolution & Environment

P77

A low frequency persistent reservoir of a genomic island in a pathogen population ensures island survival and improves pathogen fitness in a susceptible host

Helen Neale[1], Robert Laister[1], Joseph Payne[1], Gail Preston[2], Robert Jackson[3], Dawn Arnold[1]
[1]University of the West of England, Bristol, UK, [2]University of Oxford, Oxford, UK, [3]University of Reading, Reading, UK

The co-evolution of bacterial plant pathogens and their hosts is a complex and dynamic process. Plant resistance can impose stress on invading pathogens that can lead to beneficial changes in the bacterial genome to help the pathogen escape host resistance. *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) is the causative agent of halo blight in bean. The *Pph* genomic island PPHGI-1 carries an effector gene, *avrPphB*, which is recognised by the host bean and triggers a strong immune response called the hypersensitive reaction (HR). The HR creates a harsh antimicrobial environment and the resulting stress on the pathogen results in the excision of PPHGI-1 and its loss from the genome; the new pathogen genotype causes disease in the plant. However, we have never observed 100% loss of PPHGI-1 from the *Pph* population. We have developed a mathematical model to predict if the genomic island PPHGI-1 would be maintained in the population long term. We empirically tested the predictions made by the model and determined that PPHGI-1 frequency in the bacterial population drops during the HR. The island is then stably maintained in approximately 0.5% of the population over the long term. However, when a population of *Pph* that contains 0.5% cells carrying PPHGI-1 is inoculated into a bean cultivar that does not produce the HR, the proportion carrying PPGHI-1 increases rapidly suggesting that PPHGI-1 confers a fitness benefit. These results provide insights into the long term population dynamics of genomic island retention and loss influencing pathogen evolution.

Ecology, Evolution & Environment

P78

AprA peptidase production of Pseudomonas isolates from raw milk

Christopher Maier, Genia Lücking, Mario von Neubeck, Christopher Huptas, Siegfried Scherer, Mareike Wenning
Lehrstuhl für Mikrobielle Ökologie, ZIEL - Institute for Food & Health, Technische Universität München, Munich, Germany

Background
Spoilage of UHT milk or milk products is often caused by heat-resistant peptidases produced by species of the genus *Pseudomonas*. So far, one extracellular metallopeptidase (AprA) is known in *Pseudomonas*, which is encoded by a gene of the *aprA-lipA* operon. In *P. fluorescens* this operon was characterised as gene cluster comprising eight genes coding for a peptidase, an inhibitor, a type I secretion system, two putative autotransporter and a lipase.

Methods
In this study the genetic organization of the *aprA-lipA* operon was analysed in different *Pseudomonas* species. For this, whole genome sequencing of 50 milk isolates, belonging to eight *Pseudomonas* species, was performed by NGS. Furthermore, the proteolytic activity of isolates incubated at different growth conditions was quantified by an enzyme assay using azocasein as substrate.

Results
Sequence analysis revealed species- and strain-dependent differences in the presence and localisation of aprA-lipA operon genes leading to a categorisation into four main operon types. In general, isolates with the operon structure *aprAIDEF prtAB lipA2* revealed higher peptidase activity than isolates possessing other operon structures. AprA activity varied strongly in a strain-specific manner, ranging from non-proteolytic to highly proteolytic isolates. Peptidase production of most isolates increased significantly in stationary phase and was shown to be influenced by external factors like incubation time or temperature.

Conclusion
A correlation between AprA activity and *aprA-lipA* operon structure could be observed to some extend for different *Pseudomonas* milk isolates. However, regulation mechanisms on transcriptional or posttranscriptional level need to be elucidated to understand strain-specific AprA expression in *Pseudomonas* spp.

Ecology, Evolution & Environment

P79

Genome comparison of temperate phages from Cystic Fibrosis and Bronchiectasis related *Pseudomonas aeruginosa.*

Mohammad Tariq[1], Lauren Cowley[2], Giles Holt[1], Audrey Perry[3], John Perry[3], Anthony De Soyza[3,4], Darren Smith[1]
[1]Northumbria University, Newcastle upon Tyne, UK, [2]Public Health England, London, UK, [3]Freeman Hospital, Newcastle upon Tyne, UK, [4]Newcastle University, Newcastle upon Tyne, UK

Introduction: Cystic Fibrosis (CF) is associated with mutations of the Cystic Fibrosis Transmembrane Conductance Regulator gene (*cftr*) and alteration of epithelial function. Bronchiectasis (BR) is similar symptomatically to CF. *Pseudomonas aeruginosa* (Pa) is a common opportunistic respiratory pathogen that is a major driver of chronic infection correlating to lowered lung function and poor clinical outcomes. Temperate bacteriophages carried by Pa carry genes that aid bacterial selection in the lung and drive evolution through their dynamic interaction. Our aim is to characterise the genomes of the phages that can mobilise from the bacteria to disseminate these genetic traits through their Pa host range.

Method: CF (n=47) and BR (n=47) temperate bacteriophages chemically induced and sequenced. Novel use of khmer toolkit remove bacterial DNA contamination and aid assembly. Phage genomes assembled using SPAdes, contigs extended with PriceTI and annotated using prokka.

Results: 107 Pa phages were identified from the Siphoviridae class apart from H66-like phages (n=12) which were from the Podoviridae class. F10-like phages were seen with the highest frequency (n=32). The novel k-mer based separation method allowed for resolution of chimeric assemblies. PriceTI aided the extension of 27 partial phage genomes to completion.

Conclusion: Isolating and purifying temperate phages can be complex as phages that induce from their bacterial host in high numbers can be masked by secondary or tertiary phages. This study uses novel genomic approaches to overcome this problem and separate mixed phage communities using k-mer abundance. Genetic diversity of the temperate phages was illustrated using genome comparison.

Ecology, Evolution & Environment

P80

Adaptation of temperate bacteriophage in *Pseudomonas aeruginosa* isolated from Cystic Fibrosis and Bronchiectasis patients.

Mohammad Tariq[1], Francesca Everest[1], Giles Holt[1], Audrey Perry[2], John Perry[2], Mike Brockhurst[3], Anthony De Soyza[2,4], Darren Smith[1]
[1]Northumbria University, Newcastle upon Tyne, UK, [2]Freeman Hospital, Newcastle upon Tyne, UK, [3]University of Sheffield, Sheffield, UK, [4]Newcastle University, Newcastle upon Tyne, UK

Background: Cystic Fibrosis (CF) is associated with a mutations in the CFTR gene that alters epithelial cell function, giving rise to a dehydrated mucus layer, that's an ideal site for the opportunistic bacteria *Pseudomonas aeruginosa* (Pa). The infection and physiological impact of Pa infection is progressive though re-occurring inflammation, scar tissue and decreased mucocilliary clearance. Bronchiectasis (BR) is similar symptomatically to CF due to localised dilation's and inflammation. We identify here the adaptation and evolution of temperate phages in the lung through their dynamic phage-bacteria interactions but also evolution of phage genome complexity using metagenomics.

Method: Use panel of 47 CF Pa isolates and 47 BR Pa isolates stratified by time since diagnosis (BR) and patient age (CF). This study focused on three key areas: 1) adaptation and evolution of phage-host interactions, 2) role in antimicrobial resistance, 3) adaptation and evolution of gene function using metagenomics.

Results: We illustrate that bacteriophages originating from the older CF patients are more infective. We also note that infectivity of phages from CF and BR adapts and evolves over time to promote infection in the lung. These adaptation events also correspond to the accrual of genes that increase the complexity of the phage and possibly Pa that promotes selection.

Conclusion: This research illustrates that adult CF related phages were the most infective against the Pa panel and that this evolves over time. Secondly, through metagenomics we illustrate the increasing complexity Pa phage genomes over time that may promote selection in the chronic lung.

Ecology, Evolution & Environment

P81

Comparison and carriage of temperate bacteriophages genomes in clonal populations of *Pseudomonas aeruginosa* in chronic respiratory disease.

Libby Duignan[1], Mohammad Adnan Tariq[1], Giles Holt[1], Jo Fothergill[2], Craig Winstanley[2], Darren Lee Smith[1]
[1]Northumbria University at Newcastle, Newcastle, UK, [2]University of Liverpool, Liverpool, UK

Background: The complexity of the accessory genome of *Pseudomonas aeruginosa* (PA) is frequently overlooked compared to the core genome. They regularly contains temperate bacteriophage (phage) and other transposable genetic elements. PA's presence in the chronically infected lung correlates with loss of lung function particularly in cystic fibrosis (CF) and non-CF-bronchiectasis (nCFBR) patients. This study focuses in assessing the carriage of intact and remnant phages in PA variants from the same and between patients.

Methods: The temperate phages were identified from the 13 bacterial sequences from patient isolates taken at same time-point, showing two or three with different clonal lineages and morphologies using PHASTER. Comparisons thereafter were made using Gepard and MAUVE.

Results: Prophages were bioinformatically identified in the backbone of PA, their integration points and genetic variation were compared. Firstly, prophages identified in PA from the same patients were compared, and illustrated a different complement of prophages. The prophages were compared against the PA panel, each isolate bar one had at least one prophage displaying significant similarity to another from a different isolate, illustrating conservation in the lung. Integration sites of the prophages were also compared, with intergenic sites noted e.g. *PslE*. Respective core and accessory prophages genomes regions were compared to pinpoint carriage and conservation.

Conclusion:This study highlights PA isolated from the same lung, thought previously to be clonal carry a different complement of phages. These phages have the opportunity to transfer between the multiple strains, possibly spreading genes that could offer selective advantage within the lung microenvironment.

Ecology, Evolution & Environment

P82

Identification of genes involved in degradation of the toxic detergent SDS in *Pseudomonas aeruginosa*.

Gianna Panasia, Bodo Philipp
Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany

The metabolic versatility and pronounced resistance against harmful chemicals enables *Pseudomonas aeruginosa* to survive and grow in hostile environments. In this context, *P. aeruginosa* is able to use the toxic and common detergent sodium dodecyl sulfate (SDS) as a growth substrate, while aggregating as part of a survival strategy against its toxic effects [1]. Regarding the metabolic pathway, only the initial hydrolysis of the sulfate ester by the alkylsulfatase SdsA1 has been documented, whereas the following enzymes in the postulated degradation pathway are still unknown.

Based on a DNA-microarray analysis comparing SDS- and succinate-grown cells several genes with a plausible function in the degradation pathway were identified [2]. Thereby, genes of the ethanol oxidation system (*exa* system) and a so far unknown gene cluster (PA0364-PA0366) were induced. Single gene deletion mutants of the unknown gene cluster still grew with SDS but revealed a reduced SDS degradation. Furthermore, these mutants exhibited a decelerated growth with the degradation intermediate 1-dodecanol. Remarkably, the double deletion mutant PAO1 Δ*exaA* Δ0364 was not able to grow with SDS or 1-dodecanol. Complementation with either *exaA* or PA0364 restored growth similar to the respective single mutant. This indicates a significant participation of both genes in the SDS degradation.

Detailed analysis of this catabolic pathway will give insight in how *P. aeruginosa* is able to cope with SDS and potentially other similar toxic compounds.

[1] **Klebensberger J. *et al.* (2006).** Arch. Microbiol. 185:417-2.

[2] **Klebensberger J. *et al.* (2009).** Environ. Microbiol. 11(12):3073-86.

Ecology, Evolution & Environment

P83

The microevolution process of *Pseudomonas aeruginosa* in CF-lungs: genomic analysis

Jens Klockgether, Nina Cramer, Sebastian Fischer, Burkhard Tümmler
Hannover Medical School, Clinic for Ped. Pneumology, Allergology and Neonatology, Hannover, Germany

Chronic airway infections with *P. aeruginosa* typically determine the clinical course of cystic fibrosis (CF) patients. During chronic infection the bacteria undergo microevolution while adapting to the CF lung habitat and antimicrobial treatment over the years.
We analysed the *P. aeruginosa* microevolution in sequential isolates from six CF-patients with a mild and six with a severe clinical course. Serial isolates were genome sequenced to determine the genomic variations that occurred during the habitat colonisation; the isolates were also tested for several phenotypes and competitive fitness.
The sequential isolates displayed modest mutation rates unless hypermutator phenotypes manifested early in the infection course. Most mutations were nucleotide exchanges or small indels but also DNA loss or uptake could be detected. Phylogenetic trees based on the detected variations illustrated the underlying microevolution and displayed a spectrum of modes, ranging from maintenance of a single adapted strain to long-term persistence of co-existing clades with mixed types in between. Trees for severe courses isolates revealed an increased frequency of extinct clades while for mild courses higher proportions of persisting clades were seen, indicating a trend to more diversifying evolution in bacteria from mild CF courses. Differences were also detected for mutation types, as stop and frameshift mutations were more prominent in severe courses isolates. These findings provided examples for the potential influence of the patient status on bacterial microevolution in CF airways.

More results on the microevolution analysis and phenotypic characterization of serial isolates are displayed on the posters of Nina Cramer and Sebastian Fischer.

Ecology, Evolution & Environment

P84

Microevolution of the accessory genome of Pseudomonas aeruginosa

Sebastian Fischer, Jens Klockgether, Nina Cramer, Burkhard Tümmler
Hannover Medical School, Clinic for Ped. Pneumology, Allergology and Neonatology, Hannover, Germany

The accessory genome, the aggregate of all subgroup- or strain-specific DNA portions, typically accounts for 10 - 20 % of the genomic DNA of *P. aeruginosa* strains. Genomic islands (GI), prophages, other integrated mobile elements and further smaller blocks of accessory DNA are located in regions of genome plasticity (RGP) distributed over the bacterial chromosome.
During our analysis of serial isolates from CF patient airways (see posters of Nina Cramer and Jens Klockgether) we also investigated the accessory genome composition: The presence of known GI and RGP insertions from seven reference genomes was interrogated by alignment of sequencing reads, and de novo assembled contigs representing accessory DNA were checked for conservation in serial isolates.
The accessory genomes did not change much during chronic infection, but some cases of DNA loss or uptake could be monitored for most courses. The de novo assembled contigs also revealed differences between serial isolates and thereby confirmed that *P. aeruginosa* in CF airways has access to "foreign" DNA for horizontal gene transfer. Re-shaping of the accessory genome can occur during chronic infection and contributes to microevolution in this habitat.
Annotation of accessory DNA ORFs furthermore revealed some differences between isolates from mild and severe clinical courses. The proportions of functional classes differed for accessory genomes from mild and severe courses in general, but also for the subsets of ORFs affected by microevolution events. These variations affected with higher frequency genes for secreted factors and RNA processing in severe courses, in mild courses nucleotide metabolism genes instead.

Ecology, Evolution & Environment

P85

The rise and fall of an epidemic clone of *Pseudomonas aeruginosa*: 12 years of evolution during its spread in a hospital

Marie Petitjean[1,2], Paulo Juarez[2], Alexandre Meunier[1], Xavier Bertrand[1,2], Benoit Valot[2], Didier Hocquet[1,2]
[1]University Hospital, Besançon, France, [2]University of Bourgogne Franche-Comté, Besançon, France

Background. Although in-host evolution of *P. aeruginosa* is well documented, little is known about the evolution of the pathogen during its spread in a hospital. The international ST395 clone spread among more than 300 patients in the University Hospital of Besançon between 1997 and 2008. We used a comparative genomic approach to identify the origin of the outbreak, the features that could have helped its implantation in the hospital and those associated with the end of the epidemics.

Methods. The genomes of 54 isolates were sequenced by WGS. We built the phylogenetic tree, dated the Most Recent Common Ancestor, and identified chromosomal mutations. Resistance to imipenem and copper was confirmed phenotypically. The expression of *mexAB-oprM* efflux operon was assessed by RT-qPCR. LPS phenotype was determined by O-antigen serotyping.

Results. We identified a ST395-specific cluster of 6 copper transporters associated with the resistance to this heavy metal. The ancestor of the ST395 clone possibly contaminated our hospital during its construction in 1979, 5 years before its commissioning. Phylogenetic analysis revealed two parallel outbreaks. SNPs and phenotypic data showed that late isolates displayed independent genomic signatures of chronic adaptation in patients, resulting in altered LPS, mutated OprD porin, and extinction of MexAB-oprM overproduction.

Conclusion. We speculate that the ST395 epidemic clone contaminated the hospital water network during construction, possibly in relation with its resistance to copper. The end of the outbreak could be in part due to the emergence of independent epidemiological dead ends related to chronic adaptation in the patients.

Ecology, Evolution & Environment

P86

Evolution of *P. aeruginosa* rugose small-colony variants during chronic wound infections

Erin Gloag[1], Christopher Marshall[2], Vaughn Cooper[2], Daniel Wozniak[1]
[1]The Ohio State University, Columbus, OH, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA

Chronic wounds show delayed healing, attributed to the presence of biofilms, with *Pseudomonas aeruginosa* biofilms responsible for a number of such infections. Within the cystic fibrosis (CF) lung, *P. aeruginosa* evolves, forming pathoadapted variants that are more fit and persist. However, it is unclear whether *P. aeruginosa* similarly evolves in chronic wounds and the effect these variants may have on wound healing.

To determine if *P. aeruginosa* pathoadapts in response to chronic wounds we infected porcine burn wounds with wildtype *P. aeruginosa* and wound healing was monitored 7, 14 and 35 days post infection. Rugose small-colony variants (RSCVs) were isolated from punch biopsies at all timepoints, at a frequency of approximately 1% of the *P. aeruginosa* burden. Whole-genome sequencing revealed that RSCVs displayed mutations in the *wsp* system, as is seen for RSCVs from CF sputum and *in vitro* biofilms. When we infected porcine burn wounds with a *P. aeruginosa* mutant defective in biofilm formation we obtained small-colony variants at similar frequencies to wildtype infections. Whole-genome sequencing revealed these variants developed mutations in either O-antigen synthesis or type IV pili, which we predict lead to loss-of-function and hyper-piliation respectively.

Our data suggest that the selective pressures experienced by bacteria during lung or wound chronic infections are similar, leading to the evolution of similar variants. This suggests that therapies targeting variant sub-populations could be transferrable across infections where pathoadaptive variants evolve. We are currently exploring how *P. aeruginosa* variants impact chronic wound healing and advantages these variants possess in the wound.

Ecology, Evolution & Environment

P87

Contribution of increased mutagenesis to the evolution of pollutants-degrading bacteria

Tanel Ilmjärv, Ingrem Metsik, Eve Naanuri, Maia Kivisaar
Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia

Bacteria which are evolving to degrade environmental pollutants have to face strong selective pressure to survive in the presence of cell damaging effects of these compounds. It is known that bacterial population can be enriched with mutators (bacteria exhibiting elevated mutation rate) via several population bottlenecks occurring after strong selective challenges. However, mutational processes operating in natural populations of bacteria living in polluted environments are still unexplored. We have assayed for the presence of constitutive and transient mutators among 53 pollutants-degrading bacteria, many of them isolated from heavily contaminated environments. Only two strains expressed a moderate mutator phenotype and six were hypomutators, which implies that constitutively increased mutability has not been prevalent in the evolution of pollutants degrading bacteria. Nevertheless, a large proportion of the investigated strains exhibited a potential for the induced mutagenesis under the conditions of DNA damage due to the presence of error-prone DNA polymerases. A closer inspection of two Pseudomonas fluorescens strains PC20 and PC24 revealed that they harbour genes for ImuC (DnaE2) and more than one copy of genes for Pol V. However, mutation frequency increased in the presence of phenolic pollutants only in the laboratory-constructed P. putida strains carrying newly introduced phenol degradation genes but not in indigenous strains PC20 and PC24. Thus, mutagenicity of phenolic compounds could be affected by a factor of how long bacteria have evolved to use a particular pollutant as a carbon source. To test this idea, laboratory experimental evolution experiments are currently in progress.

Ecology, Evolution & Environment

P88

Biodiversity triggers the evolution of CRISPR resistance

Ellinor O Alseth, Ellie Pursey, Edze R Westra
University of Exeter, Penryn, Cornwall, UK

Due to *Pseudomonas aeruginosa*'s ability to rapidly evolve antibiotics resistance, there is a resurgence of interest in the therapeutic use of alternative antimicrobials, especially phage. However, many clinically relevant *P. aeruginosa* strains are known to possess CRISPR-Cas adaptive immune systems to combat phage infection. Here we report how the presence of other opportunistic pathogens that often co-infect with *P. aeruginosa*, namely *Staphylococcus aureus*, *Acinetobacter baumannii* and *Burkholderia cepacia*, triggers a major increase in CRISPR resistance evolution, which is virtually absent when P. aeruginosa is grown in monoculture. Our analyses demonstrate that biodiversity triggers evolution of CRISPR resistance and help to explain the apparent discrepancy between the frequently observed lack of CRISPR resistance evolution in the lab and its importance in nature.

Ecology, Evolution & Environment

P89

Just the Two of Us: Exploring a Potential Lineage of Pseudomonas Megaplasmids Through Genomics

Brian Smith, Courtney Leligdon, David Baltrus
University of Arizona, Tucson, AZ, USA

Bacteria can carry secondary replicons in the form of plasmids, megaplasmids, and chromids that vary in evolutionary rates and primary gene content. Further, these secondary replicons are known to contain genes coding for clinically relevant phenotypes like antibiotic resistance and virulence factors. We have previously characterized costs, like antibiotic sensitivity, associated with the Pseudomonas syringae megaplasmid pMPPlac107 (pMP). Here we find a Pseudomonas contig (Leaf58) isolated from an Arabidopsis leaf that shows similarities to pMP in synteny, sequence identity, gene content, and size. We use genomic comparisons to identify a second member of what may be a larger lineage of megaplasmids harbored by diverse Pseudomonads. We characterize sequence content and structure of both megaplasmids while utilizing comparative approaches to address sequence and functional similarities. Shared gene functions between the two megaplasmids include: nucleotide synthesis, recombination, and a suite of tRNAs. These megaplasmids have a similar core region surrounding the predicted origin of replication containing replication and conjugation associated genes. Despite these similarities, these genes are about 60% divergent. Furthermore, there is a 400kb cargo region in the pMP that does not match the Leaf58 contig and when deleted shows beneficial phenotypes during a 500-generation evolutionary experiment addressing amelioration of pMP in the foreign chromosomal background of P. stutzeri. With increasing sequencing and computational capabilities, it is likely large replicons will be found with increasing frequency in Pseudomonads. We report a new megaplasmid that shows resemblance to the previously identified pMP thus, creating a foundation to compare large replicons.

Ecology, Evolution & Environment

P90

Investigating the evolution of tRNA gene pools using the bacterial species *Pseudomonas fluorescens*

Zahra Khomarbaghi, Frederic Bertels, Jenna Gallie
Max Planck Institute for Evolutionary Biology, Plon, Germany

Due to their central role in protein synthesis, transfer RNA (tRNA) molecules are of fundamental importance to life. Interestingly, organisms show significant variation in both their intracellular tRNA pools and the tRNA genes that they carry. For example, the genome of *Escherichia coli* K12 encodes 86 tRNA genes, while that of *Saccharomyces cerevisiae* typically carries 286 tRNAs. We are interested in how and why these differences come to exist – what drives the evolution and divergence of tRNA gene pools? Here, we compare and contrast the tRNA gene pools of a representative sample of *Pseudomonas* strains with differing degrees of evolutionary divergence. We identify tRNAs present in all sampled genomes (core tRNAs) and those present in only a subset of genomes (accessory tRNAs). We are currently investigating the fitness effects of altering the accessory tRNA pool of individual *Pseudomonas fluorescens* genomes. Our results provide insight into the evolution of intracellular tRNA pools and our altered strains will provide a basis for future evolution experiments to study how genomes respond to modified tRNA pools.

Ecology, Evolution & Environment

P91

The interactome of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* from cystic fibrosis lung reveals a highly active *P. aeruginosa* transcriptome compared to that of *B. cenocepacia*

Jim Manos, Cali Willet, Edwina Buckle
University of Sydney, Sydney NSW Australia, Australia

Most cystic fibrosis patients succumb to *Pseudomonas aeruginosa* infection over several decades. However those infected with *Burkholderia cenocepacia* exhibit significantly reduced long-term survival. Little is known about co-infection effects on each species. Interactome analysis may help identify key interaction features.

Isolated colonies of clinical *P. aeruginosa* AES-1R and *B. cenocepacia* M2-1-67 were grown to OD_{600}=0.5 in TSB, and 100µl of a 1:100 dilution inoculated into 10ml artificial sputum medium for controls and incubated for 72hr. Co-cultures were first inoculated with M2.1.67, then inoculated with AES-1R after 24hr, before incubation for 48hr. Biofilm and RNA extraction was conducted as previously described. cDNA was synthesised and libraries prepared using OVATION (NuGen).

RNA-sequencing and EdgeR analysis showed AES-1R differentially-expressed 1346 genes in co-culture compared to monoculture while M2-1-67 differentially-expressed 151 genes ($p \leq 0.01$). Results are an average of biological triplicate sequences. 63.3% of AES-1R and 96.7% of M2-1-67 DE-genes were downregulated.

AES-1R upregulated biofilm formation gene *pelB*, suggesting enhanced biofilm formation, possibly as protection against M2-1-67; and 5-hydroxyisourate hydrolase, which hydrolyses 5-hydroxyisourate, the product of urate metabolism, thus protecting the bacterium from free radicals. Downregulated AES-1R genes included *hflK*, a negative regulator of proteolysis, and *ahcY*, involved in AHL biosynthesis. Upregulated M2-1-67 genes included *amiE*, a degradative enzyme that reduces AHL accumulation and thus quorum sensing. Downregulated genes included LPS-exporter *lptF*, indicating low LPS expression, and *yajC*, known to be downregulated by chitosan.

Overall, AES-1R differentially-expressed 9-fold more genes than M2-1-67. Both upregulated biofilm and protection genes, while down-regulating quorum sensing and virulence.

Ecology, Evolution & Environment

P92

VIM-positive *Pseudomonas aeruginosa* sink colonization dynamics in a single patient room in a Dutch tertiary care hospital

Jannette Pirzadian, Corné H. W. Klaassen, Margreet C. Vos, Juliette A. Severin
Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

Background: Since 2003, VIM-positive *Pseudomonas aeruginosa* (VPPA) circulates in our hospital, causing low-frequency but sustained outbreaks. Sinks act as persistent reservoirs. We aimed to retrospectively analyze sink colonization dynamics in one patient room between 2013-2017.

Methods: The room selected was a known "hotspot" for VPPA. For outbreak management, two actions were taken: the sink and sink environment were cultured starting in 2013, and patients admitted here were screened for VPPA on admission, at discharge, and weekly during hospitalization. Patient- and environment-derived isolates of VPPA were typed using multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA). Eight VNTRs were amplified and studied using capillary electrophoresis and BioNumerics software.

Results: Seven environmental and two patient isolates were analyzed. In January 2013, one identical clone (A) of VPPA was found in both the sink drain and siphon, and again in October 2013 from the drain. The drain was replaced and sink disinfected, eradicating the clone. In December 2013, a patient colonized by a different genotype (B) of VPPA was admitted. Environmental sampling between 2014-2015 revealed no VIM-positive *Pseudomonas*, and all other patients screened negative, but in 2016, the sink drain was found contaminated by clone B. In January 2017, a patient negative upon admission acquired clone B. An identical clone was found in the drain one week and again eight weeks later.

Conclusions: Replacing the drain eradicated clone A. We hypothesize that a patient with clone B re-colonized the sink, which formed a long-standing but low-level reservoir that disseminated the clone to another patient.

Ecology, Evolution & Environment

P93

Environmental and lichen-associated Pseudomonas syringae isolates from Iceland have plant pathogenic potential and display behavioural responses to plant matter

Auður Sigurbjörnsdóttir, Helena Sævarsdóttir, Oddur Vilhelmsson
University of Akureyri, Akureyri, Iceland

Bacteria of the genus Pseudomonas are found to be among the most competent bacteria to colonize plants and their immediate vicinity, e.g. the rhizosphere or phyllosphere. Certain species within the genus are known as plant pathogens, with Pseudomonas syringae being among the best-studied bacterial plant pathogens.
More than 270 bacterial strains of the Pseudomonas genus have been isolated from various environments in Iceland, including lichens and cold desert soils, and are stored at the Microbial Culture Collection at the University of Akureyri. Using a PCR-based screen for the type III secretion system (T3SS) component hrcC, we have found several of the isolates to possess a T3SS. A similar screen for the T3SS effector gene avrE indicated that several of these strains may have plant pathogenic potential. A further PCR screen using the P. syringae-specific Psy-PCR primers indicated that some of these strains should be considered as members of that species, even though 16S rRNA gene phylogenies indicated otherwise in some cases.
To explore the isolates' responses to plant matter, the effect of tomato leaves and potato plant extracts on growth, surface motility, biofilm formation and biosurfactanct production of the isolates were investigated. The results revealed that the presence of plant extracts enhances the biofilm formation of several Pseudomonas isolates and biosurfactants were only produced by some strains in the presence of plant extracts. Several of the strains tested for plant-dependent responses had the virulence gene that encodes important components of the type III secretion system, strongly suggesting their plant pathogenicity.

Ecology, Evolution & Environment

P94

Accelerating the evolution of plasmids: modular replicons from *Pseudomonas*

Leire Bardaji[1], Maite Añorga[1], José A. Ruiz-Masó[2], Gloria del Solar[2], Jesús Murillo[1]
[1]Departamento de Producción Agraria, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Pública de Navarra, Pamplona, Spain, [2]Molecular Biology of Gram-Positive Bacteria, Molecular Microbiology and Infection Biology, Centro de Investigaciones Biológicas (Consejo Superior de Investigaciones Científicas), Madrid, Spain

Plasmids are the main vehicle for horizontal gene transfer. Their autonomous replication is dependent on their replicon, which also defines copy number, host range and incompatibility with other plasmids. The replicon from the virulence plasmid pPsv48C from *Pseudomonas syringae* pv. savastanoi NCPPB 3335 consists of the replication initiator protein gene (*repA*) preceded by a putative replication control region spanning the promoter, a putative antisense RNA and a leader peptide. We identified a second non-homologous replicon in pPsv48C that is a chimera, containing a nearly identical control region. Blast comparisons and functional assays showed that this type of chimerism is common in at least four non-homologous replicon families from species of *Pseudomonas*, including environmental bacteria as well as plant, animal and human pathogens. Gene swapping showed that these replicons consist of two functionally separable modules corresponding to the control (REx-C module) and replication (REx-R module) regions. Only the REx-C module displayed strong incompatibility, which is overcome by a few nucleotide changes clustered in a stem-and-loop structure of the putative antisense RNA. Additionally, a REx-C module from pPsv48C conferred replication ability to a non-replicative chromosomal DNA region containing features associated to replicons. Thus, the organization of plasmid replicons as independent and exchangeable functional modules is likely facilitating rapid replicon evolution, fostering their diversification and survival, besides allowing the potential co-option of appropriate genes into novel replicons and the artificial construction of new replicon specificities.

Ecology, Evolution & Environment

P95

Investigating the functional importance of biased codon usage in *Pseudomonas fluorescens*

Anuradha Mukherjee, Frederic Bertels, Jenna Gallie
Max Planck Institute for Evolutionary Biology, Plön, Schleswig-Holstein, Germany

Biological organisms have 61 DNA codons that code for only 20 amino acids. Therefore the genetic code is redundant – some amino acids are coded for by more than one codon (a codon "set"). Importantly, usage of codons is biased towards certain codons within a set. Although codon bias is universally observed, the functional importance of preferring one codon over another is poorly understood. Here, we investigate the consequences of changing codon usage in *Pseudomonas fluorescens.* Our laboratory uses three *P. fluorescens* strains that show various differences in codon usage. Using bioinformatics techniques, we have identified a number of genes that are identical with respect to non-synonymous codon use across three *P. fluorescens* strains but exhibit different patterns of synonymous codon usage.

We show how codon usage in each gene correlates with the codon usage patterns and tRNA availability in each genome and are currently investigating how swapping genes in non-native genomic backgrounds influences phenotype. Our results demonstrate the importance of the interaction between synonymous codon usage patterns in individual genes with respect to their genome. Finally, our engineered strains will allow us to explore the evolutionary response to altering established codon usage patterns at the level of individual genes.

Ecology, Evolution & Environment

P96

Fitness effects of the GraTA toxin-antitoxin system in *Pseudomonas putida*

Rita Hõrak[1], Hedvig Tamman[1,2], Andres Ainelo[1], Sirli Luup[1], Jaanus Remme[1]
[1]Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia, [2]Biologie Structurale et Biophysique, Université Libre de Bruxelles (ULB), Gosselies, Belgium

Bacterial genomes contain many copies of potentially poisonous gene pairs encoding a toxin and its antagonist antitoxin. Several roles, mostly associated with stress tolerance, have been assigned to these toxic loci. Yet, the conflicting results from different studies indicate the actual biological importance of genomic TA loci is still unclear.
The growth-rate-affecting GraTA system is the first well-characterized TA system in pseudomonads. We have shown that GraTA encodes the toxin GraT, which is unusually mild at higher temperatures but causes a severe growth defect at lower temperatures (1). Both functional and structural studies indicate that GraT is, similar to its closest homologue HigB, a ribosome-dependent mRNase. However, differently from other mRNase toxins, it seems to have special target(s), as it inhibits ribosome biogenesis (2) and cell central metabolism. Interestingly, the toxicity of GraT involves binding to DnaK chaperone (2). GraT can influence the stress tolerance, but the GraT-mediated stress protection is costly resulting in fitness trade-offs between increasing and reducing the stress tolerance in different environments. Despite the ability of GraT to affect stress tolerance, the benefit of GraTA is still vague, as deletion of the whole system does not affect the fitness of *P. putida*. We are currently testing whether the GraTA system may act in synergy with other genomic TA systems of *P. putida*.

1. Tamman, Ainelo, Ainsaar, Hõrak. A Moderate Toxin, GraT, Modulates Growth Rate and Stress Tolerance of *Pseudomonas putida*. J Bacteriol. 2014;196(1):157-69.
2. Ainelo, Tamman, Leppik, Remme, Hõrak. The toxin GraT inhibits ribosome biogenesis. Mol Microbiol. 2016;100(4):719-34.

Ecology, Evolution & Environment

P97

Within-host evolution of multidrug resistant Pseudomonas aeruginosa in non-CF patients

Paula Ruiz-Hueso[1,2], Nuria Tormo-Palop[3], Concepción Gimeno-Cardona[3], Fernando González-Candelas[1,2]
[1]FISABIO-Universidad de Valencia, Valencia, Spain, [2]CIBERESP, Valencia, Spain, [3]Hospital General Universitario de Valencia, Valencia, Spain

Background
The in vivo evolution Pseudomonas aeruginosa has been studied mainly in the lung environment of cystic fibrosis patients. However, P. aeruginosa also causes complicated infections in different body locations in patients of all types. As a bacterium with a highly variable genome, it is important to describe how it evolves and adapts inside other types of patients.

Methods
We studied 92 samples taken at different times (average=197 days, range 9-556) and/or body locations from 15 patients (2-17 samples/patient). Isolates were sequenced with Illumina MiSeq 2x300bp. SNPs and indels in the core genome were obtained by mapping against PA01 with BWA and variant calling with Samtools. A phylogeny was obtained with RAxML with the constructed consensus genomes. In addition, each genome was assembled with SPAdes, resistance genetic profiles were obtained with ARIBA and MLST typing was done with SRST2.

Results
Most (80%) samples belonged to ST244. No identical sequences were obtained. The phylogeny indicates possible co-infection or re-infection in at least 5 patients, as the corresponding genomes conform polyphyletic groups. Within ST244, only one patient was clearly monophyletic. We observed an average of 15.3 SNPs within patient (range 2.7 - 95.0) and an average of 28.12 SNPs between ST244 isolates. We detected 57 different genes responsible for antibiotic resistance, mainly efflux pump genes with mutations, which could explain the wide variety of resistance patterns detected with phenotypic tests.

Conclusions
P. aeruginosa accumulates differences very rapidly within the infected patient and these can be colonized by divergent strains, thus facilitating the spread of resistance mutations.

Ecology, Evolution & Environment

P98

Mixed species biofilm provides associational resistance to predation by protists

Siew Herng Chan[1], Henriette L. Røder[2], Jonas S. Madsen[2], Kai Wei Kelvin Lee[1], Mette Burmølle[2], Søren J. Sørensen[2], Scott A. Rice[1,3], Diane McDougald[1,3]
[1]Nanyang Technological University, Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore, [2]University of Copenhagen, Copenhagen, Denmark, [3]University of Technology Sydney, ithree Institute, Sydney, NSW, Australia

Mixed species biofilms exhibit increased tolerance to a number of stresses compared to single species biofilms. Here we examined effects of grazing by the heterotrophic protist, *Tetrahymena pyriformis*, on a mixed species biofilm consisting of *Pseudomonas aeruginosa*, *Pseudomonas protegens* and *Klebsiella pneumoniae*. Spatial organization and quantification of fluorescently tagged strains within the biofilm was determined by confocal microscopy. Protozoan grazing significantly reduced the single species *K. pneumoniae* biofilm, and while the single species *P. protegens* biofilm also displayed sensitivity to grazing, the biofilm biovolume was not significantly affected. Unlike *K. pneumoniae* and *P. protegens* biofilms, *P. aeruginosa* biofilms exhibited grazing resistance to predation by *T. pyriformis*. Additionally, mixed species biofilms containing *P. aeruginosa* also displayed grazing resistance, indicating that the sensitive strains gained associational resistance to predation by *T. pyriformis*. Rhamnolipids produced by *P. aeruginosa* were shown to be toxic for the protist. However, a rhamnolipid-defective mutant strain of *P. aeruginosa* maintained grazing resistance, which suggests that predation protection is a multifactorial phenotype. A single species biofilm of a mutant of *P. aeruginosa* lacking rhamnolipids and the type III secretion system was grazing resistant. However, the overall grazing tolerance of the biofilm was reduced when the single and double knockout mutants of *P. aeruginosa* were introduced to the mixed species biofilm, suggesting that both play partial roles in predation resistance. This study demonstrates that residing in a mixed species biofilm can be an advantageous strategy for grazing sensitive bacterial species, as *P. aeruginosa* protects the whole community from protozoan grazing.

Ecology, Evolution & Environment

P99

Social antibiotic resistance in chronic *Pseudomonas aeruginosa* infections in cystic fibrosis

David Crosse, Melanie Ghoul, Ashleigh Griffin
Department of Zoology, University of Oxford, Oxford, UK

Antibiotic resistance is a global health crisis. As antibiotic resistance mechanisms in pathogenic bacteria can be social, social evolutionary theory needs to be applied to this problem. Secreted antibiotic degradation enzymes, such as beta-lactamase (which breaks down penicillins and related antibiotics), are public goods: cooperative producers could therefore be vulnerable to exploitation by selfish, low enzyme-producing cheats. We ask if low-producers can exploit resistant high-producers using laboratory strains of Pseudomonas aeruginosa and natural strains from chronic infections of cystic fibrosis lungs.

Using a chromogenic assay, we measure beta-lactamase production in 219 isolates collected over ten years from 17 cystic fibrosis patients. This shows that (i) there is often considerable extracellular beta-lactamase activity and (ii) that high and low producers of beta-lactamase co-exist in chronic infections. We then show that supernatant of high producers can protect the less-resistant low-producers from antibiotics. A competition assay between high-producing clinical isolates and a beta-lactamase knockout strain shows that the antibiotic-sensitive knockout can survive in normally lethal concentrations of antibiotic when mixed with resistant high-producers.

Our work shows that social interactions may be important in understanding the evolution of antibiotic resistance; indeed, the maintenance in the population of low enzyme-producers (which are themselves more vulnerable to antibiotics) could be of considerable clinical significance.

Ecology, Evolution & Environment

P100

Persistent bacterial infections are based on antibiotic resistance: Fact or Fiction?

Lea Sommer[1], Janus Haagensen[2], Jennifer Bartell[2], Søren Molin[2], Helle Johansen[1]
[1]Rigshospitalet, Department of Clinical Microbiology, Copenhagen, Denmark, [2]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark

Genomics is an important tool to understand the adaptation and evolution of bacterial populations, including the development of antibiotic resistance.

The term: ”Resistome” is widely used to describe the linkage of genotype and antibiotic resistance phenotype, with the links comprising: specific genes, mutations, and mutation combinations. However, in many infection studies, these results are based purely on the manipulation and analysis of laboratory strains, or horizontal transfer of genes from natural populations into laboratory strains. This is due to limitations in evolution-relevant collections of bacterial lineages.

Employing a collection of about 500 Pseudomonas aeruginosa isolates, covering 1-10 years of persistent colonisation and infection of more than 30 cystic fibrosis patients, we have a unique and high-resolution window into pathogen adaptation.

Using phenotypic tests and whole genome sequencing we sought to find phenotype-genotype links. Analysis with linear-mixed models and experimental assays suggests: 1) no overall correlation between the length of infection and the development of antibiotic resistance, 2) genes (e.g. mexZ) correlated with antibiotic resistance in the laboratory do not reliably induce antibiotic resistance in clinical isolates.

Despite intensive antibiotic therapy, we see no uniform phenotypic evolution of antibiotic resistance in CF patients, but we do se the expected genotypic evolution of resistance associated genes. Using this collection of isolates and linked patient information, we find that the correlation of phenotype-genotype is much more complex than one-gene-one-function and that the ability for a pathogen to induce a persistent bacterial infection is far more complex than solely its acquisition of antibiotic resistance.

Ecology, Evolution & Environment

P101

Mapping metabolic adaptation of *Pseudomonas aeruginosa* in cystic fibrosis airways using computational systems modeling

Jennifer Bartell, Janus Haagensen, Helle Krogh Johansen, Søren Molin
Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark

Understanding the relationship between genotype and phenotype is a critical step towards the use of genomics and predictive modeling in patient treatment. Here, we systematically map this relationship during the adaptation of 36 lineages of *Pseudomonas aeruginosa* in a population of young cystic fibrosis patients. We develop isolate-specific computational metabolic network models by deriving reaction constraints from mutations accumulating in 541 metabolic genes distributed across 457 clinical isolates. We evaluate connections between clonal lineages, networks of genetic mutation, and predicted phenotypic profiles, identifying 262 unique metabolic networks linked to 94 unique phenotypic profiles within our isolate collection. Clustering datasets by these profiles shows that while some lineages have unique adaptive profiles, others share similar trajectories despite diverse genetic backgrounds. Overall, 192 networks predict inhibited growth, the most common altered phenotype. By iteratively removing constraints associated with each mutation in an isolate-specific model and predicting the resulting growth recovery, we show that mutations in essential genes appear to be the main drivers of slowed growth in many of our isolates despite other accumulating mutations. We contextualize these predictions of driver mutations with an array of *in vitro* phenotype screens, filtering targeted genes by prediction accuracy, lineage distribution, and potential broader impact of the mutations suggested by other studies. Using an iterative cycle of prediction and validation, we are both identifying promising driver mutations and refining our base network model. Our results regarding inhibited growth support a reconsideration of the true objective of adaptation in these strains.

Ecology, Evolution & Environment

P102

Activation of the MexXY-OprM efflux pump has little impact on aminoglycoside resistance

Jakob Frimodt-Møller[1], Søren Molin[1], Helle Krogh Johansen[1,2]
[1]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark, [2]Dept. of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Recent work describes the frequent occurrence of patho-adaptive mutations in a small number (52) of genes thought to optimize *Pseudomonas aeruginosa* (PA) fitness during adaptation within cystic fibrosis (CF) patients; with the mexZ mutations found to be the most frequent. MexZ is a negative regulator of the *mexXY* genes, which together with oprM encode the MexXY-OprM efflux pump.

Data from 184 clinical PA mexZ mutants have shown that a *mexZ* mutation does not result in resistance to any of the tested antibiotics, including the MexXY-OprM substrates ciprofloxacin and aminoglycosides. The clinical mexZ mutations are located randomly throughout the gene, suggesting that no mutational hot-spot exists. To understand the phenotypic trait(s) resulting in the increased adaptation of *mexZ* mutants to CF lungs, the phenotypic consequences of deleting the *mexZ* gene were investigated in the laboratory strains, PAO1 and PA14. MexZ deficient PAO1 and PA14 cells are susceptible to aminoglycosides and ciprofloxacin. From trancriptomic analysis by RNA-Seq the deletion of *mexZ* only resulted in a decrease in *mexZ* expression and the expected increase in *mexXY* expression. In addition, *mexZ* deficient bacteria have the same catabolic profile (Biolog) as wild-type bacteria. However, when mexZ deficient bacteria compete against wild-type bacteria in the presence of sub-MIC of amikacin, tobramycin or ciprofloxacin, the *mexZ* deficient bacteria outcompete the wild-type bacteria.

These results suggest that *mexZ* deficient bacteria have a fitness advantage under specific selective sub-MIC growth conditions, which probably are commonly found in the lungs of antibiotic-treated CF patients.

Ecology, Evolution & Environment

P103

Pseudomonas aeruginosa persisters in patients with cystic fibrosis

Biljana Mojsoska[1], David Cameron[3], Janus Haagensen[2], Jennifer Bartell[2], Kim Lewis[3], Søren Molin[2], Helle Krogh Johansen[1]
[1]Rigshospitalet Clinical Microbiology Department, Copenhagen, Denmark, [2]The Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark, [3]Northeastern University, Boston, USA

Antibiotic susceptible bacteria surviving antibiotic treatment (persisters) are believed to contribute to the difficulties in treatment of many infections.
We have investigated a collection of 583 Pseudomonas aeruginosa (P. a) isolates systematically obtained over a period of more than 10 years from the Cystic Fibrosis clinic in Copenhagen. The fraction of persister isolates was determined by high-throughput screening of surviving isolates in stationary phase after treatment with high concentrations of Ciprofloxacin. Phenotypic observations with respect to the growth rate, lag time, biofilm forming capacity along with length of infection etc, are some of the descriptors that we have used to correlate the persistence phenomenon in these isolates. In addition, whole genome sequencing data was used to generate various data on frequency of presence of genes with impact mutations within the persister population.
Our objective is to understand why antibiotic treatment fails in CF patients infected with P.a. We have looked at the early stages of infection and based on the MIC values of all the isolates, antibiotics fail to fully eradicate the bacteria and no or marginal antibiotic resistance development is seen. We have found that approximately 25 % of the bacteria show a persister phenotype with respect to Ciprofloxacin treatment in vitro. The persister isolates display significant slower growth and extended lag times compared to non-persisters. Mutation frequencies from the genomic data reveal increased impact mutations in 37 genes.
The quantitative characterization of the persisters is providing insight into the failure of treatment of P. a infections in CF patients.

Ecology, Evolution & Environment

P104

Applying an *in vitro* model for investigations of bacterial adaptation to the airways of cystic fibrosis patients

Janus Haagensen[1], Katherine Yang[4], Alfred Spormann[3], Helle Krogh Johansen[1,2], Søren Molin[1]
[1]Novo Nordisk Foundation Center for Biosustainability, DTU, Lyngby, Denmark, [2]Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark, [3]Department of Civil and Environmental Engineering, Stanford University, Stanford, USA, [4]Department of Clinical Pharmacy, University of California San Francisco School of Pharmacy, San Francisco, USA

In patients with cystic fibrosis (CF), *Pseudomonas aeruginosa* is a dominating airway colonizer. Longitudinal investigations of bacterial isolates from the patients have shown that long-term infection by *P. aeruginosa* is associated with adaptive evolution of the bacteria, suggesting a gradual increase in their fitness properties. However, assessments of bacterial fitness cannot be performed directly in the environment from which the bacteria are derived (the patients), and we are therefore applying an *in vitro* model micro-cosmos mimicking the airway environment with a strong emphasis on the antibiotic selection pressure and the structured heterogeneity of the lung environment.

The principle is a pharmaco-kinetic/pharmaco-dynamic model system based on bacterial biofilms grown under continuous culture conditions, simulating the changing antibiotic concentrations in CF patients during intravenous dosing. The antibiotics will decay in a way similarly to what takes place in CF patients during treatment.

Our investigations using this model system will focus on determinations of fitness, the spatial distribution of bacterial sub-populations, and their persistence when exposed to relevant antibiotics. In particular we design competition experiments in which sub-lineages isolated from the patients are differentially tagged and introduced in the model system under conditions of treatment and host reactions. In later applications we will employ the model system in investigations of gene expression and metabolism in relation to the processes of adaptation and evolution in the patients.

Ecology, Evolution & Environment

P105

Persistence of *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients

Helle Krogh Johansen[1,2], Ruggero La Rosa[3], Lea Sommer[1,3], Janus Haagensen[3], Jakob Frimodt-Møller[3], Elio Rossi[1,3], Jennifer Bartell[3], Biljana Mojsoska[1,3], David Cameron[4], Søren Molin[3]
[1]Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark, [2]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [3]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark, [4]Northeastern University, Boston, USA

Most cystic fibrosis (CF) patients are colonised in their lungs with environmental *P. aeruginosa* that have a unique genotype, are susceptible to antibiotics and grow fast. The current principle for antibiotic treatment in the CF clinic is prevention or delay of chronic infection.

Based on a large collection of more than 500 genome sequenced clinical *P. aeruginosa* isolates from 41 children and young CF patients we found that most patients maintained the same clone type from the beginning of the infection and up to 10 years after first colonisation. Most of the isolates are susceptible to the antipseudomonal antibiotics that are being used in the clinic, in vitro biofilm capability does not change over time when measured in micro titre plates, and only a small fraction of bacteria has the persister phenotype. Bioinformatically it was found that some genes such as those associated with biofilm formation (loss of function), mucoidity and antibiotic resistance were among the most frequently mutated genes. Conventional susceptibility testing in the laboratory, however, do not detect these genetically resistant variants, and therefore continued treatment results in later appearance of truly resistant populations of *P. aeruginosa* bacteria. Further, the persister phenotype, which constitutes approximately 25% of the collection, is not detected by the methods employed in the clinical microbiology laboratory.

Persistence of *P. aeruginosa* lung infection cannot entirely be explained by antibiotic resistance, biofilm formation or the persister phenotype, but might be associated with a small number of pathoadaptive mutations that seem to be especially important.

Ecology, Evolution & Environment

P106

Detoxification of the Virulence Factor 2-Heptyl-4-hydroxyquinoline *N*-oxide by Environmental and Pathogenic Bacteria

Sven Thierbach[1], Franziska S. Birmes[1], Matthias C. Letzel[2], Ulrich Hennecke[2], Susanne Fetzner[1]
[1]Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany, [2]Organic Chemistry Institute, University of Münster, Münster, Germany

The opportunistic pathogen *Pseudomonas aeruginosa* produces an arsenal of extracellular metabolites and enzymes, which helps to conquer new habitats and to outcompete other microorganisms. One of the major secondary metabolites is 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), a virulence factor that inhibits respiratory electron transfer of host cells as well as microorganisms coexisting with *P. aeruginosa*. We investigated environmental and pathogenic bacteria for their capability to detoxify this compound. HPLC, MS and NMR analyses of biotransformation assays revealed a hydroxylation at C-3 of HQNO by strains of *Arthrobacter* and *Rhodococcus* spp. as well as *Staphylococcus aureus*. *Mycobacterium abscessus*, *M. fortuitum* and *M. smegmatis* methylated the *N*-oxide group of HQNO, forming 2-heptyl-1-methoxy-4-oxoquinoline as initial metabolite, whereas *Bacillus* spp. performed an initial *O*-glycosylation, leading to 2-heptyl-1-(*β*-D-glucopyranosydyl)-4-oxoquinoline. *In vivo* and *in vitro* studies assaying cellular respiration and quinol oxidase activity of membrane fractions demonstrated a reduced inhibitory effect of these new metabolites, as their EC_{50} values were up to two orders of magnitude higher than that of HQNO. Furthermore, testing HQNO and the metabolites for *in vivo* induction of oxidative stress revealed that cellular levels of reactive oxygen species were significantly lower in presence of the metabolites than under influence of HQNO. Therefore, the capability of microorganisms to transform HQNO can be assumed to be advantageous when competing with *P. aeruginosa*. Our results contribute new insight into the complexity of metabolic interactions within polymicrobial communities comprising *P. aeruginosa*.

Ecology, Evolution & Environment

P107

Molecular mechanism of *Pseudomonas aeruginosa* superinfection by the filamentous phage Pf4

Scott A. Rice[1,2], Muhammad Hafiz Bin Ismail[1], Staffan Kjelleberg[1,2], Diane McDougald[1,3]
[1]The Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, singapore, Singapore, [2]The School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, [3]The ithree Institute, The University of Technology Sydney, Sydney, Australia

The Pf4 filamentous prophage of *Pseudomonas aeruginosa* plays a significant role in biofilm development and virulence and drives small colony variant formation during biofilm formation. Many of these phenotypes have been linked to the appearance of a superinfective form of the Pf4 phage that kills the host. Production of the phage also confers a competitive advantage over a naive host.

Here, we have investigated the mechanism the leads the conversion of the Pf4 phage from the wild-type form that can not reinfect its prophage carrying host into a genetic variant that can kill its host. The generation of the superinfective phage is linked to the accumulation of specific mutations only at a hot-spot in the phage genome. The accumulation of these mutations are associated with increased oxidative stress and functional DNA repair systems. This genetic region has homology to phage repressor proteins. The Pf4 repressor protein confers immunity to the prophage carrying host and mutations in the promoter region of this gene are also linked to the superinfective phenotype. ChipSeq experiments demonstrated that the immunity protein binds to a number of genes outside of the prophage, indicating that it can regulate host genes as well as its own. These data have defined the mechanism of superinfection in filamentous phage of *P. aeruginosa* and also demonstrate that phage can modulate adaptive behaviours of their host, including biofilm formation and virulence, by exerting regulatory control over the host. This highlights that the phage-host relationship is considerably more complex than is commonly considered.

Ecology, Evolution & Environment

P108

Parasitic growth of *Pseudomonas aeruginosa* in co-culture with a chitin-degrading bacterium reveals novel metabolic control on quorum-sensing regulated virulence factor production

Nina Jagmann, Bodo Philipp
University of Münster, Institute for Molecular Microbiology and Biotechnology, D-48149 Münster, Germany

Pseudomonas aeruginosa uses its infection-related and quorum sensing (QS)-regulated virulence factors also for competing with other bacteria. Previously, we established a co-culture, in which *P. aeruginosa* exploits chitin-degradation by *Aeromonas hydrophila* in a parasitic way [1]. Parasitic growth is strictly dependent on pyocyanin and initiated by induction of a QS-response at a relatively low cell density. We employed this co-culture system for searching for novel mechanisms that could be involved in inducing a QS-response by sited-directed and by transposon mutagenesis of *P. aeruginosa*. Inactivating the stringent response by deleting *relA spoT* completely prohibited pyocyanin in the co-culture. Screening of 3,500 transposon mutants revealed that deletion of *gbuA*, which encodes a guanidinobutyrase, led to complete loss of pyocyanin production in co-cultures and to a reduced pyocyanin production in single cultures [2]. Physiological analysis suggested that accumulation of the GbuA substrate 4-guanidinobutyrate caused this effect. Transcription of the *pqsABCDE* operon was reduced in the *gbuA* mutant. Pyocyanin production was restored by adding alkylquinolone signal molecules and by PqsE overexpression.
These results identify starvation and a non-anticipated metabolic reaction as important for inducing QS at low cell density and show the value of bacterial co-cultures for identifying novel influences on virulence.

[1] Jagmann et al. (2010) Parasitic growth of *Pseudomonas aeruginosa* in co-culture with the chitinolytic bacterium *Aeromonas hydrophila*, Environ Microbiol 12: 1787-1802
[2] Jagmann et al. (2016) The guanidinobutyrase GbuA is essential for the alkylquinolone-regulated pyocyanin production during parasitic growth of *Pseudomonas aeruginosa* in co-culture with *Aeromonas hydrophila*, Environ Microbiol 18: 3550-3564

Ecology, Evolution & Environment

P109

Pseudouridine synthases TruA and RluA affect mutation frequency in *Pseudomonas putida*

Mari Tagel, Heili Ilves, Margus Leppik, Jaanus Remme, Maia Kivisaar
Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia

To understand bacterial evolution, it is essential to know the mechanisms underlying mutational processes. Mutation frequency is commonly considered as the equilibrium between replication error rate and repair pathway enzymes' activity. In our recent study we constructed and described a papillation-based assay which enables detection of changes in mutation frequency on a single colony level in bacteria from the genus *Pseudomonas* (Tagel, *et al*. 2016). In a genome-wide screen of *Pseudomonas putida* we identified numerous genes not yet associated with mutation frequency, amongst them one with highest mutation frequency was *truA*, encoding a tRNA modification enzyme. TruA catalyses the isomerization of uridine to pseudouridine in positions 38-40 of tRNA, whereas position 32 is pseudouridylated by the modification enzyme RluA. tRNAs are the most abundantly modified nucleic acids in the cell. tRNAs have numerous interaction partners crucial for their functionality and this may be the reason for the complex set of modifications. The most heavily modified are the anticodon and its close proximity - the anticodon stem-loop (ASL). We demonstrate that deficiency of either TruA or RluA causes at least fourfold rise in genomic mutation frequency in *P. putida*. We also show that this is specifically caused by the lack of pseudouridines, as mutating the catalytic aspartic acids to alanines causes the same phenotype as deleting whole genes. This enables us to hypothesize that pseudouridines in tRNA ASL affect mutation frequency via a yet unknown mechanism.

Tagel M, Tavita K, Hõrak R, Kivisaar M, Ilves H (2016). Mutation Research, 790, 41-55

Ecology, Evolution & Environment

P110

Isolation, characterization and selection of *Pseudomonas* spp. as biological control agents from a suppressive soil

CARMEN VIDA, SANDRA TIENDA, ANTONIO DE VICENTE, FRANCISCO M. CAZORLA
Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM-UMA-CSIC). DEPARTAMENTO DE MICROBIOLOGIA, UNIVERSIDAD DE MALAGA., MALAGA, Spain

Bacterial profile from a suppressive soil against *Rosellinia necatrix*, fungal pathogen of avocado roots, were obtained by 16S rRNA gene sequencing. The results revealed a significant increase in the bacterial class of *Gammaproteobacteria*, especially in some antagonistic representatives of *Pseudomonas* spp. For this reason, a collection of 246 bacterial isolates was obtained from this suppressive soil in order to identify new strains with antifungal activity against this fungal phytopathogen. First, we performed an isolation on a selective medium for *Pseudomonas*-like microorganisms. Then, we used different characterization tests in order to analyse the bacterial collection, including the identification of the general metabolic profile of glucose, the profiling of antifungals produced, both the putative production of antifungal compounds and lytic exoenzymes, as well as the evaluation of traits related with beneficial effects on plants. A final selection of representative strains resulted in antifungal isolates belonging to the genus *Pseudomonas* and related groups. These selected strains were tested for plant protection by an *in vivo* experiment using avocado and wheat plants challenged by the pathogen *R. necatrix*, showing all of them an antifungal ability and plant disease protection.

This work was supported by Plan Nacional I+D+I from MINECO (Spain) (AGL2014-52518-C2-IR) and co-financed by FEDER funds (EU). C.Vida was supported by a PhD fellowship from the FPI program of MINECO.

We would like to thank Irene Linares Rueda for her support as Laboratory technician of the group of Microbiology and Plant Pathology of the University of Malaga.

Ecology, Evolution & Environment

P111

Genomic analysis of eight native plasmids of the phytopathogen *Pseudomonas syringae*

Jose Antonio Gutiérrez Barranquero[1], Francisco Cazorla López[1], Antonio de Vicente Moreno[1], George W. Sundin[2]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Malaga, Spain, [2]2Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, Michigan, USA

The pPT23A family of plasmids (PFPs) appears to be indigenous to the plant pathogen *Pseudomonas syringae* and these plasmids are widely distributed and widely transferred among pathovars of *P. syringae* and related species. PFPs are sources of accessory genes for their hosts that can include genes important for virulence and epiphytic colonization of plant leaf surfaces. Further understanding of the evolution of the pPT23A plasmid family and the role of these plasmids in *P. syringae* biology and pathogenesis, requires the determination and analysis of additional complete, closed plasmid genome sequences. Therefore, our main objective was to obtain complete genome sequences from PFPs from three different *P. syringae* pathovars and perform a comparative genomic analysis. In this work plasmid DNA isolation, purification by CsCl-EtBr gradients, and sequencing using 454 platform, were used to obtain the complete sequence of *P. syringae* plasmids. Different bioinformatic tools were used to analyze the plasmid synteny, to identify virulence genes (*i.e.* type 3 effectors) and to unravel the evolutionary history of PFPs. Our sequence analysis revealed that PFPs from *P. syringae* encode suites of accessory genes that are selected at different levels (universal, interpathovar and intrapathovar). The conservation of type IVSS encoding conjugation functions also contributes to the distribution of these plasmids within *P. syringae* populations. Thus, this study contributes to unravel the genetic basis of the role of PFPs in different *P. syringae* lifestyles.

Ecology, Evolution & Environment

P112

Functional role of lanthanides in enzymatic activity and transcriptional regulation of PQQ-dependent alcohol dehydrogenases in *Pseudomonas putida* KT2440

Matthias Wehrmann[1], Patrick Billard[2], Audrey Martin-Meriadec[2], Asfaw Zegeye[2], Janosch Klebensberger[1]
[1]*University of Stuttgart, Institute of Technical Biochemistry, Stuttgart, Germany,*
[2]*Université de Lorraine, LIEC - CNRS UMR7360, Nancy, France*

Background: To efficiently capture and detoxify various biogenic volatile organic compounds (VOCs), many Gram-negative organisms have evolved a periplasmic oxidation system based on pyrroloquinoline quinone-dependent alcohol dehydrogenases (PQQ-ADHs). The present study was conducted to gain insight into the physiological role of the two PQQ-ADHs PedE and PedH, which are encoded in the genome of *P. putida* KT2440.

Methods: The metal cofactor dependency, substrate range and kinetic parameters were determined using purified enzymes of PedE and PedH and a colorimetric activity assay. Growth phenotypes and *pedE* and *pedH* promoter activity studies with various mutant strains under conditions of varying lanthanum availabilities were performed.

Results: The present study reports the first description of a lanthanide-dependent PQQ-ADH (PedH) in a non-methylotrophic organism. PedH exhibits enzyme activity on a similar substrate range as its Ca^{2+}-dependent counterpart PedE, however, only in the presence of different lanthanide ions. We show that for efficient growth with many VOCs, the functional production of at least one of the PQQ-ADHs is essential and that conditions exist in which both enzymes can be functionally expressed. Transcriptional reporter assays further reveal that PedH not only has a catalytic function, but is also involved in the inverse transcriptional regulation of *pedE* and *pedH* in response to lanthanide availability in a concentration dependent manner.

Conclusion: From our results we propose that functional redundancy and inverse regulation of PedE and PedH represents an adaptive strategy of *P. putida* KT2440 to optimize growth with alcoholic VOCs in response to different lanthanide availabilities in natural environments.

Ecology, Evolution & Environment

P113

Molecular characterization and phylogenetic analysis of aquatic *Pseudomonas aeruginosa* isolates from Greece implementing the Double Locus Sequence Typing (DLST) scheme

Olga Pappa[1,2], Apostolos Beloukas[3,1], Anastasia-Maria Kefala[5], Apostolos Vantarakis[4], Athena Mavridou[1], Alexis Galanis[5]
[1]Department of Medical Laboratories, Athens University of Applied Science, Athens, Greece, [2]Central Public Health Laboratory, Hellenic Centre for Disease Control and Prevention, Athens, Greece, [3]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [4]Environmental Microbiology Unit, Department of Public Health, School of Medicine, Patra, Greece, [5]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupoli, Greece

Background: Pseudomonas *aeruginosa* has been proposed to be a potential reservoir of resistance genes in a variety of aquatic habitats such as swimming pools, water tanks, main waters, freshwaters, and waste-waters. Understanding the resistant's genetic structure of environmental *P. aeruginosa* isolates is of significant importance in order to get deep insight into the complexity and ecological versatility of this opportunistic pathogen.

Methods: The recently described Double Locus Sequence Typing (DLST) scheme implemented to deeply characterize the genetic profiles of 52 environmental *P. aeruginosa* isolates with both intrinsic and acquired resistant mechanisms, derived from several aquatic habitats across Greece. OprD locus was additionally assessed in order to increase the discriminatory power of the molecular typing.

Results: DLST scheme was able not only to assign an already known allelic profile to the majority of the isolates but also to recognize two new ones (ms217-190, ms217-191) with high discriminatory power. Furthermore, oprD found to be fundamental for the molecular typing of environmental isolates given the resulted increased discrimination between the isolates. Finally, the acquisition of resistant mechanisms found to be aligned to their divergent genetic profiles

Conclusions: We observed that the combination of the DLST to oprD-typing can discriminate phenotypically and genetically related environmental *P. aeruginosa* isolates from different aquatic sites providing reliable phylogenetic analysis at a regional level.

Ecology, Evolution & Environment

P114

Pseudomonas aeruginosa from vegetables and children: a comparison of molecular typing and virulence.

Lidia Ruiz-Roldán[1], Beatriz Rojo-Bezares[1], Carmen Lozano[1], José Manuel Azcona-Gutiérrez[3], Carmen Torres[1,2], Yolanda Sáenz[1]
[1]Área de Microbiología Molecular. Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain, [2]Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain, [3]Laboratorio de Microbiología. Departamento de Diagnóstico Biomédico. Hospital San Pedro, Logroño, Spain

BACKGROUND: *Pseudomonas aeruginosa* (PA) is a ubiquitous pathogen that shows high antimicrobial resistance and virulence.

Our aim was to characterise and compare PA isolates recovered from Spanish vegetal food and children.

METHODS: 82 PA isolates [50 from vegetables (V) and 32 from children (C)] were included. The following analyses were performed: molecular typing by *SpeI*-Pulsed-field-gel-electrophoresis (PFGE), multilocus-sequence-typing and serotyping, susceptibility to 11 antibiotics by disc-diffusion method, presence of thirteen virulence markers by PCR, pigments and elastase production by biochemical methods, and biofilm production by crystal violet staining and fluorescein diacetate assay.

RESULTS: 80 PA isolates were susceptible to all antibiotics, and two were imipenem-resistant isolates. Sixty-two different PFGE patterns were detected. According to origin and PFGE, 68 PA were selected. A high variety of sequence types (STs) was detected: 39 STs and 10 new ones (ST2241, ST2242, ST2416, ST2427-ST2432 and ST2448). Eleven different serotypes were found, the most predominant being serotype 6 (41% strains). Among the virulence markers, 54 strains amplified *exoS* (21 C/33 V), 13 *exoU* (9 C/4 V) and one strain none of both genes; *lasR* gene was truncated by insertion sequences in 4 strains (1 C, 3 V). 91% of strains produced more biofilm, 64.7% pyocyanin and 78% elastase than control strain. Non-significant differences were detected in biofilm, pyocyanin and elastase production between PA of both origins.

CONCLUSION: A high clonal diversity, multiple serotypes, low antimicrobial resistance levels, and high virulence markers were detected in our PA strains. Non-significant differences were detected between origins.

Ecology, Evolution & Environment

P115

Pseudomonas aeruginosa pathoadaptation mediated by intergenic evolution

S. M. Hossein Khademi, Lars Jelsbak
Technical University of Denmark, Lyngby, Denmark

Bacterial pathogens evolve during the course of infection as they adapt to the different selective pressures that confront them inside the host. Determining the molecular basis of beneficial changes that underlies host adaptation remains a central challenge. Studies of pathogen adaptation have focused predominantly on molecular evolution within coding regions whereas the role of adaptive mutations in intergenic regions has received comparably less attention. As a consequence, the extent to which intergenic mutations contribute to bacterial host adaptation remains unclear.

Here, we analyze recurrence of evolution in intergenic regions in 44 clonal lineages of the opportunistic pathogen *Pseudomonas aeruginosa* as they adapt to their human hosts. We identify 88 intergenic regions in which parallel molecular evolution occur in multiple lineages or isolates. At the genetic level, we find that mutations in these regions under selection are most often located upstream of transcriptional start sites, and within regulatory elements. At the functional level, we show that these mutations may both create or destroy regulatory interactions in connection to transcriptional processes, and that they are directly responsible for the evolution of important pathogenic phenotypes such as reduced sensitivity to antibiotics. Importantly, we find that intergenic mutations are more likely to be selected than coding region mutations and thus contribute more to this pathogen´s host adaptation than previously realized. Furthermore, intergenic mutations enable more essential genes to become target of evolution compared to coding region mutations.

Our results highlight the evolutionary significance of intergenic mutations in creating host-adapted variants through altered regulatory interactions.

Ecology, Evolution & Environment

P116

AHL mediated quorum sensing mediates species interactions in multispecies biofilms

Sujatha Subramoni[1], Li Yi[1,2], Muhammad Zulfadhly Bin Mohammad Muzaki[2], Scott A. Rice[1,2]
[1]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore, [2]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

A major concern is to understand processes that govern functional synergy and dynamics of multispecies bacterial biofilms that are important medically, environmentally and industrially. Here, we have used a model mixed species biofilm community comprising *Pseudomonas aeruginosa* PAO1, *Pseudomonas protegens* PF5 and *Klebsiella pneumoniae* KP1 with higher biomass and tolerance to antimicrobial stress conditions such as SDS and tobramycin, compared to monospecies biofilm populations. In this study, we evaluated the function of N-acyl homoserine lactone (AHL)-dependent quorum sensing (QS) systems of *P. aeruginosa* PAO1, which are known to regulate group behavior including biofilm formation and the production of effector molecules, in determining the structure and function of this mixed species biofilm community. Mixed species biofilms containing either wild type PAO1, *lasIrhlI* (QS signal negative) or *lasRrhlR* (QS signal receptor negative) mutants were grown under continuous flow conditions and their composition was analyzed by confocal laser scanning microscopy. We observed that mixed species biofilms containing *P. aeruginosa* QS mutants had significantly altered proportions of *K. pneumoniae* and *P. protegens* populations compared to mixed species biofilms with the wild type *P. aeruginosa*. Surprisingly, the proportions of *P. aeruginosa* were the same for both the wild type and the QS mutants. We also showed that the *P. aeruginosa* QS system, through its downstream targets, alters the stress resistance of the whole community. These observations suggest that QS plays an important role in modulating community biofilm structure and physiology, affecting both interspecific interactions and community level protection provided by species specific functions.

Ecology, Evolution & Environment

P117

Pseudomonas Genome Database 2017: Improved Gene/AMR/VF/Genomic Island Annotations, Comparative Genome Analyses, and a Platform for Facilitating Public Health Genomic Epidemiology

Geoffrey L. Winsor[1], Claire Bertelli[1], Kara K. Tsang[2], Brian Alcock[2], Andrew G. McArthur[2], Fiona S.L. Brinkman[1]
[1]Simon Fraser University, Burnaby, BC, Canada, [2]McMaster University, Hamilton, ON, Canada

The Pseudomonas Genome Database provides high quality genome annotations for select *Pseudomonas* strains and computational predictions and comparative analyses for more than 3000 others. To date, curator-based and community-assisted approaches have contributed more than 7000 updates to key features. These are further complemented with numerous computational predictions including gene/protein function, subcellular localizations, orthologs and predicted drug targets. The database aims to maintain high quality annotations and analyses, but is evolving to meet a growing need for real-time genomic epidemiology investigations, including identification of virulence/antimicrobial resistance features and phylogenetic relationships from genomic data of bacterial isolates. This process includes linking with the open source Integrated Rapid Infectious Disease Analysis (IRIDA.ca) platform, now implemented within the Public Health Agency of Canada (a public version is being made available separately). Following comprehensive software-guided curation of published literature with an emphasis on efflux, antimicrobial resistance (AMR) genes from the Comprehensive Antibiotic Resistance Database (CARD) have been added and are being further expanded. Improved Genomic island (GI) predictions (>10% increase in recall/sensitivity at almost 90% precision/specificity) are available using our new IslandViewer4 predictor. A novel "IslandCompare" interface is being finalized that displays core-genome phylogenetic trees and zoomed in views of linearly-displayed genomes with clusters/groups of GIs highlighted differently. These integrated platforms aim to provide high quality genome annotations, with a user-friendly interface, for microbiologists and other researchers using genome sequence data in their investigations – including increasingly for analysis of clusters of closely related Pseudomonas isolates.

Ecology, Evolution & Environment

P118

Immense functional and genotypic diversification in *Pseudomonas aeruginosa* biofilm evolved populations

Katrina Harris[1], Kenneth Flynn[2], Vaughn Cooper[1]
[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of New Hampshire, Durham, NH, USA

Biofilms are known to be robust due to the extreme amounts of biodiversity they contain at both the species and gene levels. Experimental microbial evolution(EME) provides an opportunity to study how populations adapt to the biofilm environment over long time scales. *Pseudomonas aeruginosa*(Pa) populations have been evolved for 600 generations under biofilm conditions using a bead based laboratory model that forces the biofilm lifecycle of attachment, growth, and dispersal daily. Unlike the morphological parallelism observed in other EME studies, biofilm evolved Pa populations were composed of diverse colony morphologies, some of which are known to be associated with chronic infections such as mucoid and small colony variants. Whole genome sequencing of the three biofilm evolved populations at six time points allow tracking of 397 high-confidence mutational trajectories spreading in a series of sweeps and sways over the 90-day period. Sweeps, rapid rises in frequency that remove genetic diversity, occur in earlier generations until mutators, genotypes with defects in DNA mismatch repair, fix in the population. Once mutators fix, only sways, gradual increases in frequency, are observed implying diversity is favorable in biofilms. Previous studies have linked different colony morphologies with niche occupancy so the increased functional and genetic diversity is thought to be a representation of the ways these populations may be adapting and filling the niches provided by the structured nature of living in a biofilm. Understanding mechanisms that these populations use to adapt to growing in the biofilm may explain selective dynamics of chronic infections and improve treatment.

Ecology, Evolution & Environment

P119

Genetic characterization of *Pseudomonas aeruginosa* isolates from human, animal and water origins: Occurrence of common clones and profiles of resistance and virulence.

Asma Bel Hadj Ahmed[1], Mohamed Salah Abbassi[1], Beatriz Rojo-Bezares[2], Lidia Ruiz-Roldán[2], Abdennaceur Hassen[3], Yolanda Sáenz[2]
[1]Université de Tunis El Manar, Institut de la Recherche Vétérinaire de Tunisie, Tunis, Tunisia, [2]Area de Microbiología Molecular. Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain, [3]Laboratoire de Traitement des Eaux Usées, Centre des Recherches et des Technologies des Eaux (CERTE), Soliman, Tunisia

BACKGROUND: *Pseudomonas aeruginosa* is a ubiquitous bacteria that causes many chronic disease in human and shows high antibiotic resistance and virulence. Studies of *P. aeruginosa* from environment and animal are scarce.

The aim of this study was to investigate the antimicrobial phenotype, virulence and molecular typing of *P. aeruginosa* from different origins (patients, animals and water) and regions in Tunisia.

METHODS: Sixty-six *P. aeruginosa* isolates were collected from human patients and hospital environment (9 isolates), cow/goat milk (5), poultry meat (1), chicken/sheep faecal samples (4/7), wastewater treatment plant (water/solid samples, 28/9), thermal water (2), and seawater (1). They were confirmed by MALDI-TOF. The susceptibility to 18 antibiotics was analysed by disc diffusion method and the presence of six virulence markers by PCR. Molecular typing was analysed by SpeI-Pulsed-field-gel-electrophoresis (PFGE) and multilocus-sequence-typing (MLST).

RESULTS: Fifty-four isolates were susceptible to all tested antibiotics. Only resistance to colistin (13.6%), ticarcillin (3%) and imipenem (1.5%) were found. *exoA-lasB-rhlR-lasR* genes were positive in 60 isolates. Forty-six isolates amplified *exoS* gene, 8 *exoU* and two isolates *exoS-exoU*. The typable 65 isolates were classified in 26 PFGE pulsotypes. Interestingly, some pulsotypes encompassed isolates from different origins and regions. According to pulsotypes and origins, 12 selected isolates were typed by MLST, identifying ST233, ST244, ST275, ST641, ST773, ST792, ST1248, ST1420, ST1621, and 3 new STs.

CONCLUSIONS: *P. aeruginosa* showed low antimicrobial resistance levels, but a high number of virulence markers. Results highlight the huge variability and dissemination of some clones among different ecological niches.

Infections & Host-Pathogen Interactions

P120

Analysis of 2-(2-Hydroxylphenyl)-Thiazole-4-Carbaldehyde Production *via* the *ambABCDE* Gene Cluster and its Relationship with *Pseudomonas aeruginosa* Quorum Sensing

Ana Carolina Paiva, Matthew Fletcher, Nigel Halliday, Morgan Alexander, Paul Williams
University of Nottingham, Nottingham, UK

Pseudomonas aeruginosa is able to colonize several hosts by producing a variety of virulence factors and secondary metabolites. One of these is the anti-metabolite, L-2-amino-4-methoxy-trans-3-butenoic acid (AMB). AMB biosynthesis is directed by the gene products of the five-gene cluster *ambABCDE*. Recently, these genes have been associated with quorum sensing (QS) in *P. aeruginosa* by directing the biosynthesis of another putative QS molecule, 2-(2-hydroxylphenyl)-thiazole-4-carbaldehyde (IQS). IQS was reported to activate the *rhl* and *pqs* QS systems in an *ambB* mutant, where pyocyanin production was restored under phosphate-limited conditions. To further investigate these findings, transcriptional fusions of *ambA* and *ambB* were created and the expression of these genes was tested in a range of *amb* and QS mutants, in both low and normal phosphate conditions. Expression of these genes was not altered when synthetic exogenous IQS was added to the cultures at concentrations of 200 μM. Additionally, LCMS analysis showed that production of IQS was not altered in *amb* mutants. *phzA1* and *phzA2* transcriptional reporter fusions were constructed and the expression and production of pyocyanin in these mutants was analysed under low phosphate conditions. Pyocyanin expression and production was unaltered compared with the wild type. Since QS in *P. aeruginosa* is also involved in controlling swarming motility and biofilm development, strains carrying mutations in the *ambABCDE* locus were tested for defects. No differences among mutant and wild type strains were observed. The relationship of the *ambABCDE* genes with IQS production and of the putative IQS signal molecule to QS is therefore unclear.

P121

Manipulating the T6SS tip to shuttle effectors

Sarah Wettstadt, Alain Filloux
Imperial College London, London, UK

The bacterial type VI secretion system (T6SS) is a nanomachine involved in the transport of effectors into competing microorganisms or eukaryotic host cells. It is composed of a cytosolic contractile sheath loaded with Hcp hexamers, on top of which is the so-called puncturing device consisting of a VgrG-trimer tipped by a sharpening PAAR-protein.
Previous studies showed that effectors are specifically recognised by the C-terminus of a VgrG protein, while adaptor proteins can be involved. Once bound to VgrG, the effector is delivered upon sheath contraction that propels Hcp and VgrG components out of the cell.
In this study, we further studied the C terminus specificity by performing swapping experiments using the Pseudomonas aeruginosa VgrG4b and VgrG5 and monitoring recognition of their substrates PldA and PldB. We also showed that heterologous secretion from P. aeruginosa does not occur using Tde from Agrobacterium tumefaciens. We then designed strategies to modulate the H1-T6SS VgrG tip to achieve effector delivery from a heterologous host.
Using bacterial competition, secretion and translocation assays we could follow the fate of the T6SS effectors. Results from these experiments shed light on the specificity between the VgrG spike, PAAR or adaptor proteins and cognate effectors while demonstrating how the modularity at the T6SS tip is controlled. Based on these data we developed a model about how effectors are organised around VgrG, so that the T6SS tip could be crafted for "a la carte delivery" of any effector of interest.

Infections & Host-Pathogen Interactions

P122

The cystic fibrosis airways microbial metagenome

Katarzyna Pienkowska, Patricia Moran Losada, Marie Dorda, Frederik Behr, Silke Hedtfeld, Rebecca Hyde, Lutz Wiehlmann, Burkhard Tuemmler
Medizinische Hochschule Hannover, Hannover, Germany

Deep metagenome sequencing was performed on nasal lavage, throat swabs and induced sputa collected on several occasions from 142 patients with cystic fibrosis (CF). We typically identified less than ten DNA viruses or fungi and several hundred bacterial taxa in samples from children, adolescents and adults. Bacteria made up more than 99% of the microbial community. The spectrum ranged from a normal flora via an intermediate stage when the normal community is perturbed by *Haemophilus influenzae* or *Staphylococcus aureus* to a final stage of a low-diversity community dominated by *Pseudomonas aeruginosa*. This shift from a normal highly diverse metagenome indistinguishable from that of a healthy subject to the CF-typical end-stage of an almost pure culture of *P. aeruginosa* was correlated in our patient cohort with disease severity, but not with age. *P. aeruginosa* was identified in all specimens from exocrine pancreas-insufficient (PI) patients suggesting the ubiquitous presence of *P. aeruginosa* in respiratory secretions of PI CF patients. The clonal composition of the *P. aeruginosa* communities in CF airways was determined from the frequency distribution of SNPs in the metagenomes. The *P. aeruginosa* communities consisted of one or two major clone types making up 96 -100 % of the consortium. Within-clone variation was detectable for the most prevalent clone which split up in two to five discriminable clonal variants. The rare clones and clonal variants constitute a low copy genetic resource which could rapidly expand as a response to habitat alterations such as antimicrobial chemotherapy or invasion of novel microbes.

Infections & Host-Pathogen Interactions

P123

P. aeruginosa blood stream infection isolates from patients with recurrent blood stream infection: Is it the same genotype?

Kate McCarthy[1], Tim Kidd[1,2], David Paterson[1]
[1]University of Queensland, Brisbane, Queensland, Australia, [2]Queen's University Belfast, Belfast, Northern Ireland, UK

Introduction

Pseudomonas aeruginosa isolate typing in the setting of recurrent *P. aeruginosa* blood stream infection (BSI) has not been studied. This study aimed to further characterise the genotypic relationship between the BSI isolates in this setting.

Methods

Patients with a primary and recurrent *P. aeruginosa* BSI were identified retrospectively from the 1st of January 2008 to the 1st of January 2013 from 5 different laboratories. The laboratories serviced eight tertiary care and one secondary care institution. Isolates were genotyped by iPLEX *MassARRAY MALDI-TOF MS genotyping*. If a clonal MLST type was found, DiversiLab genotyping was subsequently performed. Epidemiological, clinical and microbiological data was obtained on the BSI episodes.

Results

It was found that a recurrent BSI from *P. aeruginosa* was more commonly from a genotypically related strain of *P. aeruginosa*. Relapse due to a genotypically related bacterial isolate occurred earlier in time than a relapsing infection from an unrelated bacterial isolate. Line related infections were found to be the most common source of suspected BSI. Almost half the BSI episodes were associated with neutropenia.

Conclusion

In summary bacterial isolates giving rise to recurrent BSI in the setting of *P. aeruginosa* BSI are more likely to be genetically related, particularly if relapse occurs soon after the primary BSI episode. Gut translocation may be another important source found in the setting of neutropenia.

P124

Virulence genes in the setting of early patient death in *P. aeruginosa* blood stream infection isolates

Kate McCarthy[1], Amy Jennison[2], Alexander Wailan[3], David Paterson[1]
[1]University of Queensland, Brisbane, Queensland, Australia, [2]Forensic and Scientific Services, Queensland Health, Brisbane, Queensland, Australia, [3]Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, UK

Introduction

We have recently characterised the epidemiology of *P. aeruginosa* blood stream infection (BSI) in a large retrospective multicentre cohort study. Utilising corresponding patient BSI isolates we aimed to characterise the genotypic virulence profile of the *P. aeruginosa* isolates that were associated with rapid death in the non-neutropenic host.

Methods

Five *P. aeruginosa* BSI episodes were identified from a larger cohort of *P. aeruginosa* BSI episodes previously described by McCarthy *et al*. These episodes were in patients who were non-neutropenic and had experienced rapid death. These isolates underwent Illumina whole genome sequencing and were de novo assembled using Spades VX. Annotation was performed by the NCBI prokaryotic Genome Annotation Pipeline. A comprehensive suite of virulence genes was determined by utilising the Pseudomonas Genome Database (http://www.pseudomonas.com/). For the HigB toxins and the ExoU protein, primer sequences were obtained and blasted to identify the gene product. For a gene to be considered present searching required 100% of the gene to be present in the BLAST output.

Results

There was extensive conservation of virulence genes across all five of the BSI isolates studied. The *higA* and *higB* genes were detected in all isolates. Two of the isolates contained the *exoU* gene. The *exlA* gene was not detected in any isolates.

Conclusion

These findings may reflect that to cause both a blood stream infection and rapid death that it is only the highly virulent *P. aeruginosa* isolate that succeeds. Further phenotypic correlation is required.

Infections & Host-Pathogen Interactions

P125

The natriuretic peptide hormones prevent *Pseudomonas aeruginosa* biofilm formation through a specific bacterial target.

Florie Desriac[1], Thomas Clamens[1], Thibaut Rosay[1], Jeremy Enault[1], Lucille Rocquigny[1], Alexis Bazire[2], Alain Dufour[2], Pierre-Jean Racine[1], Pierre Cornelis[1], Emeline Bouffartigues[1], Sylvie Chevalier[1], Marc Feuilloley[1], Olivier Lesouhaitier[1]
[1]Laboratory of Microbiology Signals and Microenvironment EA 4312, Univ. Rouen, Evreux, France, [2]LBCM, IUEM, EA 3884, Univ. Bretagne-Sud, Lorient, France

We have shown that the C-type Natriuretic Peptide (CNP), a peptide produced in the lung, reduces *Pseudomonas aeruginosa* biofilm formation. Herein, we clarified the effect of CNP and studied the effect of two other members of the natriuretic peptide family, the atrial natriuretic peptide (ANP) and the brain natriuretic peptide (BNP) on *P. aeruginosa* biofilm.
We observed that exposure of *P. aeruginosa* to graded concentrations of ANP, CNP or BNP (10^{-9} to 10^{-6} M) induced a dose-related inhibition of biofilm formation under dynamic conditions. In parallel, we showed that static biofilms formed by *P. aeruginosa* are impacted by BNP and CNP but more modestly as compared with the dynamic conditions. An in silico study comparing protein sequences of human natriuretic peptide receptors and *Pseudomonas* proteins revealed that the bacterial protein AmiC could be the sensor for natriuretic peptides. Using MicroScaleThermophoresis, we showed that both CNP and ANP bind AmiC with a K_D of 2 µM, whereas BNP has no affinity for AmiC. Interestingly, an *amiC* mutant strain was not impaired in biofilm formation in the presence of CNP at 10^{-7} M or less, while it remained sensitive to BNP.
We demonstrate that the natriuretic peptides strongly prevent biofilm formation through different mechanisms. Indeed, we showed that CNP and ANP which are the more expressed peptides in the lung act specifically through the AmiC sensor, whereas the BNP peptide seems to possess a non specific activity. This discrepancy should have major consequences to design drugs for biofilm treatment.

Infections & Host-Pathogen Interactions

P126

The involvement of the ami operon in *Pseudomonas aeruginosa* virulence regulation and biofilm formation reveals new functions for the amidase AmiE.

Thomas Clamens[1], Florie Desriac[1], Thibaut Rosay[1], Alexandre Crepin[2], Alain Dufour[2], Pierre Cornelis[1], Emeline Bouffartigues[1], Sylvie Chevalier[1], Marc Feuilloley[1], Olivier Lesouhaitier[1]
[1]Laboratory of Microbiology Signals and Microenvironment EA 4312, Univ. Rouen, Evreux, France, [2]LBCM, IUEM, EA 3884, Univ. Bretagne-Sud, Lorient, France

We have previously shown that the C-type Natriuretic Peptide (CNP), a peptide produced by the lung, prevents *Pseudomonas aeruginosa* biofilm formation. In the present study, we identified AmiC as the bacterial target explaining CNP effects, and we studied the involvement of the aliphatic amidase AmiE in these effects.
Comparison of 3D structures of human natriuretic peptide receptors and *Pseudomonas* proteins revealed that the bacterial protein AmiC shows significant similarity with the human C-type natriuretic peptide receptor (hNPR-C). Recombinant protein AmiC was purified and protein/peptide interactions assessed using MicroScale Thermophoresis. Results showed that both CNP and hNPR-C agonists bind the AmiC protein. The *amiC* gene belongs to the *ami* operon. This operon also encodes the aliphatic amidase AmiE which hydrolyses short-chain aliphatic amides to their corresponding organic acids. We investigated AmiE potential alternative functions in *P. aeruginosa*. We observed that over expression of AmiE protein altered biofilm formation, bacterial motilities and quorum sensing molecules production. Using several infection models, we demonstrated that AmiE over-production led to a strong decrease in *P. aeruginosa* virulence both *in vitro* and *in vivo*, suggesting that in addition to its carbon-nitrogen metabolic process activities, AmiE would have multiple other functions.
We demonstrate that the bacterial protein AmiC is an ortholog of the eukaryotic receptor hNPR-C, acting as a CNP sensor in *P. aeruginosa*. Our data show that the whole ami operon has new functions in bacteria, allowing to modulate the switch between chronic and acute infection depending on exposition to host factors.

Infections & Host-Pathogen Interactions

P127

Characterization of the *P. aeruginosa* clinical volatile metabolome

Heather D. Bean[1], Christiaan A. Rees[2], Jane E. Hill[2]
[1]*Arizona State University, Tempe, AZ, USA,* [2]*Dartmouth College, Hanover, NH, USA*

Pseudomonas aeruginosa is nearly ubiquitous, well known to occupy a multitude of environmental niches and cause human infections at a variety of bodily sites, due to its metabolic flexibility, secondary to extensive genetic heterogeneity at the species level. Because of its dynamic metabolism and clinical importance, we sought to perform a comparative analysis on the volatile metabolome (the 'volatilome') produced by *P. aeruginosa* clinical isolates. In this study, we analyzed the headspace volatile molecules of 24 *P. aeruginosa* clinical isolates grown *in vitro*, using two-dimensional gas chromatography - time-of-flight mass spectrometry (GC×GC-TOFMS). We identified 391 non-redundant compounds that we associate with the growth and metabolism of *P. aeruginosa* (the 'pan- volatilome'). Of these, 70 were produced by all 24 isolates (the 'core volatilome'), 52 by only a single isolate, and the remaining 269 volatile molecules by a subset. Sixty-five of the detected compounds could be assigned putative compound identifications, of which 43 had not previously been associated with *P. aeruginosa*. Using the accessory volatile molecules, we determined the inter-strain variation in the metabolomes of these isolates, clustering strains by their metabotypes. Additionally, using rarefaction and accumulation curves, we were able to approximate the sizes of the *P. aeruginosa* clinical core and pan-volatilomes, respectively, under the experimental conditions used in this study. Assessing the extent of metabolomic diversity in *P. aeruginosa* through an analysis of the volatile molecules that it produces is a critical next step in the identification of novel diagnostic or prognostic biomarkers of *P. aeruginosa* infections.

Infections & Host-Pathogen Interactions

P128

Oxygen and pH Alter the Targets of Antimicrobial Therapy in Cystic Fibrosis

Robert Quinn, William Comstock, Jamie Morton, Doug Conrad, Rob Knight, Pieter Dorrestein
University of California at San Diego, San Diego, CA, USA

The field of environmental microbiology has long known that basic chemistry, such as pH, oxygen and redox potential, drive the structure and function of microbial communities. Many human infections also involve a community of microorganisms, such as those that exist in the cystic fibrosis (CF) lung, but how these communities are shaped by similar chemical gradients is poorly understood. Here, we demonstrate that pH and oxygen also drives the structure and function of the CF lung microbiome using a novel culture-based system that mimics a mucus-plugged bronchiole. Changes of pH and oxygen concentration result in non-pathogenic species outcompeting *Pseudomonas aeruginosa,* the notorious pathogen of CF. Under low pH and low oxygen conditions, anaerobes flourish and *P. aeruginosa* does not survive. The physiology of the CF microbiome is also altered through these chemical gradients. Fermentation metabolites from anaerobes are produced in low oxygen and low pH, while virulence factors from *P. aeruginosa*, such as phenazines and rhamnolipids, are undetectable under these conditions. The effect of these gradients results in altered efficacy of antimicrobial therapy. Deep in the anoxic mucus anaerobes survive treatment with the anti-Pseudomonas drug tobramycin, while in the aerobic mucus the pathogen *Stenotrophomonas maltophilia* replaces *P. aeruginosa*. This study demonstrates that slight changes in basic chemistry at the site of a polymicrobial infection can change treatment outcomes. Though *P. aeruginosa* is a formidable pathogen with an array of virulence factors and resistance mechanisms, in certain chemical conditions it is overwhelmed by less infamous pathogens rendering its virulence repertoire futile.

Infections & Host-Pathogen Interactions

P129

Generation of lipopolysaccharide biosynthesis mutants of plant-associated *Pseudomonas* strains

Alexander Kutschera[1], Nicolas Gisch[2], Ann-Katrin Gerullis[1], Stefanie Ranf[1]
[1]Technical University of Munich, School of Life Sciences, Chair of Phytopathology, Freising, Germany, [2]Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Division of Immunochemistry, Borstel, Germany

In order to successfully establish an infection in plants, bacteria have to overcome various chemical and physical barriers as well as plant immune responses. Lipopolysaccharide (LPS), the major component of the outer membrane (OM) of gram-negative bacteria, confers stability to and restricts permeability of the OM. In mammals, the O-polysaccharide (OPS) of LPS is crucial to fend off cationic antimicrobial peptides and other defense agents targeting the bacterial cell envelope. To investigate the role of OPS in pathogenicity of plant-adapted bacteria we conducted bioinformatic analyses to determine the genetic background which facilitates the biosynthesis of their LPS structures. This revealed that several LPS biosynthesis genes, documented in *Escherichia coli* and *Pseudomonas aeruginosa*, are conserved in the genomes of *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 and related plant-associated Pseudomonas strains. By these means however, we also found that parts of the biosynthesis pathway are yet unknown in plant-adapted bacteria. We further used the information from the bioinformatic analysis to identify single gene targets to create knockout mutant strains of *Pst* DC3000 incapable of synthesising OPS. Such *Pst* DC3000 rough LPS strains were then further characterized for swarming motility and pathogenicity on Arabidopsis plants.

Infections & Host-Pathogen Interactions

P130

Pseudomonas aeruginosa Biofilm Recognition by Host C-type Lectins

Mohammad Alshahrani[1,2], Sonali Singh[1], Tamanna Rahman[1], Yasir Almuhanna[1,3], Yasuhiko Irie[4], Daniel Mitchell[5], Paul Williams[1], Miguel Camara[1], Luisa Martinez-Pomares[1]
[1]University of Nottingham, Nottingham, Nottinghamshire, UK, [2]King Khalid University, Abha, Aseer Province, Saudi Arabia, [3]Shaqra University, Shaqra, Riyadh Region, Saudi Arabia, [4]University of Dayton, Dayton, Ohio, USA, [5]University of Warwick, Warwick, West Midlands, UK

Pseudomonas aerugionosa (PA) establishes chronic infections (e.g. in cystic fibrosis (CF)) by forming biofilms embedded in exopolysaccharide (EPS) matrix. Biofilms show increased resistance to antibiotics and the immune system. The immune receptors mediating PA biofilm recognition are still unknown. We hypothesised that C-type lectin receptors (CLRs, sugar-binding receptors) such as the mannose receptor (MR) and DC-SIGN may play a role in immune recognition of PA biofilms by binding two major EPS components - Psl and Pel.

MR and DC-SIGN binding to purified EPS and biofilms from PA wild-type, *psl* and/or *pel*-deficient mutants, and CF isolates was tested. Our results show (i) MR binds PA biofilms through its CTLD4-7 domain in a Psl-dependent manner. (ii) DC-SIGN binds to PA biofilms significantly better than MR. (iii) DC-SIGN binds multiple ligands, including Psl, in planktonic and biofilm PA.

Thus, MR and DC-SIGN mediate PA biofilm recognition by immune cells. We are currently identifying additional DC-SIGN ligands, especially in planktonic PA, and are investigating EPS modulation of PA biofilm recognition by human myeloid cells expressing CLRs. Understanding CLR-EPS interactions may yield new therapeutic targets for biofilm eradication and management of chronic PA infections in CF.

Infections & Host-Pathogen Interactions

P131

Characterizing the interaction of host-derived sphingosine with the *Pseudomonas aeruginosa* transcriptional regulator SphR.

Lauren A. Hinkel, Annette E. LaBauve, Matthew J. Wargo
University of Vermont Larner College of Medicine, Burlington, VT, USA

Pseudomonas aeruginosa's ability to detect and protect against the antimicrobial lipid sphingosine, via the AraC-family transcriptional regulator SphR, plays an important role in establishing successful infections. SphR induces genes in a sphingosine-dependent manner. The goal of our research is to determine the mechanism of sphingosine recognition by SphR and how this impacts DNA binding. We are focused on three questions: (i) What chemical and structural features of sphingosine are important for SphR-dependent transcriptional induction?; (ii) How does sphingosine impact SphR dimerization and what domain(s) is responsible?; and (iii) What amino acid residues are needed for SphR detection of sphingosine? Using SphR-dependent transcriptional reporters, our data suggest that the polar head group of sphingosine plays the largest role in SphR ligand recognition, particularly the amine group on carbon 3. Using co-expression of differentially tagged SphR constructs in *E. coli*, and subsequent pull-down assays, we determined that the N-terminus of SphR is not sufficient to bind ligand but is sufficient for dimerization. Lastly, we have designed a gain-of-function mutagenic screen to determine important amino acid residues for SphR detection of sphingosine and plan to report results at the conference.

Infections & Host-Pathogen Interactions

P132

Vitronectin is produced in the lung upon infection by respiratory pathogens, and is utilized to evade the innate immunity

Magnus Paulsson[1], Karlhans Che[2], Jonas Ahl[3], Margaretha Smith[4], Ingemar Qvarfordt[4], Yu-Ching Su[1], Anders Lindén[2,5], Kristian Riesbeck[1]
[1]Department of Translational Medicine, Lund University, Malmö, Sweden, [2]Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [3]Infectious Diseases Research Unit, Department of Translational Medicine, Lund University, Malmö, Sweden, [4]Institute of Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, [5]Lung Allergy Clinic, Karolinska University Hospital New Karolinska Solna, Stockholm, Sweden

Bacterial extracellular vesicles (EVs) are shed during growth by the respiratory pathogens *Pseudomonas aeruginosa* and *Haemophilus influenzae*. Vesicles trigger release of antimicrobial compounds and proteins of the complement, which are partly regulated by vitronectin. We hypothesized that vitronectin is elevated in the lungs during pneumonia, that respiratory epithelial cells produce vitronectin upon bacterial stimulation and that vitronectin is utilized by bacteria for increased virulence.

Vitronectin-concentrations were measured by ELISA in bronchoalveolar lavage fluid (BALF) from patients with pneumonia (n=8) and from healthy volunteers (n=13) with or without pulmonary endotoxin instillation. Elevated vitronectin concentrations were found in BALF collected during pneumonia compared to healthy individuals (p=0.0063) and in endotoxin-challenged pulmonary segments compared to control segments (after 12h: p=0.031; 48h: p=0.016). Flow cytometry revealed that bacteria captured vitronectin from BALF onto their surface and subsequently became less sensitive to killing by serum compared to controls (*P. aeruginos*a p=0.016, *H. influenzae* p=0.011). Increased levels of VTN mRNA after one hour (p=0.022) and increased surface bound vitronectin after 24h (p<0.001) were observed with type II bronchial alveolar epithelial cells (A549) after stimulation with EVs.

In conclusion, elevated vitronectin concentrations were found in BALF from patients with pneumonia and in healthy volunteers after pulmonary endotoxin instillation. Cellular experiments confirmed vitronectin production upon EV stimulation *in vitro.* Bacteria captured vitronectin from BALF on their surface to evade lysis by complement in serum. Hence, vitronectin is produced by epithelial cells upon bacterial infection and utilized by respiratory pathogens to persist in the respiratory tract.

Infections & Host-Pathogen Interactions

P133

Genomic and phenotypic insights into severe contact lens-associated eye infections caused by *Pseudomonas aeruginosa*

Yasmin Hilliam[1], Emily Richardson[2], Janet Wong[1], Joshua Quick[2], Carmen Martinez-Rodriguez[1], Timothy Neal[3], Stephen Tuft[4], Jo Fothergill[1], Stephen Kaye[3], Nick Loman[2], Craig Winstanley[1]
[1]University of Liverpool, Liverpool, UK, [2]University of Birmingham, Birmingham, UK, [3]Royal Liverpool University Hospital, Liverpool, UK, [4]Moorfields Eye Hospital, London, UK

Bacterial infections of the cornea are a major cause of vision loss world-wide, 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), and have linked clinical metadata, including contact lens use.

In this study, we used whole genome sequencing (WGS) of these isolates in order to comprehensively characterize the UK *P. aeruginosa* keratitis-associated population. Genomic DNA was extracted from each of the *P. aeruginosa* isolates, and barcoded sequencing libraries were prepared and sequenced on the Illumina MiSeq platform.

Core genome SNP phylogeny of the keratitis isolates, alongside a reference set of a similar number of genomes from isolates obtained from a range of other infections, revealed two major clusters (Group 1, which includes PAO1 and Clone C; Group 2, which includes PA14). Amongst the wider *P. aeruginosa* population, Group 1 is dominant, but the keratitis isolates were distributed more evenly between the two major clusters, confirming a statistically significant association between keratitis isolates and Group 2. Group 1 and Group 2 were almost exclusively associated with carriage of *exoS* and *exoU* respectively, but there was no association between contact lens (CL) use and either Group 1 or Group 2. Further phenotypic analysis of the CL-associated isolates indicated variations in resistance to CL disinfection solutions and susceptibility to phages.

We gratefully acknowledge funding from Fight for Sight, UK.

Infections & Host-Pathogen Interactions

P134

The effect of cigarette smoke and electronic cigarette vapour on *Pseudomonas aerouginosa*.

Deirdre Gilpin[1], Katie-Ann McGown[1], Jose Bengoechea[1], Kevin Gallagher[1], Stuart Elborn[2], Michael Tunney[1]
[1]Queen's University Belfast, Belfast, UK, [2]Imperial College London, London, UK

Introduction: Chronic colonization with *Pseudomonas aeruginosa* is associated with clinical decline in chronic lung diseases with established links to smoking. There is currently little data regarding the effect of e-cigarettes on lung health. This study aimed to determine the effect of cigarette smoke extract (CSE) and e- cigarette extract (ECSE) on *P.aeruginosa*.

Methods: The growth of *P. aeruginosa* reference isolates (n= 2: ATCC 27853, PA01), clinical isolates (n=2, COPD; n=1 bronchiectasis) and one veterinary isolate (n=1) were determined in MHB +/- CSE/ECSE planktonically and in biofilm. Virulence in *Galleria melonella* was determined. Pyoverdin and pyocyanin secretion, in n=5/n=3 isolates respectively, +/- CSE/ECSE, was determined by spectrophotometry. IL-8, TNF-α and IL-1β levels were determined following infection of A549 cells with *P.aeruginosa* +/- CSE/ECSE.

Results: Neither CSE or ECSE inhibited the growth of any *P.aeruginosa* isolate. Biofilm formation in MHB vs. CSE or ECSE was significantly increased (p= <0.001) in 5/5 and 3/5 isolates respectively. *G. mellonella* survival was significantly decreased in isolataes + CSE (p= <0.001) or ECSE (p= <0.001). Pyoverdin and pyocyanin secretion increased significantly in all CSE-exposed isolates and in 2/5 (Pyoverdin) and 2/3 ECSE (Pyocyanin) exposed isolates (p=0.02). IL-8, TNF-α and IL-1β were all significantly increased in following infection by CSE and ECSE-exposed *P.aeruginosa* compared to non-exposed control.

Conclusions. Exposure of *P.aeruginosa* to CSE and ECSE resulted in changes in phenotype which may impact virulence, persistence and establishment of chronic lung infection. Further work aims examine differences in transcriptome +/-CSE/ECSE, and how this might relate to clinical outcomes.

Infections & Host-Pathogen Interactions

P135

The single sequence type pandemic of clinical isolate Pseudomonas aeruginosa in a university hospital, Japan.

Atsushi Kainuma[1], Koichi Akiyama[1], Yoshifumi Naito[1], Kyoko Momiyama[2], Keita Inoue[1], Mao Kinoshita[1], Hideya Kato[1], Teiji Sawa[1]
[1]Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Kyoto pharmaceutical university, Kyoto, Japan

Background: Pseudomonas aeruginosa (P. aeruginosa), a gram-negative bacillus, is common cause of epidemics. The aim of this study was to characterize the genetic diversity of multidrug resistant (MDR; defined as resistance to ≧ 3 antimicrobial agents) P. aeruginosa strains isolated at a university hospital of Kyoto, Japan.

Methods: We investigated MDR P. aeruginosa isolates collected prospectively between 2005 and 2014. Antimicrobial resistance was tested using a disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute to select for MDR P. aeruginosa isolates were selected. The PCR-based genotyping of type Ⅲ secretory virulence (*exoS* and *exoU*) was conducted, and genetic diversity was examined using multilocus sequence typing (MLST) technique.

Results: Forty-three *P. aeruginosa* strains were isolated from urine, sputum, skin, blood, and a catheter tips. A total of 41 multilocus sequence types (ST) were identified. Thirty-seven isolates were *exoS-/exoU+*; of these, 34 were ST357, and three were ST235. Five isolates were *exoS+/exoU-*; the ST of these isolates was ST186, ST244, ST314, ST508, and ST512. Two isolates were *exoS-/exoU-*; the ST of these isolates could not be detected. Ten isolates were carbapenem-resistant; of these, seven were ST357 and the remaining isolates were ST 235, ST244 and *exoS-/exoU-* type. Antibiotic resistance can vary within the same ST group.

Conclusions: This study demonstrates the widespread prevalence of ST357 *exoS-/exoU+* strains in our hospital over ten years. Further research is required to identify the causes of the pandemic and the underlying resistance mechanisms in order to control hospital infection.

Infections & Host-Pathogen Interactions

P136

Comparison of culture and quantitative PCR for bacterial quantification in CF sputum.

Clodagh McGettigan[1], Elinor Johnston[1], Stuart Elborn[1], Damian Downey[2], Michael Tunney[1], Deirdre Gilpin[1]
[1]Halo Research Group, Belfast, UK, [2]Belfast Health and Social Care Trust, Belfast, UK

Background: Detection and quantification of bacteria in sputum samples largely relies on culture-dependent techniques. Quantitative real time – PCR (qPCR) offers the potential for microbial quantification without the reliance on culture.

Methods: Expectorated sputum samples (n=45) were collected from adult CF patients. TVCs were determined by serial dilution on anaerobic blood and cetrimide agars to determine the total bacterial and PA load, respectively (cfu/g). DNA was extracted from frozen (-80°C) sputum samples and targets (16SrRNA; *oprL*) amplified by qPCR (LightCycler®). Eight samples were sequenced on the Illumina MiSeq platform following a standard protocol for verification of the oprL qPCR assay in detecting PA.

Results: Bacteria were detected in 45/45 samples by both culture and 16SrRNA qPCR.In each sputum sample, no significant difference was observed between total cfu/g and 16SrRNA copies/ml (p = 0.3848, Wilcoxon signed rank test). PA was detected in 31/45 samples by culture on cetrimide agar vs. 38/45 by qPCR. The sensitivity and specificity of qPCR compared to culture on cetrimide agar was determined as 93.5% and 35.7%, respectively (Fisher' exact test). Furthermore, a significant difference (p = <0.0001) in PA cfu/g vs *opr*L copies/ml was observed in 29/45 samples positive by both methods. Next generation sequencing (NGS) showed that PA was the most abundant species in qPCR positive samples and absent or present at <10% in qPCR negative samples.

Conclusion: qPCR has the potential for rapid, high-throughput microbial identification and quantification from CF sputum.

Infections & Host-Pathogen Interactions

P137

The stringent stress response as a therapeutic target to reduce virulence in a *Pseudomonas aeruginosa* cutaneous abscess model

Robert E.W. Hancock, Daniel Pletzer
Centre for Microbial Diseases and Immunity Research, Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

One of the greatest health crises facing the world today are bacterial infections that have evolved resistance to commonly used clinical antibiotics; compounds generally targeting cellular processes. A promising approach to reduce selective pressure for resistance development are compounds that aim to disarm bacterial invaders rather than killing them. The stringent stress response has recently been identified as such a potential drug target. We have found that stringent response mutants of *Pseudomonas aeruginosa* PAO1 and LESB58 were viable in a cutaneous mouse abscess model of high-density, chronic infections, however, they led to significantly reduced abscesses and cutaneous lesions. Intriguingly, our experiments revealed that treatment of *P. aeruginosa* infections with anti-biofilm peptides, which promote degradation of the stringent response nucleotide mediator ppGpp, also significantly reduced abscess sizes while improving animal welfare. Conversely, a stringent response mutant complemented with *relA* (a ppGpp synthase) reverted back to wild-type phenotype and was able to withstand peptide treatment, providing evidence that the stringent stress response is indeed targeted *in vivo*. Next we investigated the potential of using synthetic peptides in combination with antibiotics to treat bacterial abscesses. Treatment of LESB58 infections with a combination of ciprofloxacin and peptide (1018/DJK-5) significantly decreased bacterial burden inside the abscess by more than 100-fold compared to saline-treated controls while stand-alone treatment caused only a 5- to 10-fold reduction. Our findings show that stringent response inhibitors are promising new therapeutic agents and work synergistically *in vivo*, which could dramatically reduce the use of prescribed antibiotics to treat high-density bacterial infections.

Infections & Host-Pathogen Interactions

P138

Pseudomonas aeruginosa in vitro response to novel antibacterial and biological burn wound bandage formulation

Alexandre Luscher[1], Paris Jafari[2], Thissa Siriwardena[3], Tamis Darbre[3], Thilo Köhler[1], Jean-Louis Reymond[3], Damien Maura[4], Alicia Ballok[4], Lee Ann Applegate[2], Wassim Raffoul[2], Laurence Rahme[4], Christian van Delden[1]
[1]Department of Medical Specialties, Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [2]Plastic and Hand Surgery Service, Unit of Regenerative Therapy, Lausanne University Hospitals, Lausanne, Switzerland, [3]Department of Chemistry and Biochemistry, Bern, Switzerland, [4]Department of Surgery, Innovation and Bioengineering, Massachusetts General Hospital and Harvard Medical School, Boston, USA

Background: Burn wound infections are a major issue in the management of burn patients. *Pseudomonas aeruginosa* is one of the main pathogens involved in burn wound infections, causing high morbidity and costs. The Swiss Transmed-funded platform B[5] (Biological, Biodegradable, anti-Bacterial Burn wound Bandages) aims to design novel bandage formulation, combining within a collagen matrix: i) a novel type of antimicrobial peptide (AMP), ii) a quorum-sensing inhibitor (QSi), and iii) wound-healing enhancing progenitor cells (PG). As a member of this platform, our goal was to investigate in vitro the anti-pseudomonas efficiency of this bandage.

Material/methods: Anti-pseudomonas bandage efficacy was assessed by performing growth inhibition assays on agar plates, using different bandage formulations. PG-containing-bandages were soaked in PBS containing sub-cytotoxic concentrations of the antibacterial and QSi compound prior to being deposited on a lawn of *P. aeruginosa* bacteria.

Results: Untreated collagen matrix was partially degraded by *P. aeruginosa*, after 20 h of incubation at 30°C. This degradation was prevented by addition of AMP or polymyxin B. Both AMP and polymyxin B impaired bacterial growth around the matrix, the latter showing the largest zone of inhibition (5mm). QSi alone showed no growth inhibition and did not prevent matrix degradation, but efficiently inhibited production of the QS-regulated virulence factor pyocyanin. Lastly, the AMP showed a faster killing kinetic than polymyxin B**.**

Conclusions: Our data suggest that AMP and polymixin B combined with QSi in a collagen matrix effectively restrict *P. aeruginosa* growth and virulence factor production, while being compatible with progenitor cells.

Infections & Host-Pathogen Interactions

P139

Genomic diversity of bacteraemia-associated *Pseudomonas aeruginosa*

Samuel Kidman[1], Josephine Bryant[1], Kim Judge[1], Sharon Peacock[1,2], Julian Parkhill[1]
[1]The Wellcome Trust Sanger Institute, Cambridge, UK, [2]Department of Medicine, Addenbrooke's Hospital, University of Cambridge; Cambridge University Hospitals NHS Foundation Trust, Cambridge; London School of Hygiene and Tropical Medicine,, London, UK

Multi-drug resistant (MDR) *Pseudomonas aeruginosa* bacteraemia infections are becoming increasingly common worldwide and are associated with a 20-50% mortality rate.

In this study, 293 *P. aeruginosa* samples were isolated from bacteraemia patients and whole-genome sequenced. Of these, 79 isolates were obtained from a systematic survey of UK hospitals by the British Society for Antimicrobial Chemotherapy between 2001 and 2011, and selected for a MDR phenotype. The remaining 213 isolates were consecutive available isolates from Cambridge University Hospitals between 2006 and 2013, and selected irrespective of drug resistance. All isolates were subjected to drug susceptibility testing.

The genomic data revealed a diverse population structure. 75% of Multi-Locus Sequence Types (ST) were represented only once, and only a few over-represented STs were identified. Of these successful STs, all are well-characterised, globally-disseminated "high risk" clones, linked with extensive resistance. 35% of the 293 isolates were defined as MDR and 3% of isolates were resistant to all but one antibiotic. 32% of all isolates showed evidence of resistance to one or more of the carbapenems, the current drug of last resort. The genetic cause of the carbapenem resistance was identified as loss-of-function mutations in the OprD porin, consistent with previously published results.

This study indicates a non-clonal population structure of *P. aeruginosa*, with no widespread bacteraemia-associated epidemic clones. With MDR *P. aeruginosa* becoming more common, an understanding of population structure and resistance mechanisms will prove helpful when designing interventions to prevent transmission, and therapies and treatments to combat MDR infections.

Infections & Host-Pathogen Interactions

P140

Psl produced by mucoid *Pseudomonas aeruginosa* contributes to the establishment of biofilms and immune evasion

Christopher Jones, Daniel Wozniak
Ohio State University, Columbus, OH, USA

Despite years of research and clinical advances, chronic pulmonary infections with mucoid *Pseudomonas aeruginosa* remain the primary concern for cystic fibrosis patients. Much of the research on these strains has focused on the contributions of the polysaccharide alginate, however it is becoming evident that the neutral polysaccharide Psl also contributes to biofilm formation and the maintenance of chronic infections. Here, we demonstrate that Psl produced by mucoid strains has significant roles in biofilm structure and evasion of immune effectors. Though mucoid strains produce less Psl than nonmucoid strains, Psl that is produced is functional since it mediates adhesion to human airway cells and epithelial cell death. Additionally, Psl protects mucoid bacteria from opsonization and killing by complement components in human serum. Psl production by mucoid strains stimulates a proinflammatory response in the murine lung, leading to reduced colonization. To determine the relevance of these data to clinical infections, we tested Psl production and biofilm formation of a panel of mucoid clinical isolates. We demonstrated three classes of mucoid isolates, those that produce Psl and form robust biofilms, those that did not produce Psl and have a poor biofilm phenotype, and EPS redundant strains. Collectively, these experiments demonstrate that Psl contributes to the biofilm formation and immune evasion of many mucoid strains. This is a novel role for Psl in the establishment and maintenance of chronic pulmonary infections by mucoid strains.

Infections & Host-Pathogen Interactions

P141

Mixed communities of mucoid and non-mucoid *Pseudomonas aeruginosa* exhibit enhanced resistance to host antimicrobials

Sankalp Malhotra[1,2], Dominique Limoli[3], Anthony English[2], Daniel Wozniak[1]
[1]The Ohio State University College of Medicine, Columbus, OH, USA, [2]The Ohio State University, Center for Microbial Interface Biology, Columbus, OH, USA, [3]Geisel School of Medicine at Dartmouth, Department of Microbiology and Immunology, Hanover, NH, USA

Mucoid and nonmucoid revertants of *Pseudomonas aeruginosa* (*P.a.*) are often co-isolated from the cystic fibrosis (CF) lung, suggesting a selective advantage for the co-existence of these variants. Here, we hypothesized that in consortia, mucoid/non-mucoid *P.a.* may be differentially susceptible to neutrophil products, exhibiting heightened resistance to host factors.
We found that a clinical mucoid *P.a.* isolate (FRD1) was 10-fold more resistant to a cationic antimicrobial peptide, LL-37, compared to isogenic, non-mucoid variants of FRD1. However, FRD1 was significantly more sensitive to hydrogen peroxide (H_2O_2) than FRD2, a non-mucoid revertant of FRD1 with a mutation in the sigma factor gene, *algT*. This phenotype was conserved in other clinical mucoid *P.a.* isolates and their isogenic *algT* revertants. Filter-sterilized supernatants from FRD2 were sufficient to protect FRD1 from H_2O_2-stress; this protection was abrogated with supernatants derived from a FRD2 catalase (*katA*) mutant. A FRD1 non-mucoid variant with a mutation in *algR*, a transcription factor downstream of *algT*, also exhibited enhanced resistance to H_2O_2. *katA* transcript and catalase protein activity was increased in both FRD1*algT* and *algR* mutants, suggesting a novel *katA* repression mechanism. Furthermore, the release of catalase into the extracellular space was dependent on *lys*, which encodes a phage endolysin implicated in autolysis/eDNA release.
Importantly, co-cultures of mucoid/non-mucoid variants of FRD1 exhibited greater resistance to both H_2O_2 and LL-37 than in mono-culture, suggesting an advantage for multi-variant *P.a.* communities in evading neutrophil products. These data provide an important rationale to study the interaction of mucoid/non-mucoid variants as contributors to CF pathology.

Infections & Host-Pathogen Interactions

P142

"A gut feeling"; why *Pseudomonas aeruginosa* stands up and fights

Stephanie Flynn[1], F. Jerry Reen[1], Stephen Stick[3,4], Sarath Ranganathan[5], Fergal O'Gara[1,2]
[1]BIOMERIT Research Centre, School of Microbiology, University College Cork- National University of Ireland, Cork, Ireland, [2]School of Biomedical Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth, Australia, [3]Division of Clinical Sciences, Telethon Institute for Child Health Research and School of Paediatrics and Child Health, University of Western Australia, Perth, Australia, [4]Department of Respiratory Medicine, Princess Margaret Hospital for Children, Perth, Australia, [5]Department of Respiratory Medicine, Royal Children's Hospital, Melbourne, Australia

Pseudomonas aeruginosa is a leading cause of nosocomial infections, particularly in patients suffering from Cystic Fibrosis (CF). Infection with this key respiratory pathogen, where it dominates the lung microbiome, is the leading cause of morbidity and mortality in the CF cohort. However, the host factors that trigger the dominance of this opportunistic pathogen remain unclear. Bile aspiration, a consequence of gastro-esophageal reflux, has recently emerged as a major co-morbidity in CF. A large prospective study has demonstrated that bile is a central host factor capable of shaping the CF lung microbiome and promoting the emergence of proteobacterial pathogens including *P. aeruginosa*. Aspirated bile was correlated with increased inflammation and pathogen dominated microbiomes. A virtual metagenomics approach revealed an enrichment of virulence related functions potentially explaining the dominance of *P. aeruginosa* within the CF lung.
P. aeruginosa has been shown to undergo extensive genomic adaptation in order to persist in the CF lung environment and we have found that bile can act as a significant selective pressure for genetic adaptation further enhancing its competitive advantage within the lung microbiome. Bile also increases antibiotic tolerance and biofilm formation consistent with the transition towards a chronic lifestyle. This is underpinned by metabolic flux and second messenger signaling within the cell. The aspiration of bile in respiratory patients has potential as a new clinical biomarker for the development of targeted therapeutics. Early intervention could reduce the onset of chronic infections in at risk patients.

Infections & Host-Pathogen Interactions

P143

Artificial tear solution increases virulence determinants of *Pseudomonas aeruginosa* but at a fitness cost.

Victoria Rimmer, Carole Maldonado-Codina, Philip Morgan, Curtis Dobson, Bianca Price
The University of Manchester, Manchester, UK

The tear film is an important barrier against *Pseudomonas aeruginosa* keratitis. This study aimed to determine the effects of an artificial tear solution (TF) on the growth and virulence of PA01.

TF comprised a complex salt solution supplemented with tear proteins including lactoferrin, lysozyme mucin and IgG. Growth curves in TSB and TF were performed and cultures were harvested mid-stationary phase (5 hours in TF and 9 hours in TSB) for protease and haemolysis assays. Attachment and MBEC assays were also performed.

Growth attenuation of 2.97 logs was observed for PA01 in TF compared to TSB. Average protease and haemolysis activity (cm^2/cell) in TSB were measured at $6.2x10^{-10}$ and $3.2x10^{-10}$ respectively. This significantly increased in TF to $4.7x10^{-7}$ and $2.3x10^{-7}$ respectively ($P<0.0001$). PA01 was found to display both α and β-haemolysis. Attachment and MBEC (OD_{590}/cell) in TSB were measured at $2.0x10^{-9}$ and $8.01x10^{-10}$ respectively, compared to $9.1x10^{-8}$ and $6.0x10^{-8}$ in TF. Further assays were performed for PA9027 with similar results, however no β-haemolysis was observed.

We conclude that a combination of the nutrient limited medium and tear proteins induces increased virulence determinants despite attenuated growth. The antimicrobial proteins have little or no effect on *P. aeruginosa* in these conditions, likely due to their low concentration. Indeed, they are the only carbon source to support growth and this may be reflected by increased protease production. These data support the assertion that exposure to TF does not inhibit *Pseudomonas* but primes it for invasion of the cornea.

Infections & Host-Pathogen Interactions

P144

Host-pathogen metabolic signature of P. aeruginosa: diagnostic value and insight into pathogenesis

Jane Hill[1], Mavra Nasir[1], Heather Bean[2], Edith Zemanick[3], Alix Ashare[1], Alex Gifford[1], Agnieszka Smolinksa[4]
[1]Dartmouth College, Hanover, NH, USA, [2]Arizona State University, Tempe, USA, [3]University of Colorado, Aurora, USA, [4]Maastricht University, Maastricht, The Netherlands

P. aeruginosa (Pa) is common Gram-negative bacterium responsible for various diseases in plants and animals. Pa is metabolically very flexible and thus it dominates in many polymicrobial infection scenarios. The continuing rise of multi-drug-resistant Pa has led to its status as a high priority pathogen around the world, particular in the context of lung infection.

In part 1 of the presentation, we focus on the context of cystic fibrosis. We characterized the volatile metabolites in breath, sputum, and bronchoalveolar lavage fluid from persons with cystic fibrosis (combined n > 300; cross-sectional and longitudinal samples) infected with Pa and other organisms. Pa-specific features were identified across sample types, with exhaled breath being the richest in putative biomarkers. We determined the identity of approximately 50 core volatile molecules. 30 of these volatile molecules (dominated by hydrocarbons, ketones, and alcohols) hint at metabolic endpoints or excess in the lung ecosystem. These molecules could be considered biomarkers of Pa, which could then be used to rapidly detect P. aeruginosa in the clinic as a faster, non-invasive alternative to poorly correlated oropharyngeal swab cultures as well as generate gross insight into the lung ecosystem during infection.

In part 2 of the presentation, we report on a preliminary analysis of the potential for in vitro-derived volatile metabolites from clinical isolates (from multiple sampling sites e.g., lung, skin, urinary tract) to systematically identify clinically-relevant patterns of antibiotic resistance. We report on and contextualize possible links to biochemical pathways in Pa.

Infections & Host-Pathogen Interactions

P145

Pseudomonas aeruginosa PAO1 is impaired in anaerobic growth and infectivity due to a single point mutation in class III ribonucleotide-reductase promoter region Pseudomonas aeruginosa PAO1 is impaired in anaerobic growth and infectivity compared to clinical isolates due to a single point mutation in the class III ribonucleotide reductase promoter region.

Anna Crespo[1], Joan Gavaldà[2], Esther Julián[3], Eduard Torrrents[1]
[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, [2]Infectious Diseases Research Laboratory. Vall d'Hebron Hospital., Barcelona, Spain, [3]3Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Bellaterra, Spain

Pseudomonas aeruginosa strain PAO1 has become the reference strain in many laboratories. One enzyme that is essential for its cell division is the ribonucleotide reductase (RNR) enzyme that supplies the deoxynucleotides required for DNA synthesis and repair. *P. aeruginosa* is one of the few microorganisms that encodes three different RNR classes (Ia, II and III) in its genome, enabling it to grow and adapt to diverse environmental conditions, including during infection.

In this work, we demonstrate that a lack of RNR activity induces cell elongation in *P. aeruginosa* PAO1. Moreover, RNR gene expression differs among *P. aeruginosa* strains, with class III highly expressed in *P. aeruginosa* clinical isolates relative to the laboratory *P. aeruginosa* PAO1 strain. A single point mutation was identified in the class III RNR promoter region that disrupts its anaerobic transcription by the Dnr regulator. The induction of class III RNR expression allows *P. aeruginosa* PAO1 anaerobic growth and increases its virulence to resemble that of clinical strains. Therefore, our results demonstrate that *P. aeruginosa* PAO1 is adapted to laboratory conditions and is not the best reference strain for anaerobic or infection studies.

Infections & Host-Pathogen Interactions

P146

Aminoglycoside efficacy against *Pseudomonas aeruginosa* is potentiated by conditioned medium of three-dimensional lung epithelial cells

Aurelie Crabbe[1], Lisa Ostyn[1], Simon Daled[2], Maarten Dhaenens[2], Charlotte Rigauts[1], Dieter Deforce[2], Tom Coenye[1]
[1]Ghent University - Laboratory of Pharmaceutical Microbiology, Ghent, Belgium, [2]Ghent University - Laboratory of Pharmaceutical Biotechnology, Ghent, Belgium

Novel treatment approaches against *Pseudomonas aeruginosa* are needed. We previously demonstrated that several aminoglycosides showed an increased efficacy at inhibiting *P. aeruginosa* PAO1 biofilm formation in an *in vivo*-like three-dimensional (3D) lung model, as compared to on plastic.

The aim of the present study was to gain insights in the host factor(s) that enhance the efficacy of aminoglycosides. Our results indicate that conditioned medium of 3D lung epithelial cells (3D-CM) improves the efficacy of aminoglycosides against *P. aeruginosa* PAO1 biofilm formation, suggesting the involvement of (a) secreted host compound(s). In contrast, conditioned medium of 2D (monolayer) cells did not potentiate aminoglycosides. Tobramycin activity was also potentiated by 3D-CM for 6/8 (75%) tested *P. aeruginosa* strains (including the CF isolates LESB58 and AA44), and for 3/3 (100%) strains belonging to other *Pseudomonas* spp. (including *P. putida* KT2440). In contrast, only 2/7 (30%) non-*Pseudomonas* strains were more susceptible to tobramycin or gentamicin in the presence of 3D-CM.

Preliminary characterization indicates that the compound(s) are heat- and proteinase K sensitive and are smaller than 3kDa. The nature of the compound(s) that caused an increased aminoglycoside efficacy against *P. aeruginosa* biofilm formation is currently under investigation.

Infections & Host-Pathogen Interactions

P147

Multiple post-segregational killing systems prevent the accumulation of high-frequency deletion events in virulence plasmids of *Pseudomonas syringae*

Maite Añorga[1], Leire Bardaji[1], Myriam Echeverría[1], Cayo Ramos[2], Jesús Murillo[0]
[1]Departamento de Producción Agraria, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Pública de Navarra, Pamplona, Spain, [2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias, Ca, Málaga, Spain

The *Pseudomonas syringae* complex includes several species of gram negative bacteria causing economically relevant diseases in practically all cultivated plants. *P. syringae* pv. savastanoi NCPPB 3335 contains two virulence plasmids that are essential to cause aerial tumours on olive (*Olea europaea*). From these, pPsv48C (42 kb) is extremely stable, contains two independent functional replicons (*repA* and *repJ*) and 29.5% of its sequence occupied by putative mobile elements. This plasmid spontaneously suffers the deletion of an 8.3 kb fragment, with a frequency higher than 10^{-3}, by recombination between two direct copies of the miniature transposable element MITE*Psy2*. We also showed that insertion sequence IS*801* promotes the occurrence of deletions in pPsv48C by one-ended transposition with an average frequency higher than 10^{-4}, half of them resulting in the loss of a virulence gene. These deletion derivatives were maintained in the population by replication mediated by *repJ*, which is adjacent to IS*801*. We demonstrated that IS*801* also promotes deletions of variable size in the other virulence plasmid from strain NCPPB 3335, either by recombination or failed transposition. The accumulation of these type of deletions *in vivo* is prevented by the occurrence in these plasmids of several functional post-segregational killing systems. Together, our results indicate that maintenance of pathogenicity genes in bacterial populations of *P. syringae*, and their allocation to plasmids, results from the combination of diverse antagonistic evolutionary forces that are unrelated and independent of the pathogenicity process.

Infections & Host-Pathogen Interactions

P148

Genomic analysis of a multidrug resistant Pseudomonas aeruginosa outbreak in a tertiary hospital in Spain

Paula Ruiz-Hueso[1,2], Victoria Sánchez-Hellín[3], Melanie Abreu[3], Fernando González-Candelas[1,2]
[1]FISABIO-Universidad de Valencia, Valencia, Spain, [2]CIBERESP, Valencia, Spain, [3]Hospital General Universitario de Elche, Elche, Alicante, Spain

Background
Nosocomial outbreaks of multidrug resistant strains of P. aeruginosa represent a serious problem. We have used WGS to characterize one such outbreak to facilitate the adoption of control measures.

Methods
We analyzed 52 samples (47 clinical, 5 environmental) taken between 12/2014 and 2/2016 at Hospital Universitari d'Elx. The isolates were sequenced by Illumina MiSeq 2x300bp. MLST typing and search for resistance genes were performed with SRST2. Reads were mapped to a nearby reference genome (strain H27930) using BWA and Samtools and, from the alignment of the genomic core, a first phylogenetic tree was obtained with RAxML.

Results
42 samples (including the 5 environmental samples) belong to ST175, and the rest are from ST1212 (n=2), ST235 and ST357, and to new STs (n=6). The phylogenetic tree constructed groups the new STs within ST175, leading to an outbreak of 43 patients. We detected 1,576 SNPs among the outbreak sequences (core genome), and 27,651 SNPs between this clade and the nearest samples, the two 34 of ST1212. In addition, these two samples, obtained the same day from different patients, differed in only 142 SNPs.

Conclusions
The genomic analysis of outbreak-related isolates was instrumental in delimiting its extent and it also revealed a substantial variation, possibly indicating the presence of several foci of infection with slightly different strains. This is information is very valuable in the adoption of control measures.

Infections & Host-Pathogen Interactions

P149

Phenotypic diversity within *Pseudomonas aeruginosa* populations causing urinary tract infections

John Newman[1], Rachel Floyd[2], Jo Fothergill[1]
[1]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

Urinary tract infections (UTIs) are the most common healthcare acquired infection. A small but significant portion of UTIs, both healthcare acquired and community associated, are caused by *Pseudomonas aeruginosa*. *P. aeruginosa* is more frequently detected in complicated UTIs and shows a higher prevalence of resistance to carbapenems than other UTI-causing pathogens. The biofilm lifestyle of *P. aeruginosa* is believed to play a critical role in its antibiotic resistance. Studies of clinical *P. aeruginosa* in chronic lung infections have discovered remarkable within-patient phenotypic and genotypic diversity however bacterial populations during UTI have not been studied in detail. Therefore, the aim of this research was to study the heterogeneity within clinical samples collected from 10 patients suffering from uropathogenic *P. aeruginosa* infections.

A representative population of 40 isolates were collected from each of the ten patient samples and subjected to phenotypic assays including pyocyanin assays, hypermutability assays, minimal growth assays and antimicrobial susceptibility tests (ASTs). Random amplification of polymorphic DNA (RAPD) PCR was conducted on all isolates to determine whether there was genotypic homogeneity within samples in spite of their phenotypic heterogeneity. Samples tested exhibited homogenous genotypes (as determined by RAPD PCR) while displaying heterogeneous phenotypes. Natural populations will be recreated in vitro using artificial urine medium to determine the contribution of co-existing phenotypic variants. Phenotypic variation within samples could play a crucial role aiding the adaptation of mature *P. aeruginosa* biofilms to the urinary tract. Increased understanding of UTI dynamics may lead to more effective treatment regimens for recurrent UTIs.

Infections & Host-Pathogen Interactions

P150

Naturally-acquired mutations in *pmrB* confer increased *in vivo* fitness via resistance to host antimicrobials and modulation of the host environment.

Laura Bricio-Moreno[1], Elaine Waters[1], Victoria Sheridan[1], Adrita Charaborty[1], James Dunn[1], Stuart Armstrong[2], Craig Winstanley[1], Aras Kadioglu[1], Dan Neill[1], Jo Fothergill[1]
[1]Department of Clinical Infection, Microbiology & Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

Pseudomonas aeruginosa persistence is a key feature of lung infections. The upper airways may play an important role as a silent reservoir of P. aeruginosa, for adaptation during chronic infection, leading to persistence despite immune pressures and intensive therapy.

We have previously described a murine inhalation model of chronic respiratory infection which can be used to study early adaptation events. Here, we characterise a naturally acquired genetic change in pmrB, which confers an advantage in the lungs. Mutations in pmrB, encoding the sensor kinase protein of the PmrAB two-component system, are acquired early in infection and result in phenotypic changes leading to increased resistance to the host antimicrobial lysozyme and increased susceptibility to a variety of antibiotics. Mutations in pmrB were also found in isolates from patient samples and gave rise to the same altered phenotype. Proteomics data on wild type (WT) and a pmrB knock-out (KO) strains revealed significantly altered protein profiles. Proteins involved in LPS, lipid A biosynthesis and phenazine production were upregulated in the WT whereas proteins involved in adherence, lysozyme resistance and CFTR inhibition were upregulated in the KO.

pmrB is a global regulator in P. aeruginosa and acquisition of a single nucleotide change leads to a significantly altered phenotype which confers a competitive advantage in vivo during lung infection. These findings suggest that naturally-acquired mutations in pmrB may confer the ability to modulate the host environment through CFTR inhibition and to resist host immune responses but with an associated cost via enhanced susceptibility to antibiotics.

Infections & Host-Pathogen Interactions

P151

Alkyl-quinolone-dependent quorumsensing controls prophage activation, autolysis and antibiotic resistance in *Pseudomonasaeruginosa* biofilm

Giulia Giallonardi[1], Marco Messina[1], Emanuela Frangipani[1], Stephan Heeb[2], Miguel Càmara[2], Fabio Polticelli[1], Paolo Visca[1], Livia Leoni[1], Giordano Rampioni[1], Paul Williams[2]
[1]University Roma Tre, Departement of Science, Rome, Italy, [2]University of Nottingham, Centre for Biomolecular Sciences, Nottingham, UK

The *pqs* quorum sensing (QS) system of *Pseudomonas aeruginosa* directs the production of diverse alkyl-quinolones including 2-heptyl-4-hydroxyquinoline (HHQ), 2-heptyl-3-hydroxy-4-quinolone (PQS) and 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO). While HHQ and PQS act as QS signals, HQNO has no effect on *P. aeruginosa* transcriptome and its physiological role is unclear. Interestingly, a *P. aeruginosa pqsL* mutant, impaired in HQNO synthesis, shows autolysis plaques when grown as colony biofilm. This phenotype is worth studying in detail, because it is frequently found in *P. aeruginosa* clinical isolates.

Here we show that HHQ accumulation as a result of mutating *pqsL*, a gene required for HQNO biosynthesis, induces transition of the Pf4 prophage from the lysogenic to the lytic phase. The consequent autolysis increases the antibiotic resistance of *P. aeruginosa* PAO1 biofilms, likely as a consequence of increased release of extracellular DNA. Colony biofilms formed by 126 *P. aeruginosa* clinical isolates were tested, and about 40% exhibited an autolytic phenotype. In the majority of these isolates the *pqsL* gene was deleted or mutated. The wild type phenotype could be restored in the mutated strains by complementation with the wild type *pqsL* gene.

Overall, these data indicate that PqsL-mediated synthesis of HQNO might serve as a sink to avoid HHQ accumulation and consequent autolysis in wild type *P. aeruginosa*. However, the autolytic phenotype is associated with biofilms and increased antibiotic resistance and this could confer an advantage during chronic infections. Hence, loss of *pqsL* expression or functionality might represent a pathoadaptative mutation in *P. aeruginosa* clinical isolates.

Infections & Host-Pathogen Interactions

P152

Hormone interactions with *Pseudomonas aeruginosa* urinary tract infections

Hussain Ebrahim[1], Rachel Floyd[2], Jo Fothergill[1]
[1]1Department of Clinical Infection, Microbiology and Immunology, Institute of Infection & Global Health, University of Liverpool, UK, Liverpool, UK, [2]2Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, UK, Liverpool, UK

Introduction: Urinary tract infections (UTIs) are the second most common infections around the world causing significant morbidity and mortality. These infections are classified into uncomplicated and complicated, with the latter associated with higher levels of multi-drug resistance, particularly in infections caused by *Pseudomonas aeruginosa*. Studies have shown that hormones such as estrogens exacerbate *P. aeruginosa* infections in cystic fibrosis patients and are involved in modulating exopolysaccharides. Thus, an investigation into the impact of hormones on UTI *P. aeruginosa* pathogenesis is warranted.

Methods: Gene expression of a panel of *P. aeruginosa* virulence factors was studied after exposure of bacteria to 10nM estradiol and testosterone In addition, 15 *P. aeruginosa* UTI isolates were examined by confocal microscopy to study the effects of hormones on biofilm architecture. The biofilms were grown statically for 48h with the addition of 10 nm estradiol, testosterone or progesterone. Biofilms were stained with Baclight® kit and 3D Images were produced using Imaris. Biofilm architecture was analysed using Comstat.

Results: These results suggest that the effect of hormones on expression of genes involved in biofilm formation, quorum sensing and pathogenicity is strain and hormone dependent. Analysis of the 3D images showed reduction of roughness-coefficient by progesterone.

Conclusions: Hormones modify virulence gene expression and biofilm architecture of some populations of UTI *P. aeruginosa*. Due to the observed variations in isolates, some patients maybe more susceptible than others to recurrent and persistent infections. Understanding the factors that may increase bacterial persistence and pathogenesis might lead to alternative management and treatment strategies.

Infections & Host-Pathogen Interactions

P153

Emergence and maintenance of extrachromosomal elements within chronic cystic fibrosis infections

Cristina Amador, Anders Norman, Lars Jelsbak
Technical University of Denmark, Kongens Lyngby, Denmark

Chronic cystic fibrosis (CF) airway infections offer unique opportunities for fundamental investigations related to microbial evolutionary dynamics, diversity and interactions within a natural polymicrobial ecosystem. CF patients are predisposed to airway infections from a wide range of microbial species - with Pseudomonas aeruginosa as the major contributor to morbidity and mortality.

In our laboratory, we have previously described two highly persistent and transmissible P. aeruginosa lineages among CF patients - namely DK1 and DK2. Here we focus on a sub collection of 30 P. aeruginosa isolates sampled from a particular CF patient over a 25-year time span. Whole-genome sequencing allowed phylogeny reconstruction and the discovery of a new clone type exhibiting a mosaic genome. This novel clone type, named DK1.5, consists of a DK2 backbone with a 216-Kb region acquired by horizontal gene transfer from the DK1 clone.

Moreover, we identified a 25-kb duplication in four DK2 isolates. We performed specific PCR to discriminate between lineal or circular DNA which, followed by molecular purification, evidenced that the duplication was provided as an extrachromosomal feature. The element comprises 19 genes, including the operon opp –that has been proven to be involved in the uptake of peptidyl nucleoside antibiotics in P. aeruginosa PA14 and PAO1. We hypothesize that an additional copy of the encoded genes might be associated with antibiotic resistance.
Intriguingly, no replication cassettes have been found so far –despite the element persisted for four years. The mechanisms for replication and transmission of this extrachromosomal element will be thereby further discussed.

Infections & Host-Pathogen Interactions

P154

The role of Type VI Secretion System on the competitiveness of *P. aeruginosa* clinical isolates

Silvia D'Arcangelo[1], Irene Bianconi[1], Jo Fothergill[2], Thomas Wood[3], Alain Filloux[3], Craig Winstanley[2], Olivier Jousson[1]
[1]Centre for Integrative Biology, University of Trento, Trento, Italy, [2]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [3]Imperial College London, London, UK

Pseudomonas aeruginosa infection in Cystic Fibrosis (CF) occurs via primary colonization of the airway followed by chronic infection with loss of function mutations.

In previous studies, two CF isolates from the same patient, one isolated early in the infection (TNCF_23), and a clone isolated three years later (TNCF_175), plus VrPa97, an acute-infection isolate phylogenetically closely related to the CF isolates, were analyzed. We found that TNCF_175 carried a stop mutation in *tssK3*, part of the H3-T6SS cluster encoding a Type VI Secretion System (T6SS).

To gain insight on the role of T6SS impairment in the adaptive process within the CF lung, we set out experimental approaches and further characterized the contribution of the H3-T6SS in bacterial competition and infection. An assay involving *E. coli* strain DH5α as a target of the T6SS machinery was performed to assess its survival rate when co-cultured with the three strains; TNCF_23 and VrPa97 killed *E. coli*, but TNCF_175 was unable to do so, indicating impairment of the T6SS. Strains were then grown alone and in pairs in Artificial Sputum Medium mimicking CF lung environment. When TNCF_175 was co-cultured with VrPa97 its growth rate dramatically decreased. TNCF_175 survived when cultured with TNCF_23 but its growth was reduced. In addition, the killing of *Caenorhabditis elegans* by TNCF_175 was remarkably reduced compared to TNCF_23 and VrPa97.

Our data indicate a role for the T6SS in the competitiveness of CF isolates. To elucidate the mechanisms underlying the observed phenotypes, clean mutants in *tssK3* have been created.

Infections & Host-Pathogen Interactions

P155

Killing of *Staphylococcus aureus* by a soluble factor secreted by a *Pseudomonas aeruginosa wspF* mutant

Bartosz Gdaniec[1], Thilo Köhler[1], Mikaël Tognon[1], Alexandre Luscher[1], Angus Buckling[2], Christian van Delden[1]
[1]Department of Medical Specialties, Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [2]Environment and Sustainability Institute, University of Exeter, Penryn, UK

Background: Pulmonary disease is the primary cause of morbidity and mortality in patients with cystic fibrosis (CF). CF airway infections are polymicrobial, *Pseudomonas aeruginosa* and *S. aureus* being the most prevalent organisms. How *P. aeruginosa* adapts to other co-colonizing species has not been studied in detail.

Methods: Genomes of *P. aeruginosa* populations having evolved for 15 days in presence and absence of *S. aureus* were sequenced and compared to the ancestor genome. Killing assays were performed on 10E6 bacterial cells and culture supernatants mixed in a 1:1 (vol/vol) ratio. CFU were counted after 24h static incubation at 37 °C.

Results: We developed an *in vitro* co-evolution assay, in which we evolved *P. aeruginosa* in the presence and absence of *S. aureus* for 15 days. In both conditions, the most frequent mutation occurred in the Wsp signaling system of *P. aeruginosa*. Both a *wspA* and a *wspF* mutant showed a > 10E4 reduction in *S. aureus* CFU. Substantial killing (> 100-fold reduction in CFU) was also observed with *S. epidermidis* and several Gram-negative bacteria. The killing activity is an extracellular soluble factor, which is resistant to heat and Proteinase K treatments. Deletion of the Quorum sensing (QS) regulator genes *lasR*, *rhlR* and *mvfR* (*pqsR*), abolished the killing activity of the *wspF* mutant, suggesting a QS-dependent process.

Conclusion: WspF mutants, obtained during an in vitro evolution assay, showed killing activity against *S. aureus* in culture supernatants. The factor is heat and proteinase K resistant and regulated by all three QS-systems.

Infections & Host-Pathogen Interactions

P156

Targeting host proteins: a strategy for treating antibiotic-resistant *Pseudomonas aeruginosa* infections

Jehan Alrahimi, Rahaf Issa, Mark Thomas, Peter Monk
The University Of Sheffield, Sheffield, UK

Abstract

Background

Infection of host tissue is commonly initiated by adherence of bacteria, such as *Pseudomonas aeruginosa,* to cell surface membrane proteins, sugar or lipids. One class of proteins known to play a major role in cellular processes, such as adhesion and migration are tetraspanins, specifically CD9, CD81 and CD151. Prevention of this process could provide a new therapeutic strategy for treating infectious diseases. This work aims to investigate the potential of using recombinant tetraspanin extracellular domains (EC2), anti-tetraspanin antibodies and EC2-derived peptides as anti-adhesives in treating *P. aeruginosa* infections.

Methods and results

Expression profiles of different tetraspanins in human epithelial cell lines, A549 and HEC-1-B, were quantified using flow cytometry. Monolayers of A549 and HEC-1-B were pre-treated with the tetraspanin reagents for 30 minutes, before infection with GFP-tagged *P. aeruginosa* (PAO1) for 1 hour. Bacterial adhesion was determined by counting the number of bacteria per infected cells with fluorescence microscopy. Analysis revealed the anti-adhesive properties of these tetraspanins (anti-tetraspanin antibodies and recombinant proteins). In particular, anti-CD9, CD63 and CD81 reduced PAO1 adhesion to A549 cells by approximately 45%, 40% and 52%, respectively. Of the peptides tested, three reduced bacterial attachment by 70-90%. Furthermore, these tetraspanin-derived agents reduced the cytotoxicity of PAO1.

Conclusions

In conclusion, anti-tetraspanin antibodies, recombinant proteins and EC2-derived peptides have the potential to inhibit adherence of *P. aeruginosa*.This work has identified the role of tetraspanins in bacteria-host cell adhesion, and the possible use of tetraspanin reagents as therapeutics for treating *P. aeruginosa* infection.

Infections & Host-Pathogen Interactions

P157

The *Pseudomonas aeruginosa* PUMA3 cell-surface signalling system is induced in response to the host

Joaquín-Rodrigo Otero-Asman[1], José Miguel Quesada[1], Cristina Civantos[1], Kinki Jim[2], Alain Ocampo-Sosa[3], Astrid Van der Sar[2], Wilbert Bitter[2], Marian Llamas[1]
[1]CSIC, Granada, Spain, [2]VuMC, Amsterdam, The Netherlands, [3]HUMV-IDIVAL, Santander, Spain

The pathogenicity of the human pathogen *Pseudomonas aeruginosa* largely depends on the ability of the bacterium to sense and respond to the host. The cell-surface signalling (CSS) system known as PUMA3 was earlier proposed to play a role in this. By genomic analyses we showed that PUMA3 controls the expression of two secretion systems (T2SS and T5SS) needed for *P. aeruginosa* virulence. The PUMA3 system is constituted by the VreA receptor, the VreR anti-sigma factor and the σ^{VreI} extracytoplasmic function sigma factor. The genes encoding these three proteins are arranged in an operon whose expression is induced in phosphate (Pi) starvation conditions in a PhoB-dependent manner. Recently, we have demonstrated that this transcriptional regulator is not only required for PUMA3 gene expression but also to modulate the activity of σ^{VreI}. In fact, presence of this regulator is an absolute requirement for σ^{VreI} to complex the DNA and initiate transcription of the PUMA3 regulon.
In this work we have analysed the activity of the PUMA3 system in vivo using both zebrafish embryos and the A549 lung epithelial cell line as *P. aeruginosa* hosts. Using red fluorescence reporter constructs we have observed induced expression of the PUMA3 regulon during *P. aeruginosa* infection. This induction depends on PUMA3 since it is completely abolished in a vreI sigma factor mutant. Our results demonstrate for the first time that the PUMA3 CSS system allows *P. aeruginosa* to detect and respond to animal hosts, which has important clinical implications.

Infections & Host-Pathogen Interactions

P158

The effectiveness of organic acids at inhibiting growth and biofilm formation in *Pseudomonas aeruginosa*

Francesca Bushell[1], Gareth Hughes[1,2], Peter Lund[1]
[1]University of Birmingham, Birmingham, UK, [2]University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

Acetic and citric acid are being used increasingly as topical treatments against opportunistic infection in burn wounds, but detailed studies on their efficacy are rare and the potential of other organic acids remains unexplored. In this study, we have compared the effectiveness of eight organic acids on growth and biofilm formation of four strains of *Pseudomonas aeruginosa*: lab strain PA01 and clinical isolates PA1054, PS919 and PS1586. All strains were grown in either supplemented M9 media or Iso-sens media, plus each organic acid at concentrations from 5mM to 20mM across a decreasing pH range. For all strains MIC and MBIC were determined. For PA01 and PA1054, growth kinetics were analysed using a mathematical model and biofilm was quantified. There was an organic acid-dependent response, with some acids being more effective at inhibiting growth and biofilm formation than others. As expected, the effect was highly pH dependent, with all organic acids becoming more effective at inhibiting growth and biofilm as the pH decreases. Biofilm formation increased as overall growth decreased, until growth was completely inhibited at which point biofilm formation was also inhibited. There was also a strain dependent response, with the clinical isolate PA1054 being more tolerant of organic acids at mild pH than the lab strain PA01. Our data support the use of organic acids on burn wounds as topical anti-microbials at lower concentrations than currently used in clinical practice. The potential for biofilm elimination, and the importance of strain variation, need to be considered in designing further studies.

Infections & Host-Pathogen Interactions

P159

Functional analysis of the Hxc-Type II Secretion System of *Pseudomonas aeruginosa* and its role in the bacterial virulence during infection in the lungs of cystic fibrosis patients

Philemon Gyasi-Antwi[1], Nora Wolff[2], Farah Hussasin[1], James Lazenby[3], Stephan Heeb[1], Miguel Camara[1], Luisa Martinez-Pomares[1]
[1]School of Life Sciences, University of Nottingham, Nottingham, UK, [2]Academic Medical Center (AMC), Amsterdam, The Netherlands, [3]University of Technology Sydney, Sydney, Australia

Pseudomonas aeruginosa (PA) virulence is multifactorial; combining a variety of cell-associated structures and extracellular secretions. Aside the well characterised conventional Xcp Type II secretory system (T2SS) in PA, the bacterium possesses another T2SS known as Hxc (**H**omologous to **xc**p) system. While the Xcp T2SS secretes diverse exoproteins such as elastase, lipase, phospholipases or exotoxin A, the Hxc T2SS is known to transport one product, low molecular weight alkaline phosphatase (LapA) under phosphate-limiting conditions.

Using standard procedure of allelic exchange, we have constructed an in-frame deletion mutant lacking the hxc-operon (Δ*Hxc*) in a PAO1-L(Lausanne) background. Our phenotypic studies indicate that bacterial growth, biofilm formation and pyocyanin expression are not detectably affected by the Hxc-T2SS deletion. Addition of exogenous phosphate to culture medium significantly increased bacterial growth as well as biofilm formation, but pyocyanin secretion was markedly reduced in both the mutant and the wild type PAO1-L.

Using an in vitro infection model, we show that the PAO1-LΔ*Hxc* mutant grows better than the wild type after contact with differentiated and undifferentiated human bronchial epithelial cells at a multiplicity of infection of 50. This finding indicates how phosphate limitation or acquisition by PA may contribute to bacterial fitness during infection. Cytotoxicity detection as well as cytokine multiplex assays are being employed to respectively analyse lactate dehydrogenase (LDH) activity and cytokine profile in the supernatants of wt PAO1-L and the PAO1-LΔ*Hxc* mutant after infection. This work presents new insights into how PA modulates its pathogenesis under phosphate limiting conditions.

Infections & Host-Pathogen Interactions

P160

Regulation of IL-1β secretion by IFN-γ in Human Macrophage-Neutrophil co-cultures During *Pseudomonas aeruginosa* Infection.

Sirina Muntaka[1], Yasir Almuhanna[1,2], Miguel Cámara[1], Luisa Martínez-Pomares[1]
[1]School of Life Sciences, University of Nottingham, Nottingham, UK, [2]College of Applied Medical Sciences, Shaqra University, Shaqra, Saudi Arabia

Pseudomonas aeruginosa (PA) can cause chronic infection in patients with Cystic Fibrosis. These patients have been proposed to have a T-helper (Th)-2 and Th-17 biased immune response; suggesting either the absence of Th-1 and/or over exuberant Th-17 might contribute to chronic PA infection with a deterioration of lung function.
This work describes the development of a human macrophage/neutrophil infection assay and its use to model Th1 (IFN-γ)- and Th17 (IL-17A)-driven microbicidal activity and inflammatory potential upon infection. Results show that IFN-γ and IL-17A have opposite effects on the killing ability of macrophages/neutrophils co-cultures; bacterial killing was reduced by IFN-γ and promoted by IL-17A. Interestingly, the addition of neutrophils to cultures of macrophages infected with PA promotes secretion of IL-1α and IL-1β. IL-1β secretion is dependent on caspase-1 activity, is not linked to cell death and is reduced in the presence of IFN-γ compared to IL-17A. Other cytokines such as TNF-α and MCP-1 were not affected by neutrophil addition. Thus phagocyte co-cultures unveil an unappreciated collaboration between macrophages and neutrophils in the promotion of IL-1-mediated inflammation that can be controlled by IFN-γ. Current work focuses on the mechanism(s) underpinning enhanced IL-1β production in the presence of neutrophils and the inhibitory effect of IFN-γ on IL-1β release.

Infections & Host-Pathogen Interactions

P161

Role of 2-Alkyl-4-Quinolone-Dependent Quorum Sensing during *Pseudomonas aeruginosa* Infection of Differentiated Human Bronchial Epithelial Cells

Yi-Chia Liu, Farah Hussain, Ola Negm, Ana Paiva, Sirina Muntaka, Nigel Halliday, Sonali Singh, Jean Dubern, Lee Wheldon, Jennifer Luckett, Paddy Tighe, Cynthia Bosquillon, Paul Williams, Miguel Cámara, Luisa Martínez-Pomares
Nottingham University, Nottingham, UK

Pseudomonas aeruginosa causes both acute and chronic infections in patients with compromised epithelial barrier function. *P. aeruginosa* synthesises 2-alkyl-4(1*H*)-quinolones (AQs), including the quorum-sensing (QS) signalling molecule 2-heptyl-3-hydroxy-4(1*H*)-quinolone (PQS), that integrate with the *N*-acylhomoserine lactone QS systems to coordinate virulence gene expression. AQs are detectable in the lungs of individuals with cystic fibrosis who are infected with *P. aeruginosa* and AQ-dependent QS contributes to virulence regulation in *in vivo* disease models and modulates inflammatory signalling in immune cells. However, whether AQ-dependent QS influences infection of the human lung epithelium is not known. This work evaluated the contribution of the AQ-QS to *P. aeruginosa* infection of differentiated human bronchial epithelial cells. Results show that human bronchial epithelial cells support *P. aeruginosa* growth and AQ production, are susceptible to *P. aeruginosa*-induced cytotoxicity and orchestrate a robust pro-inflammatory response to infection in an AQ-independent manner but these effects were not affected by lack of endogenous PQS production. A possibility is that the effect of AQ-QS on the interaction of *P. aeruginosa* with bronchial epithelial cells can only become apparent in more complex models that enable exposure of bronchial epithelial cells to PQS prior infection. This hypothesis is currently being investigated.

Infections & Host-Pathogen Interactions

P162

Drivers of virulence evolution in *Pseudomonas aeruginosa*

Elisa Granato[1], Christoph Ziegenhain[2], Rolf Kümmerli[1]
[1]University of Zurich, Zurich, Switzerland, [2]Ludwig-Maximilians University Munich, Munich, Germany

Background. Opportunistic bacterial pathogens, like *Pseudomonas aeruginosa*, are a major cause of morbidity in immunocompromised patients. Yet, we know little about how these opportunists adapt to the different environments they inhabit inside and outside the host, and how adaption affects virulence, and thus damage to the host.

Methods. To address this issue, we let *P. aeruginosa* PAO1 evolve in the presence or absence of its model host *Caenorhabditis elegans*, in two different environments differing in their level of spatial structure. Following experimental evolution, we used a combination of virulence assays, virulence factor screens and whole-genome sequencing to identify the targets of selection and the resulting changes in virulence.

Results. We found that virulence significantly dropped in unstructured environments both in the presence and absence of the host, but remained unchanged in spatially structured environments. The observed virulence decline seemed to be driven by two interacting forces: accidental effects, where bacteria became deficient for virulence traits not needed outside the host; and social effects, where mutants that lost the ability to produce shareable virulence factors (siderophores, proteases and toxins) outcompeted the ancestral wildtype through social exploitation.

Conclusion. Our study shows that accidental non-host effects and social conflicts over virulence factor production can drive rapid virulence evolution in an opportunistic pathogen. In a clinical context, our findings suggest that disrupting spatial structure in *P. aeruginosa* infections could help to steer pathogen evolution towards lower virulence.

Infections & Host-Pathogen Interactions

P163

The impact of low oxygen tensions on the exoproteome of *Pseudomonas aeruginosa*

Annika Schmidt[1], Elena Schäfer[1], Christine Rother[1], Irina Droste-Borel[2], Boris Macek[2], Sandra Schwarz[1]
[1]Interfaculty Institute of Microbiology and Infection Medicine Tuebingen (IMIT), University of Tuebingen, Tuebingen, Germany, [2]Proteome Center Tübingen, University of Tuebingen, Tuebingen, Germany

Hypoxic and anoxic niches are found in the lungs of pediatric cystic fibrosis (CF) patients, indicating that low oxygen tensions affect the interaction of *Pseudomonas aeruginosa* with the host from the early stages of infection. To gain an insight into the response of *P. aeruginosa* to anoxic conditions, we performed a global exoproteome analysis of PAO1 and RP73, which was isolated after 16.9 years of chronic lung infection in a CF patient. For this, the bacteria were grown under aerobic and anaerobic conditions in the presence of 15 mM KNO3. The culture supernatants were precipitated with TCA and analyzed by label-free quantitative LC-MS/MS. A total of 23 proteins were significantly more abundant in the supernatant of PAO1 under anoxic conditions in all three biological and technical replicates. Interestingly, 15 of these proteins were also repeatedly detected at significantly higher levels in the supernatants of the isolate RP73 grown in the absence of oxygen. Among these proteins is an uncharacterized protein with similarity to a protease. Labeling of the protein using a chromosomal Strep-tag fusion and Western blot analysis confirmed the oxygen-dependent abundance in the supernatant. In addition, we showed that hypoxic conditions, i.e. 6% oxygen, induce secretion of the protein. Furthermore, when overexpressed in *E. coli*, the protein can be detected by Western blot using sera from CF patients indicating that *P. aeruginosa* produces the protein during infection of the lung of CF patients. The activity of the protein and its role in host interactions is currently under investigation.

Infections & Host-Pathogen Interactions

P164

Biochemical, bioinformatics and functional analysis of amino acids chemoreceptor of Pseudomonas syringae pv. tomato DC3000

Jean Paul Cerna-Vargas[1], Diego López-Mateos[1], José Juan Rodríguez-Herva[1,2], Pablo Rodríguez-Palenzuela[1,2], Abdealoui Daddaoua[3], Tino Krell[3], Emilia López-Solanilla[1,2]
[1]Centro de Biotecnología y Genómica de Plantas (CBGP). Universidad Politécnica de Madrid-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Parque Científico y Tecnológico de la UPM. Campus de Montegancedo. 28223 Pozuelo de Alarcón, Madrid, Spain, [2]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agrónoma, Alimentaria y de Biosistemas, UPM, 28040, Madrid, Spain, [3]Departamento de Protección Ambiental, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Prof. Albareda 1, 18008, Granada, Spain

During infection, chemotaxis enables foliar bacteria to move towards an optimal environment. Chemoreceptors constitute a key component of this process.

Chemoreceptors known as MCPs (Methyl accepting Chemotaxis Proteins) are transmembrane proteins with a cytoplasmic domain, involved in signaling, and a periplasmic domain, also known as ligand binding domain (LBD), involved in binding.

Based on its amino acid similarity with chemoreceptors from other Pseudomonas which bind GABA (γ amino-butyrate), a ubiquitous amino acid also present in plants, we chose ADG-0002525 as a putative chemoreceptor of GABA in Pseudomonas syringae pv. tomato DC3000 (PsPto). We first cloned and purified the LBD of ADG-0002525 and performed two binding assays: thermal shift and ITC. Both assays showed that this LBD doesn´t bind GABA, but it can bind a series of both L and D amino acids. We performed bioinformatics analysis that allowed us to determine the amino acid residues involved in the binding. Site-directed mutagenesis on these residues will allow us to corroborate the binding and to determine its impact during the infection process.

Other two GABA putative chemoreceptors from PsPto are under analysis through biochemical and bioinformatics approaches to find out the chemoreceptor involved in the perception. Moreover mutant altered in these MCPs are under construction and their functional characterization in vivo will give us new clues about the role of GABA perception during infection.

Infections & Host-Pathogen Interactions

P165

Light perception orchestrates pathogenesis in *Pseudomonas syringae* pv. tomato

Saray Santamaría-Hernando[1], José Juan Rodríguez-Herva[1,2], Pedro Manuel Martínez-García[3], Isabel Río-Álvarez[1], Pablo Rodríguez-Palenzuela[1,2], Gwyn Beattie[4], Emilia López-Solanilla[1,2]

[1]Centro de Biotecnología y Genómica de Plantas. Universidad Politécnica de Madrid (UPM) - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Campus Montegancedo UPM 28223-Pozuelo de Alarcón (Madrid), Spain, [2]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, UPM, 28040-Madrid, Spain, [3]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), CSIC-Universidad de Sevilla-Universidad Pablo de Olavide, 41092-Sevilla, Spain, [4]Department of Plant Pathology & Microbiology, Iowa State University, 50011 Ames, Iowa, USA

Pseudomonas syringae pv. tomato DC3000 (*PsPto*) is a hemibiotrophic pathogen that can survive as an epiphyte on leaf surfaces without causing disease. When *PsPto* enters the plant leaf apoplast through wounds or natural openings like stomata, it multiplies and can induce symptoms. In the phyllosphere, *PsPto* is exposed to the diurnal cycle and thus to changes in the light regimen. *PsPto* perceives light using a blue light-sensing LOV domain protein and two red light-sensing bacteriophytochromes. We have previously demonstrated that *PsPto* light perception affects motility and attachment to plant leaves, and that light regulation of these traits on leaves affects virulence. To explore the effect of light perception on *PsPto* at a global level, we carried out microarray hybridization experiments. We found that light induces a gene expression reprogramming in *PsPto* primarily characterized by changes in pathogenicity-related genes. This finding is consistent with our prior evidence that exposure of cells to distinct types of light before inoculation strongly affects virulence. Our results showed a priming effect in which cells exposed to darkness prior to inoculation at subjective dawn exhibit an increased invasiveness. The pathogenic behavior of mutants altered in the *PsPto* photoreceptors further supports this key role for light perception during the initial stages of infection. These results highlight new questions regarding how cells integrate the prevalent monochromatic wavelengths of light over the course of a day and how this perception influences their gene expression and resulting behavior.

Infections & Host-Pathogen Interactions

P166

Contribution of the metabolism of phenolic compounds to the virulence of *Pseudomonas savastanoi* in woody hosts

Eloy Caballo-Ponce[1], Regina Michaela Wittich[2], Pieter van Dillewijn[2], Cayo Ramos[1]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias., Malaga, Spain,[2]Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), Granada, Spain

Most strains of the *Pseudomonas syringae* complex isolated from the trunks of woody plants and belonging to phylogroups (PGs) 1 and 3 share a genomic region, named WHOP (from woody host and *Pseudomonas*), which is partially conserved in some PG2 strains. Annotation of the 14 ORFs encoded within this region, which is absent in the genomes of P. syringae strains infecting herbaceous hosts, yielded functions possibly involved in the metabolism of phenolic compounds. The genetic organization of this region was analyzed by RT-PCR in *Pseudomonas savastanoi* pv. savastanoi (Psv), the causative agent of olive knot disease. The WHOP region is organized in four operons (*antABC, catBCA, ipoABC* and *dhoAB*) and three genes transcribed independently (*antR, benR* and PSA3335_3206). HPLC analyses confirmed that the *antABC* and *catBCA* operons mediate the catabolism of anthranilate and catechol, respectively, through the β-ketoadipate pathway. In addition, oxygenase activity on aromatic compounds, tested as the conversion of indole into indigo, was assigned to the *ipoABC* operon. Pathogenicity tests revealed that deletion of *antABC, catBCA* or *ipoABC* in Psv caused reduced symptoms in woody olive plants, while such phenotype was not observed in young micropropagated (non-woody) plants. Similarly, the *catBCA* and *dhoAB* operons and the PSA3335_3206 gene (encoding a putative aerotaxis receptor) are also required for full bacterial fitness exclusively in woody olive plants. At present, we are addressing the role in virulence of the orphan *benR* gene encoded in the WHOP region.

Infections & Host-Pathogen Interactions

P167

Comparative Analysis of the Type III Secretion System Effector Repertoires of *Pseudomonas savastanoi* Pathovars Pathogenic on Woody Hosts

Alba Moreno-Pérez[1], M. Pilar Castañeda-Ojeda[1], Jesús Murillo[2], Emilia López-Solanilla[3], Pablo Rodríguez-Palenzuela[3], Cayo Ramos[1]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea «La Mayora», Universidad de Málaga-CSIC, Área de Genética, Universidad de Málaga, Malaga, Spain, [2]Departamento de Producción Agraria, ETS de Ingenieros Agrónomos, Universidad Pública de Navarra, Pamplona, Spain, [3]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid – Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Parque Científico y Tecnológico de la UPM, Madrid, Spain

The species *Pseudomonas savastanoi*, a member of the *Pseudomonas syringae* complex, includes four pathovars causing knots or excrescences in woody hosts: *P. savastanoi* pv. savastanoi (Psv), pv. fraxini (Psf), pv. nerii (Psn) and pv. retacarpa (Psr), comprising isolates from olive, ash, oleander and broom plants, respectively. Pathogenicity of *P. savastanoi* is dependent, among other factors, on the type III secretion system (T3SS) and its effector (T3E) repertoire. Furthermore, a putative role in the interaction with woody hosts has been suggested for several of these T3E. The recent availability of the genome sequences of several *P. savastanoi* strains isolated from different hosts has facilitated bioinformatics predictions of their T3SS genes and T3E pools, the study of their distribution in other strains of the *P. syringae* complex isolated from woody hosts and the functional analysis of several of these secreted proteins. As previously reported for Psv, Psn and Psf, here we show that pathogenicity of Psr ICMP16945, is also dependent on the T3SS. Psv strains NCPPB 3335, ICMP4352 and PseNe107 share a core set of at least 22 T3E, 18 of which are also encoded in Psn ICMP16943, Psf ICMP7711 and Psr ICMP16945. However, these three strains encode truncated versions of 1-2 of these 18 T3E and, Psr ICMP16945 contains three pathovar-specific T3E. Our results also show that several T3E, including HopAO1, are phylogenetically clustered across the *P. syringae* complex according to the woody/herbaceous nature of their host of isolation, suggesting host specialization of these effectors in this complex.

Infections & Host-Pathogen Interactions

P168

Intramacrophage phase for *Pseudomonas aeruginosa*: MgtC shows the way

Preeti Garai, Laurence Berry, Malika Moussouni, Anne Blanc-Potard
CNRS, Montpellier, France

Intracellular bacteria use a broad range of molecular pathogenicity determinants to manipulate host cell processes and adapt to the intracellular environment. The MgtC protein has been shown to be important for the replication of various intracellular bacteria inside macrophages. An MgtC related protein is encoded by the extracellular pathogen *Pseudomonas aeruginosa*. The recent phenotypic analysis of a *P. aeruginosa mgtC* mutant in various models, including the zebrafish embryo infection model, revealed a role for P. aeruginosa MgtC in an intramacrophage phase (Belon et al., 2015, PLoS Pathogens 11(6): e1004969). We are currently investigating the intramacrophage fate of *P. aeruginosa*, which has been poorly documented so far. Strikingly, for both intracellular pathogens and *P. aeruginosa*, MgtC expression is induced inside macrophages. Furthermore, we recently showed that MgtC modulates the expression of known *P. aeruginosa* virulence factors inside macrophages. Based on these recent findings, we propose that the study of *mgtC* mutant is a tool to decipher the relevance of an intramacrophage phase during *P. aeruginosa* infection.

Infections & Host-Pathogen Interactions

P169

Response to fungal exudates of the rhizosphere isolate *Pseudomonas* sp. UMAF110 involves a GGDEF/EAL domain-containing protein.

Adrian Pintado[1], Isabel M. Aragon[1], Isabel Perez-Martinez[1], Clara Pliego[2], J. Ignacio Crespo-Gomez[1,3], Antonio de Vicente[3], Francisco M. Cazorla[3], Cayo Ramos[1]
[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Área de Genética, Universidad de Málaga, Malaga, Spain, [2]IFAPA, Centro de Churriana (CICE Junta de Andalucía), Malaga, Spain, [3]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Departamento de Microbiología, Universidad, Malaga, Spain

Pseudomonas sp. UMAF110, isolated from rhizosphere soil in Spain, display *in vitro* antagonism towards the pythopathogenic fungus *Rosellinia necatrix* and is able grow in fungal exudates (BM-RE medium). A transposon mutant library of this strain was constructed and several mutants were selected by their reduced competitiveness in BM-RE medium. *Pseudomonas* sp. UMAF110-G3, which contains the transposon into a gene encoding a putative REC/PAS/GGDEF/EAL protein, was selected for further characterization. Blastn searches using the sequence of the gene interrupted by the transposon in UMAF110-G3, here called *cmpA* (*c-di-GMP Metabolizing Protein*), yielded a single positive hit (98% cover, 78% identity) with a gene from a terpene-degrading *Pseudomonas* sp. strain isolated from soil. Context analysis of the *cmpA* gene in *Pseudomonas* sp. UMAF110 showed that this gene is located downstream from several genes involved in flagellar motility/chemotaxis. RT-PCR experiments further confirmed that *cmpA* form a transcriptional unit with the *che* gene cluster. Expression analysis of *cmpA* by qRT-PCR clearly showed upregulation of this gene after transfer of *Pseudomonas sp.* UMAF110 cells to BM-RE medium, suggesting a role for this operon in response to fungal exudates. Deletion of *cmpA* in *Pseudomonas* sp. UMAF110 did not affect the ability of the strain to form biofilms under the conditions tested. However, overexpression of wild type CmpA in *Pseudomonas putida* KT2440 negatively regulated biofilm formation in this strain. Together, these results suggest that CmpA could be involved in signal transduction pathways regulating flagellar motility/chemotaxis in response to fungal exudates.

Infections & Host-Pathogen Interactions

P170

Quantifying iron-regulated *Pseudomonas aeruginosa* alkyl-quinolone production in complex polymicrobial environments using real-time PCR and liquid chromatography-tandem mass spectrometry

Luke K. Brewer, Angela T. Nguyen, Jace W. Jones, Maureen A. Kane, Amanda G. Oglesby-Sherrouse
University of Maryland Baltimore, Baltimore, Maryland, USA

Pseudomonas aeruginosa is an antimicrobial-resistant bacterial pathogen that causes life-threatening acute and chronic infections in immune-compromised patient populations. These infections result in severe damage to surrounding host and bacterial cells, due in part to secretion of antimicrobial 2-alkyl-4(1H) quinolones (AQs). AQs are involved in quorum sensing and the regulation of virulence factors by *P. aeruginosa,* thus contributing to the liberation of iron from host and bacterial cells. Our lab has demonstrated that production of some AQs by *P. aeruginosa* is increased in iron-deplete conditions, and that AQ production is restored in AQ-deficient *P. aeruginosa* strains when grown in co-culture with *Staphylococcus aureus*. These data suggest AQs are critical to *P. aeruginosa's* ability to acquire iron during poly-microbial infections, like those seen in the lungs of CF patients. Understanding regulation of AQ production may provide valuable insight into how *P. aeruginosa* interacts and competes with host cells and co-colonizing pathogens during infection. Here, we have developed a method for quantifying genomic DNA sequences that are specific to *S. aureus* and *P. aeruginosa,* using real-time PCR (qPCR). This method will allow us to normalize AQ concentrations determined by LC-MS/MS to the amount of viable *P. aeruginosa* cells present in co-culture, while simultaneously determining the impact of these metabolites on *S. aureus* viability. We are applying this method to laboratory strains and clinical CF isolates to understand how co-culture with *S. aureus* promotes AQ production by *P. aeruginosa*. These studies are critical for understanding how AQs contribute to iron acquisition during polymicrobial infections.

Infections & Host-Pathogen Interactions

P171

Understanding the ecology and evolution of polymicrobial wound infections

Ana C. da Silva[1], Tim Sloan[2], Stephen P. Diggle[3], Paul Williams[1]
[1]*Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK,*
[2]*Department of Microbiology, Nottingham University Hospitals Trust, Nottingham, UK,*
[3]*School of Biological Sciences, Georgia Institute of Technology, Atlanta, USA*

Chronic wound ulcers in diabetic patients often become colonised with polymicrobial infections of *Staphylococcus aureus* (Sa) and *Pseudomonas aeruginosa* (Pa). Polymicrobial infections have been suggested to be more virulent, antibiotic resistant and result in poor patient outcomes, but the reasons why remain unclear. Mechanisms of interactions between Pa and Sa have been identified and studied but mainly using well characterized laboratory strains. However, interactions occurring between bacterial species during actual infection are likely to be hugely different than interactions between reference strains.
Reference strains of Pa effectively kill co-cultured Sa, but studies have shown that a change in Pa metabolism present in a dominant chronic cystic fibrosis (CF) lineage allows a commensal-like interaction where Pa benefits from interacting with Sa. Here we focus on understanding how the ecology of chronic wounds shapes cooperation and conflict between different species. We isolated bacterial populations from five patients and six chronic wounds and found that one was colonized with *P. aeruginosa* but not with *S. aureus*, and the other five had both species. We found that Sa and Pa strains isolated from the same wound can often co-exist when grown in synthetic wound fluid. In contrast, when *P. aeruginosa* and *S. aureus* strains are isolated from different wounds, *P. aeruginosa* often outcompetes *S. aureus*. Our findings are an important first step in understanding how ecology influences the evolution of different species within actual wound infections and how this contributes to antibiotic resistant polymicrobial infections.

Infections & Host-Pathogen Interactions

P172

Pseudomonas aeruginosa EoxS and ExoT block ROS production in neutrophils

Hyunkeun Kim, Arne Rietsch
Case Western Reserve University, Cleveland, USA

Background: *P. aeruginosa* uses its type III secreted effectors ExoS and ExoT, to subvert neutrophil function and establish an infection. The goal of this project is to identify the targets of the ExoS- and ExoT ADP-ribosyltransferase (ADPRT) activities, through which these effectors inactivate of neutrophils.

Methods: ROS-production was monitored using luminol. Activation of the PI3-kinase pathway was monitored by Western blot. The Ras-PI3K interaction was tested by affinity chromatography. Intracellular survival was assayed by gentamicin protection assay. Ras(R41K) was introduced into neutrophils by fusing the protein to the Tat cell permeating peptide. A new target of ExoT in neutrophil lysates was identified using biotinylated NAD as a substrate, and mass-spectrometry. ADP-ribosylation was confirmed using protein purified from *E. coli*.

Results: ExoS and ExoT independently block ROS production in human neutrophils in an ADPRT-dependent manner. ExoS ADP-ribosylates Ras, which blocks binding of Ras to PI3-kinase. Preventing ADP-ribosylation of Ras by introducing Tat-Ras(R41K) into neutrophils, partially reverses the ExoS-dependent block in ROS production and increases killing of phagocytosed bacteria. We also identified gelsolin, which is involved in the calcium-dependent maturation of phagosomes in neutrophils, as a new target of the ExoT ADPRT activity. ExoT only ADP-ribosylates the calcium-bound, active form of gelsolin.

Conclusion: *P. aeruginosa* uses ExoS and ExoT to block ROS production in neutrophils. This promotes intracellular survival by phagocytosed bacteria and is important for acute infections. By the same token, loss of type III secretion could lead to a more fulminant inflammatory response in chronic infections by neutrophils left unchecked.

Infections & Host-Pathogen Interactions

P173

The role of antibody in killing of *Pseudomonas aeruginosa* by healthy control serum.

Emma Sheehan[1], Timothy Wells[2], Adam Cunningham[3], Anna Schager[1], Anthony De Soyza[4,5], James Chalmers[6], Ian Henderson[1]
[1]Institute of Microbiology and infection, University of Birmingham, Birmingham, UK, [2]Diamantina Institute, University of Queensland, Queensland, Australia, [3]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK, [4]Institute of Cellular Medicine, Newcastle University, Newcastle, UK, [5]Adult Bronchiectasis service, Freeman Hospital, Newcastle, UK, [6]Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Dundee, UK

Pseudomonas aeruginosa is an opportunistic pathogen that can cause acute or chronic infections in immunocompromised patients. Well known for causing infections in severe burns, the bacteria also causes other acute infections including keratitis and urinary tract infections. Chronic *P. aeruginosa* lung infections occur in patients suffering from cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary disorder (COPD). Such infections lead to chronic inflammation, deteriorating lung function and increased morbidity and mortality.

Antibodies normally protect individuals against infection. However, recently we identified that some patients with non-CF bronchiectasis and chronic *Pseudomonas* infection produce O-antigen specific IgG2 that actually protects the colonising bacteria from killing by the immune system. These 'inhibitory antibodies' prevent both serum and cell-mediated killing of the infecting bacterium. During these studies we found that all healthy control serum (HCS) used could kill chronic *Pseudomonas* strains even when those strains were expressing high levels of O-antigen. Here we investigate how HCS kills *Pseudomonas* strains isolated from the environment, acute infections and chronic infections. We found that HCS could kill all chronic strains tested, however acute and environmental isolates were much more variable in their serum resistance profile. HCS was found to have specific antibody, in particular towards outer membrane proteins, for *P. aeruginosa* strains and specific antibody was necessary for the killing of O-antigen positive strains isolated from acute infections. Finally, O-antigen specific antibody purified and concentrated from HCS could inhibit the killing of normal HCS.

Infections & Host-Pathogen Interactions

P174

Stressed Yet Infectious: Biosynthesis of Alpha-Glucan in Pseudomonads

Stuart Woodcock, Karl Syson, Jacob Malone, Stephen Bornemann
John Innes Centre, Norwich, UK

Pseudomonas spp. are significant pathogens of both humans and plants. An important aspect of bacterial infection is the ability to withstand external stresses. The production of trehalose has been implicated in the tolerance of many stresses, in particular osmotic shock. The disruption of the TreY/TreZ and TreS trehalose biosynthetic operons reduces pathogenicity *in planta*, illustrating the importance of trehalose during plant infection. A metabolic pathway that utilises enzymes previously thought to synthesise trehalose was found instead to metabolise trehalose into α-glucan, suggesting additional complexity than what was previously suspected. We therefore sought to investigate the function of α-glucan in pseudomonads, by genetically dissecting the trehalose and α-glucan pathways in *P*. *syringae* and *P*. *aeruginosa.* We show that α-glucan can indeed be synthesised from trehalose, and that α-glucan is involved in the tolerance of desiccation stress in both Pseudomonas species, a phenotype that is apparently independent of trehalose levels.

Infections & Host-Pathogen Interactions

P175

FleQ determines invasion of ExoS-producing *Pseudomonas aeruginosa* strains

Abby Kroken[1], Camille Chen[1], David Evans[1,2], Timothy Yahr[3], Suzanne Fleiszig[1]
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Touro University California, Vallejo, CA, USA, [3]University of Iowa, Iowa City, IA, USA

Background: Intracellular survival of *Pseudomonas aeruginosa* has been demonstrated by multiple investigators, however *P. aeruignosa* is usually considered an extracellular pathogen due to type III secretion system (T3SS) effectors with anti-phagocytic activity. Previously we reported that the antiphagocytic effector ExoS paradoxically plays multiple roles by enabling *P. aeruginosa* replication in a membrane bleb-niche inside epithelial cells. Here we addressed how ExoS does not universally inhibit invasion by strains natively encoding it.

Methods: T3SS expression versus bacterial location was examined in four *P. aeruginosa* isolates (PAO1, PAK, PA103, PA14) and their T3SS effector or *fleQ* mutants using microscopy, gentamicin protection, and qPCR in several epithelial cell lines.

Results: All T3 effector null mutants invaded cells, with PA103 being the only one that lost that capacity when ExoS was expressed exogenously. Wild-type PAO1 demonstrated T3SS activation after bacteria entered cells. In contrast, PA103 expressing ExoS were located extracellularly when activated, and inhibited uptake of otherwise invasive strains. PA103 does not express flagella due to a mutation in *fleQ*: restoring *fleQ* in PA103 increased invasion frequency ~10-fold even in the presence of ExoS, whereas deleting *fleQ* in PAO1 reduced invasion ~10-fold despite ExoS delivery.

Conclusions: Together, these results suggest internalization of *P. aeruginosa* by epithelial cells is efficiently inhibited by T3SS effector translocation only in *fleQ* mutants such as wild type PA103. How *fleQ* impacts either host cell phagocytosis or T3SS triggering remains to be determined. Nevertheless, our data reconcile differing viewpoints about *P. aeruginosa* invasion and the roles of ExoS.

Infections & Host-Pathogen Interactions

P176

Whole genome sequencing of geographically and historically diverse isolates of thePseudomonas aeruginosa Liverpool Epidemic Strain

Matthew Moore[1], David Williams[2], Steve Paterson[2], Irena Kukavica-Ibrulj[3], Iain Lamont[4], Nicholas Tucker[5], Dervla Kenna[6], Jane Turton[6], Julie Jeukens[3], Luca Freschi[3], Nicholas Loman[7], David Taylor-Robinson[8], Simon Holden[9], Suzanne Manzoor[10], Peter Hawkey[10], Kevin Southern[11], Martin Walshaw[12], Roger Levesque[3], Joanne Fothergill[1], Craig Winstanley[1]

[1]Institute of Infection and Global Health, Liverpool, UK, [2]Institute of Integrative Biology, Liverpool, UK, [3]Universite Laval, Quebec, Canada, [4]University of Otago, Otago, New Zealand, [5]University of Strathclyde, Strathclyde, UK, [6]Public Health England, London, UK, [7]Birmingham University, Birmingham, UK, [8]University of Liverpool Public Health and Policy, Liverpool, UK, [9]Nottingham University Hospital, Nottingham, UK, [10]Birmingham Heartlands Hospital, Birmingham, UK, [11]Alder Hey Children's Hospital, Liverpool, UK, [12]Liverpool Heart and Chest Hospital, Liverpool, UK

Chronic lung infections with Pseudomonas aeruginosa remain the major cause of morbidity and mortality associated with Cystic Fibrosis (CF). Once of the best studied transmissible clones of P. aeruginosa is the Liverpool Epidemic Strain (LES), first discovered in a children's CF Unit in Liverpool, but since identified as the most common single clone amongst CF isolates from patients in the UK, and associated with greater patient morbidity in both the UK and Canada. The complete genome of the earliest known LES isolate (LESB58 from 1988) was published in 2009, since which a number of other LES genomes from both Liverpool and Canada have been published.

The aim of this study was to genome sequence a larger collection of LES isolates from multiple centres throughout the UK, and analyse alongside the existing datasets in order to characterise the intraclonal diversity of a major CF transmissible strain in the context of geographical and temporal dissemination.

Analysis using core genome SNP phylogeny indicates that LES isolates form two distinctive clusters (UK and Canadian isolates), with LESB58 lying between. Within the UK isolates, further geographical clustering was evident. By mapping to the genome of LESB58, we were able to identify loss of function adaptive mutations, with the most common occurring in genes such as oprD, lasR, mexB and mucA. Some mutations were associated with specific geographical clusters. Pangenome analysis indicated variations in the carriage of prophages and genomic islands.

We would like to acknowledge the Cystic Fibrosis Trust for funding this research.

Infections & Host-Pathogen Interactions

P177

Rewiring of a sigma factor regulatory network in P. aeruginosa by a naturally occurring single nucleotide polymorphism

Eva Kammer Andresen[1], Grith M M Hermansen[1], Cristina Isabel Amador[1], Denitsa Eckweiler[2], Sebastian Schulz[2], Susanne Haussler[2], Tino Krell[3], Maher Abou Hachem[1], Lars Jelsbak[1]
[1]Department of Biotechnology and Biomedicine, DTU Bioengineering, Technical University of Denmark, Kgs. Lyngby, Denmark, [2]Department of Molecular Bacteriology, Helmholtz Centre for Infection Research, Braunschweig, Germany, [3]Department of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

Sigma factors confer specificity of promoter recognition to the RNA core polymerase and regulate a large array of genes simultaneously while reflecting and integrating internal and external signals. The impact of naturally occurring polymorphisms within sigma factors during adaptation is yet to be characterized.
In the current study we investigated the effect of a single polymorphism, resulting in an amino acid change, occurring in RpoN during chronic infection of cystic fibrosis patients. Alterations of transcriptional networks and binding profiles were identified by RNAseq and Chipseq analyses of wildtype and mutant RpoN, respectively, expressed in the PAO1 genetic background.
The single polymorphism resulted in differential expression of nearly 2 % of all open reading frames. As the polymorphism is found in the DNA interacting domain of RpoN it was no surprise that many regions displayed reduced binding affinity and transcription. More interestingly, however, is the fact that the RpoN mutant displayed a unique positive regulatory effect on the tad locus, which is involved in biofilm formation, colonization and pathogenesis. Changes to biofilm formation, colony morphology and pyocyanin production were also identified. Moreover, the polymorphism induced sigma factor cross-talk with an increased expression of the RpoS regulon.
In conclusion, a single sigma factor polymorphism caused an extensive rewiring of regulatory networks with altered binding and transcription profiles reflected in phenotypic changes. This study highlights the central role of regulatory networks during long-term adaptation to a host environment.

Infections & Host-Pathogen Interactions

P178

Phospholipase A PlbF of *Pseudomonas aeruginosa* and its posable role for membrane remodelling

Muttalip Caliskan[1], Christoph Heinrich Strunk[1], Florian Bleffert[1], Joachim Granzin[2], Meike Siebers[3], Stephan Schott[4], Peter Dörmann[3], Laurence Rahme[5], Holger Gohlke[2,4], Renu Batra-Safferling[2], Karl-Erich Jaeger[1,6], Filip Kovacic[0]
[1]Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Duesseldorf, Germany, [2]Institute of Complex Systems, ICS-6: Structural Biochemistry, Forschungszentrum Jülich GmbH, Juelich, Germany, [3]Institute of Molecular Biotechnology, IMBIO, Universität Bonn, Bonn, Germany, [4]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Duesseldorf, Germany, [5]Department of Microbiology and Immunology, Harvard Medical School, Boston, Germany, [6]Institute of Bio- and Geosciences IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Juelich, Germany

The Gram-negative bacterium *Pseudomonas aeruginosa* is one of the leading causes of nosocomial pneumonia, preferentially in immunocompromised patients. Among many virulence factors produced by *P. aeruginosa* are several phospholipases contributing to damage of host cell membranes and modulation of lipid signalling in eukaryotic cells. Fatty acid derivatives are linked to virulence of *P. aeruginosa*; however, their biosynthesis and signalling pathways are largely unknown.

We have identified PlbF, a novel phospholipase A of *P. aeruginosa* which can hydrolyse *in vitro* bacterial phospholipids resulting in release of fatty acids. *In vivo* comparison of membrane lipids of the wild-type and a *plbF*-deletion strain of *P. aeruginosa* by quantitative mass-spectrometry revealed significant differences. Additionally, the subcellular localisation of PlbF in the cytoplasmic membrane suggested that the enzyme has access to phospholipids of the inner membranes.

The *P. aeruginosa plbF*-deletion strain produced significantly less biofilm than the wild-type strain, and furthermore, it showed attenuated virulence in a *Drosophila melanogaster* infection assay. Pull-down experiments with PlbF revealed several putative interaction partners, i.e. proteins linked to biofilm formation and lipid signalling. Their precise role is currently investigated.

To study the molecular mechanism of PlbF-mediated virulence, we have solved the crystal structure of PlbF which revealed a dimeric structure with a unique transmembrane helix-helix dimerization motif and fatty acids bound into the active site. Apparently, fatty acids can inhibit the enzymatic activity of PlbF and trigger the dimerization. Molecular dynamics simulations with PlbF in a phospholipid membrane suggest a mechanism for monomerisation-induced PlbF which might be important for membrane lipid hydrolysis.

P179

Intra and inter-patient phenotypic diversity of Cystic Fibrosis clinical isolates of *Pseudomonas aeruginosa*

Natasha Wierre-Gore, Jane Davies, Jake Bundy, Huw Williams
Imperial College London, London, UK

Introduction

Pseudomonas aeruginosa is the main pathogen of the Cystic Fibrosis lung, chronically infecting up to 60% of patients. Within the lung the bacteria undergo selection pressures leading to the existence of co-lineages of strains within patients.
We tested the hypothesis that intra-patient phenotypic diversity of *P. aeruginosa* strains is greater than inter-patient diversity and that intra-patient diversity increases with length of infection.

Methods

641 clinical isolates from 18 chronically infected and 7 recently infected patients were isolated, with on average 24 single isolates per patient sample. These isolates were phenotypically characterised using high-throughput assays such as growth rate, motilities, biofilm formation, hydrogen cyanide, pyocyanin and protease production.

Results

To analyse the data a nested ANOVA approach was used and the comparison of the F statistic and its significance was used to determine the extent of the diversity for the phenotypes tested. The data showed both significant intra and inter-patient variation. For all phenotypes, the intra-patient variation increased between the early and chronic stages of infection. Nevertheless, the overall inter-patient diversity was over 40 fold greater than the intra-patient diversity, contrasting what had been previously reported for *P. aeruginosa* epidemic strains isolates.

Conclusion

While intra-patient diversity increases from early to chronic infection stages, it never exceeds inter-patient diversity. Although the specific lung environment and co-infecting bacteria will be expected to influence the phenotypic diversity of *P. aeruginosa*, our data suggests that the phenotype of the initial infecting strain has a sustained influence on the ensuing phenotypic adaptive trajectory.

Infections & Host-Pathogen Interactions

P180

Paradoxical antibody: The scope and treatment of antibody that exacerbates Pseudomonas aeruginosa infection in bronchiectasis.

Timothy J. Wells[1], Emma Sheehan[2], Suren Kanagasundaram4[4], Gavin Spickett[5], James Chalmers[7,6], Anthony De Soyza[3], Ian R. Henderson[2]
[1]The University of Queensland, Queensland, Australia, [2]University of Birmingham, Birmingham, UK, [3]Newcastle University, Newcastle, UK, [4]Renal Medicine, Freeman Hospital, Newcastle, UK, [5]Department of Clinical Immunology, Freeman Hospital, Newcastle, UK, [6]University of Dundee, Dundee, UK

Chronic Pseudomonas aeruginosa lung infections are found in patients suffering from bronchiectasis, cystic fibrosis (CF) and chronic obstructive pulmonary disorder (COPD) and once colonisation is established, it is difficult to remove by current methods. Recently, we identified a subset of patients with bronchiectasis and chronic P. aeruginosa infection had excess IgG2 specific to their cognate bacterial O-antigen. In contrast to the serum bactericidal effect normally associated with antibody, this IgG2 inhibited complement-mediated killing of the infecting strain. Crucially, patients with impaired serum killing had worse lung function than patients with normal serum killing. Two critically ill patients with this IgG2 were treated with plasmapheresis in an attempt to remove the inhibitory antibody. Both patients had immediate benefit from this treatment with a significant drop in hospitalisations, antibiotic use and markers of inflammation. Both patients lost culturable P. aeruginosa in their sputum for up to four months after treatment. Return of the inhibitory antibody in patients coincided with bacteria in their sputum and degrading health. Finally, we investigated the prevalence of this inhibitory antibody in bronchiectasis associated with P. aeruginosa infection via an ELISA-based screen using a panel of P. aeruginosa O-antigen serotypes removing the need for patient isolates. 14% of over 200 patients with bronchiectasis had high titres of IgG2 to at least one O-antigen serotype. These findings indicate that inhibitory anti-O-antigen IgG2 may be a significant problem in bronchiectasis, and that finding ways to remove or counteract this antibody could lead to improvement in health.

Mechanisms: Signalling, Systems & Synthetic

P181

Structure-function and regulation of *Pseudomonas aeruginosa* porins

Pierre Cornelis[1], Sylvie Chevalier[1], Emeline Bouffartigues[1], Josselin Bodilis[1], Olivier Maillot[1], Olivier Lesouhaitier[1], Marc Feuilloley[1], Nicole Orange[1], Alain Dufour[2]
[1]University of Rouen, Laboratory of Microbiology Signals and Microenvironment LMSM, Evreux, Normandy, France, [2]Université de Bretagne-Sud (UEB), Laboratoire de Biotechnologie et Chimie Marines, Lorient, Bretagne, France

Background: In contrast to *E. coli*, *Pseudomonas aeruginosa* does not have large diffusion porins in the outer membrane, but two small outer membrane porins, OprG and OprH, one major porin, OprF, and several specialized porins, including the 19 belonging to the OprD (Occ) family. The aim of this presentation is to present a review of the recent literature on *P. aeruginosa* porins.

Methods: For this review, we realized a comprehensive literature review and analyzed the results of transcriptomic and proteomic data in order to get more data and new insights into the regulation of porin genes expression.

Results: Besides the abundant literature on OprF, the major porin, there are some unexpected data concerning OprH porin regulation via two different two-component systems (PhoP-PhoQ and CasS-CasR/BqsS-BqsR, which we found to be encoded by the same genes) and the BrlR regulator. We also suggest, based on recent published data, that OprG could be involved in the uptake of Fe^{2+}. Another unexpected finding is the involvement of several extracytoplasmic σ factors in the regulation of porin genes, including those belonging to the OprD family. Our analysis also included a search for the conservation of porin genes among *P. aeruginosa* isolates and other *Pseudomonas* species.

Conclusion: Our literature survey (Chevalier *et al*., 2017) revealed a multitude of functions for *P. aeruginosa* porins and a highly structured and complex regulatory network, in contrast to their supposed role as constitutive channels.

Reference: Chevalier *et al*. (2017) *FEMS Microbiology Reviews* (in press)

Mechanisms: Signalling, Systems & Synthetic

P182

Specific chemoreception and chemotaxis of *Pseudomonas aeruginosa* toward α-Ketoglutarate

David Martin-Mora[1], Alvaro Ortega[1], Jose A. Reyes-Darias[1], Vanina Garcia[2], Diana Lopez-Farfan[1], Miguel A. Matilla[1], Tino Krell[1]
[1]Estación Experimental del Zaidín - CSIC, Granada, Spain, [2]Centre for Biomolecular Sciences, Nottingham, UK

Chemoreception signalling pathways allow bacteria to sense and respond to environmental signals. Among these responses is chemotaxis, which in some species is related to the capacity of a pathogen to adhere and infect hosts. *Pseudomonas aeruginosa* has 26 chemoreceptors, of which most are of unknown function. Here, we report the identification of McpK that mediates chemotaxis toward α-ketoglutarate. Using high-throughput thermal shift assays and isothermal titration calorimetry, we found that the recombinant ligand binding domain (LBD) of McpK recognized specifically α-ketoglutarate with positive cooperativity (Kd1 = 301 μM, Kd2 = 81 μM). Ultracentrifugation studies revealed that α-ketoglutarate binding induced and stabilized McpK-LBD dimerization, decreasing the dimer self-dissociation constant approximately tenfold (from 55 μM to 5.9 μM). Quantitative capillary chemotaxis assays showed that McpK mediated chemotaxis to α-ketoglutarate concentrations from 5 μM to 5 mM, with maximal responses being recorded at 500 μM. Deletion of the *mcpK* gene abolished α-ketoglutarate chemotaxis, and complementation restored wild type like chemotaxis. Chemotaxis to Krebs cycle intermediates in *P. putida* KT2440 is mediated by the broad range receptor McpS and the citrate specific McpQ. *P. aeruginosa* employs a different chemotactic mechanism that is based on McpK and the malate-specific PA2652 receptor. The existence of a chemoreceptor specific for α-ketoglutarate may be due to its central metabolic role as well as to its function as signaling molecule. This work expands the range of known chemoreceptor types and underlines the important physiological role of chemotaxis toward tricarboxylic acid cycle intermediates.

Mechanisms: Signalling, Systems & Synthetic

P183

Quorum sensing-mediated extracellular activation of proteases in *Pseudomonas aeruginosa*

Joon-Hee Lee, Xi-Hui Li, Jungmin Oh, Soo-Kyoung Kim, Tae-Hyeon Kim
Pusan National University, Busan, Republic of Korea

Pseudomonas aeruginosa secretes multiple proteases extracellularly and most of them are quorum sensing (QS)-regulated virulence factors. The most important proteases for virulence are protease IV (PIV), elastase A (LasA), and elastase B (LasB). We found that the activity of PIV is severely reduced in culture supernatant (CS) of QS mutant, even when overexpressed to high level. PIV purified from the QS mutant (M-PIV) had much lower activity than the PIV purified from wild type (P-PIV). We found that the propeptide cleaved from prepro-PIV was always co-purified with M-PIV, but never with P-PIV. Since the activity of M-PIV was restored by adding the CS of QS-positive strain, we hypothesized that the propeptide binds to and inhibits PIV. In fact, the CS of the QS-positive strain was able to degrade the propeptide, we tested QS-dependent proteases of *P. aeruginosa* and found that LasB can degrade the propeptide and activate M-PIV. We purified the propeptide of PIV and confirmed that the propeptide can bind to and inhibit PIV. We suggest that PIV is post-secretionally activated through the extracellular degradation of the propeptide by LasB, a QS-dependent protease. Interestingly, we also found that the activities of LasA and LasB are also much reduced in culture supernatant of QS mutant. By adding active LasB or PIV (P-PIV) that is purified from wild type, the activity of LasA was restored, like M-PIV. We suggest that some QS-dependent factors may initially trigger the activation of LasB extracellularly and the activated LasB subsequently activates PIV and LasA.

Mechanisms: Signalling, Systems & Synthetic

P184

Rewiring c-di-GMP signaling to control biofilm formation and motility

Martina Valentini[1], Benoit-Joseph Laventie[2], Joana Moscoso[1], Urs Jenal[2], Alain Filloux[1]
[1]Imperial College London, London, UK, [2]Biozentrum, Basel, Switzerland

The molecular basis of second messenger signaling relies on an array of proteins that synthesize, degrade or bind the molecule to produce coherent functional outputs. Cyclic di-GMP (c-di-GMP) has emerged as a eubacterial nucleotide second messenger regulating a plethora of key behaviors, like the transition from planktonic cells to a biofilm community. The striking multiplicity of both c-di-GMP control modules and regulated cellular functions raised the question of signaling specificity. Are c-di-GMP signaling routes exclusively dependent on a central hub or can they be locally administrated? In this study, we show an example of how c-di-GMP signaling evolved in *Pseudomonas aeruginosa* to gain output specificity. The HptB pathway controls biofilm formation and motility by involving the anti-anti-sigma factor HsbA. We observed that the *hptB* operon has been genetically reorganized in *P. aeruginosa* upon the emergence of a newly identified c-di-GMP synthase-encoding gene, *hsbD*. The rewiring of c-di-GMP signaling into the HptB cascade relies on the original physical interaction between HsbD and HsbA in order to coordinate, biofilm and motility behaviors. HsbD is also demonstrated to localize to the cell pole where important motility machineries sit. The c-di-GMP specific action is therefore achieved through both spatial and temporal organization of HsbD.

Mechanisms: Signalling, Systems & Synthetic

P185

The post-transcriptional Rsm system controls the overproduction of pyocyanin in Pseudomonas aeruginosa ID4365

Miguel Cocotl-Yañez, Christian Manoell Santos-Medellin, Luis Servín-González, Gloria Soberón-Chávez
Instituto de Investigaciones Biomédicas - Universidad Nacional Autónoma de México, Ciudad de México, Mexico

Pseudomonas aeruginosa produce and release a large arsenal of virulence factors such as elastase, alkaline protease, rhamnolipids and pyocianin. The synthesis of these virulence factors is controlled by the quorum sensing systems, the two-component system GacS/A, and by the post-transcriptional regulatory system Rsm. The environmental strain P. aeruginosa ID4365 overproduce pyocyanin about 40 fold compared with PAO strain. The aim of this work is to have a more complete picture of the role of Rsm system in pyocyanin production in P. aeruginosa, particularly in strain ID4365.

We measured the transcription of the genes involved in the pyocyanin production, phzA1, phzA2 and phzM and found that its expression is higher at 37 °C than 30 °C, however the major production of pyocyanin was at 30 °C than 37 °C. Thus, the possibility of that the Rsm system is involved in to regulate the synthesis of this compound was raised. Overexpression of RsmA, but not RsmE, reduced pyocyanin production about 75% and overexpression of RsmY had a positive effect, producing 35% more pyocyanin compared with the strain carrying the empty plasmid. Additionally, the rsmA mutant strain IDrsmA was constructed and the pyocyanin production was measured at 30 °C and 37 °C, and compared with the wild type strain ID4365 (in LB and PPGAS medium). The results showed that IDrsmA strain has a higher production both in both media and temperatures compared with the wild type.

In conclusion, the Rsm system is involved in to regulate the pyocyanin synthesis in P. aeruginosa ID4365.

Mechanisms: Signalling, Systems & Synthetic

P186

Pseudomonas aeruginosa ATCC9027, an atypical case of the Pseudomonas Quinolone Signal system

Selene García-Reyes, Estefanía Morales-Ruíz, Miguel Cocotl-Yáñez, Gloria Soberón-Chávez
Instituto de Investigaciones biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

The pqsABCDE synthesize alkyl-quinolones (AQ) molecules such as PQS and HHQ which bind to the regulator PqsR in order to positively regulate its own expression and to regulate the pyocyanin synthesis; this process is called Pseudomonas Quinolone Signal (PQS). Pseudomonas aeruginosa ATCC9027 is a natural pqsR mutant and therefore is unable to produce AQ. However, this strain synthesizes pyocyanin in low phosphate medium (PPGAS) but not in Luria Bertani medium (LB). We investigated the pyocyanin production by the PQS system in P. aeruginosa ATCC9027 in both media.
To determine if the lack of pqsR is the responsible for the lack of pyocyanin production in LB, P. aeruginosa ATCC9027 was complemented with pqsR in trans and AQ and pyocyanin was measured in LB. As expected, the synthesis of both metabolites was restored. Since PqsE has been shown to play a role in pyocyanin production, P. aeruginosa ATCC9027 was also complemented with pqsE, and AQ and pyocyanin was measured, the results showed that PqsE only restored pyocyanin production, but no AQ were produced. Our hypothesis is that in LB medium, the pqsABCDE operon is not expressed due to the lack of PqsR and consequently limits the pqsE expression and thus AQ and pyocyanin production. However, in PPGAS our hypothesis is that PqsE can be expressed independently of the PqsR presence by an unknown regulation system, but related to the low phosphate concentration in PPGAS. To verify whether pqsE is transcribed in PPGAS, but not in LB medium, RT-PCR assays will be performed.

Mechanisms: Signalling, Systems & Synthetic

P187

Connecting amino acid metabolism with c-di-GMP levels and associated phenotypes in *Pseudomonas putida* KT2440

Laura Barrientos Moreno, María Isabel Ramos González, Manuel Espinosa Urgel
Estación Experimental del Zaidín (CSIC), Granada, Spain

C-di-GMP is an important intracellular second messenger that regulates bacterial lifestyles, in particular biofilm development. In previous studies, the response regulator with diguanylate cyclase activity CfcR of *Pseudomonas putida* KT2440 was shown to increase the levels of intracellular c-di-GMP when expressed from its own promoter in multicopy. This causes a pleiotropic phenotype that includes crinkly colony morphology. A broad genomic screen revealed that two genes in the arginine biosynthesis pathway were relevant for the crinkly colony morphology phenotype. Our research is focused on the role of amino acids in c-di-GMP levels, associated phenotypes and biofilm regulation in *P. putida* KT2440.

We have combined qualitative and quantitative analyses of intracellular c-di-GMP using a biosensor, and biofilm formation assays in the presence of different concentrations of aspartic acid or arginine added to the culture medium. Aspartic acid caused a decrease in c-di-GMP levels and a reduction in biofilm formation whereas arginine had the opposite effect. The crinkly colony phenotype is lost in the presence of elevated concentrations of aspartic acid, while it requires high concentrations of arginine for its appearance. The role of CfcR in the response to these amino acids was also analysed in terms of gene expression and c-di-GMP quantification. Our data suggest that CfcR is not the only element involved in c-di-GMP turnover that responds to the arginine/aspartic acid balance, since a residual response is still observed in a *cfcR* mutant. The regulatory elements involved in the connection between amino acid metabolism and c-di-GMP are currently being investigated.

Mechanisms: Signalling, Systems & Synthetic

P188

The regulatory complexity of surfing motility: A novel adaptation exhibited by *Pseudomonas aeruginosa* under host-like conditions

Evelyn Sun, Erin E Gill, Robert EW Hancock
University of British Columbia, Vancouver, Canada

Surfing motility is a complex adaptation in *Pseudomonas aeruginosa* that is dependent on the presence of the glycoprotein, mucin, a major component of cystic fibrosis lung sputum. Surfing is a rapid form of surface motility that results in a colony containing two genetically and phenotypically distinct populations of cells wherein electron microscopy has revealed larger cells in the pigmented centre of the colony and a mixture of large and smaller cells at the white edge. Little is known about its regulation. We focused on identifying and characterizing the roles of major regulators responsible for this adaptation. Using a strain PA14 transposon mutant library, 192 genes necessary for mediating surfing motility were identified. Of these, 44 were identified as known or putative regulators including major transcriptional regulators such as *fleQ, rhlR, lasR, pqsR* and two-component systems like *gacA/S*, *cbrA/B*, and *fleR/S*. The importance of quorum sensing was confirmed using mutant and homoserine-lactone supplementation studies. Using RT-qPCR, the transcriptional levels of each of these 44 regulators were measured in the transposon mutants of each of these regulators in order to elucidate possible hierarchies of regulation. Analysis revealed that *gacA, PA1463*, *PA1458*, *pfeS*, and *rpoC* were higher up in the hierarchy, whereas certain flagellar biosynthesis regulators such as FlgM and motor regulators like MotB were at the bottom of the hierarchy. Identifying the major genes involved in surfing motility will potentially explain its complex characteristics including differential cell types in a surfing colony and adaptive multiple antibiotic resistance.

Mechanisms: Signalling, Systems & Synthetic

P189

The underlying concepts of the T6SS baseplate

Sara Planamente, Alain Filloux, Paul Freemont
Imperial college, London, UK

The Type VI secretion system (T6SS) is a supra-molecular bacterial complex that fires toxins into target cells1. The T6SS involves about 13 core proteins and resembles phage tails. The current model of the structural organization of the T6SS suggests that its core components are assembled in several sub-complexes. A first set of T6SS components forms a trans-envelope complex which consists of the membrane proteins TssM, TssL and TssJ2. A second group of T6SS proteins forms the bacteriophage tail-like structure. It includes the Hcp protein and the sheath-like structure made of the two proteins TssB and TssC, which are structurally related to the gp19 and gp18 proteins of the T4 phage, respectively. The remaining T6SS core components are likely to form what is known as the baseplate structure in the phage. Yet, functional and structural information about such basal platform as well as its assembly within the T6SS, remain poorly described.

Mechanisms: Signalling, Systems & Synthetic

P190

Phenotypic analysis of *Pseudomonas putida* for industrial biotechnology applications.

Ainsley Beaton, Walid El Bestawy, Nicholas Tucker
University of Strathclyde, Glagsow, UK

Pseudomonas putida is increasingly being used for a variety of industrial biotechnology applications due to its metabolic diversity and tolerance to a wide variety of otherwise toxic platform chemicals and solvents.

The omnilog phenoarray system facilitates high-throughput phenotypic analysis of microorganisms on the basis of nutrient utilisation and chemical sensitivity. In this work the phenoarray system has been employed to confirm the base line phenotypic fingerprint of *P. putida* KT2440 and compare this output to that of the same strain under the stress of industrially relevant solvents. Phenotypic arrays have also been used to determine the tolerance of *P. putida* to a range of stresses including antimicrobial, chemical, osmotic and pH shock. We are also engaging in promoter engineering in *P. putida* and it is important to determine the output of these synthetic promoters under a range of stress conditions that are relevant to fermentation and industrial bioprocessing.

In depth knowledge of the effects of certain stresses on the basic metabolism of an organism with known tolerance abilities will facilitate better utilisation of this organism and the genes involved in it's metabolic pathways can be used to enhance other strains.

Mechanisms: Signalling, Systems & Synthetic

P191

Mapping the resistance and metabolic pathways associated with preservative exposure in an industrial *Pseudomonas aeruginosa* strain

Angharad Green[1], Alejandro Amézquita[2], Yvan Le-Marc[2], Jason Papin[3], Anna Blazier[3], Thomas Connor[1], Eshwar Mahenthiralingam[1]
[1]Cardiff School of Biosciences, Cardiff University, Cardiff, South Wales, UK, [2]Unilever, Safety and Environment Assurance Centre (SEAC), Bedford, England, UK, [3]Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia, USA

Pseudomonas aeruginosa is a common contaminant relating to product-recall in the home and personal care (HPC) industry. Preservation systems prevent spoilage and protect consumers; however, there is limited knowledge of resistance mechanisms utilised in response to preservatives. The aim of this research was to predict key functional pathways involved in preservative resistance. Industrial *P. aeruginosa* strain 7 was sequenced using PacBio technology and a genome-scale metabolic network reconstruction (GENRE) was built from a mathematically validated PA14 model. RNA-Seq analysis was implemented following exposure of strain 7 to preservatives and HPC products; results were used to further constrain the GENRE to accurately represent gene expression dynamics.

Strain 7 had a large 7.8 Mb genome with approximately 1,400 more coding-sequences than PA14 and included two plasmids (5.6 and 1.5 Kb). The constructed GENRE accounted for the function of 1,329 genes, 1,656 reactions and 1,461 metabolites. Genes unique to strain 7 were associated with 166 new reactions, with 'Xenobiotic Biodegradation and Metabolism' genes representing the greatest number of new reactions. Transcriptomic fold-changes integrated into the GENRE, identified membrane transport and xenobiotic biodegradation reactions as essential during exposure to industry relevant conditions.

The combination of whole-genome sequencing, transcriptomic-profiling and GENRE mechanistic modelling with an industrial *P. aeruginosa* strain provides key insights into genetic determinants and metabolic pathways involved in preservative resistance. The ability to predict *P. aeruginosa's* response when exposed to industry-relevant conditions will lead to the development of targeted preservation systems, to minimise the use of excessive antimicrobials whilst preserving HPC products safety.

Mechanisms: Signalling, Systems & Synthetic

P192

Roles of the Hfq and Crc global regulators on iron homeostasis in *Pseudomonas putida*

Dione L. Sánchez-Hevia, Fernando Rojo, Renata Moreno
Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain

Pseudomonas putida can colonize diverse environments such as soils, the rhizosphere or aquatic systems, and it is a very important model system in biotechnology. It has a great metabolic versatility, a trait that requires not only a large set of catabolic pathways, but also an appropriate coordination of their induction to avoid metabolic imbalance. The Hfq and Crc proteins are key regulatory elements that help controlling the uptake and metabolism of carbon sources, organizing the most appropriate metabolic configuration for cell needs. They work in a coordinated fashion, inhibiting translation initiation at selected mRNAs. Although mostly studied in connection with the catabolite repression control that assures a hierarchical and sequential assimilation of carbon sources, Hfq and Crc are believed to be involved in the control of other processes. To gain insight into other functions of these regulatory proteins, we compared the transcriptome and proteome of Hfq-null and Crc-null strains with that of the wild type strain under conditions of exponential growth in a minimal salts medium containing succinate as the sole carbon source. We found that the absence of Hfq had a much greater influence than the lack of Crc. The most prominent effect was a deregulation of iron homeostasis. Several of the affected genes showed clear binding sites for Hfq/Crc at their mRNAs, but in other instances the effect was apparently indirect, or related to the action of the PrrF1/PrrF2 small RNAs, which control post-transcriptionally several genes. Our current understanding of the underlying regulatory events will be presented.

Mechanisms: Signalling, Systems & Synthetic

P193

Insights into the role of LptE in LPS transport and cell physiology in *Pseudomonas aeruginosa*

Alessandra Lo Sciuto[1], Alessandra Martorana[2], Regina Fernández-Piñar[1], Alessandra Polissi[2], Francesco Imperi[1]
[1]Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Rome, Italy, [2]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

Lipopolysaccharide (LPS) is an essential structural component of the outer membrane (OM) of most Gram-negative bacteria. In the model organism *Escherichia coli*, LPS transport to the OM requires seven essential proteins (LptABCDEFG) that form a continuous bridge across the cell envelope. The LPS transport (Lpt) pathway is crucial for the growth of the majority of Gram-negative pathogens and, thus, represents a promising target for the design of novel antimicrobials. In *Pseudomonas aeruginosa* we have recently demonstrated the essentiality of LptH, the periplasmic component of the *P. aeruginosa* Lpt system. Surprisingly, independent high-throughput transposon mutagenesis studies identified viable insertion mutants in the *P. aeruginosa lptE* gene, suggesting that it might be dispensable in this bacterium. To verify this hypothesis, we generated an *lptE* conditional mutant in *P. aeruginosa* PAO1. LptE appears to be not essential for *P. aeruginosa* viability as its depletion only slightly impaired *in vitro* growth. Conversely, LptE was found to be important for cell-envelope stability, antibiotic resistance and infectivity in an insect model. Interestingly, in LptE-depleted cells the levels of the OM component LptD are significantly reduced, suggesting that in *P. aeruginosa*, as in *E. coli*, LptE is required for the correct OM localization and/or stability of LptD. Experiments are in progress to verify the amount and localization of LPS in LptE-depleted cells. Overall, these data imply that *P. aeruginosa* can tolerate an LptE-independent LPS transport system, indicating that the Lpt machinery might not work in a conserved manner in all Gram-negative bacteria.

Mechanisms: Signalling, Systems & Synthetic

P194

Quorum sensing and putisolvin biosynthesis in *Pseudomonas putida* IsoF

Steven Higgins[1], Gerardo Cárcamo-Oyarce[2], Leo Eberl[1]
[1]*Institute of Plant and Microbial Biology, University of Zürich, Zürich, Switzerland,*
[2]*Department of Biological Engineering, MIT, Cambridge, USA*

Putisolvin I and II are cyclic lipodepsipeptides which can inhibit fungal growth, disperse bacterial biofilms, reduce surface tension and facilitate swarming motility. It has been established that *psoABC* codes for the putisolvin peptide moiety and that this operon is under the control of an acyl homoserine-lactone (AHL) quorum sensing (QS) system. The QS system of *Pseudomonas putida* consists of an AHL synthase (PpuI), response regulator (PpuR) and QS repressor (RsaL), which are homologous to the *las* QS system of *Pseudomonas aeruginosa*.

Although *psoABC* codes for the putisolvin peptide moiety it is unclear how the molecule becomes *N*-acylated. Sequence analysis shows no fatty acid coenzyme A (CoA) encoded in the vicinity of *psoABC*. We speculate that a putative long chain fatty acid CoA ligase (*ppuA*), located downstream of *ppuR*, could be involved in the biosynthesis of the activated fatty acid donor.

We have assessed the role PpuA plays in putisolvin biosynthesis by examining swarming motility and anti-fungal activity in a *ppuA* mutant strain. Reduced swarming and antifungal activity was observed suggesting *ppuA* is required for putisolvin dependent phenotypes. It is likely that insufficient fatty acid CoA is available from central metabolism to compensate for the *ppuA* mutation, rendering putisolvin non-functional.

Using a GFP reporter fusion the regulation of P*ppuA* was analysed in a range of QS mutants. Putisolvin production is co-dependent on the *psoABC* upstream regulator *psoR* hence, P*ppuA* activity was also examined in a *psoR* mutant background, allowing us to develop a model of how QS regulates putisolvin biosynthesis.

Mechanisms: Signalling, Systems & Synthetic

P195

The extraordinary role of PqsE within the quorum-sensing network of *Pseudomonas aeruginosa*

Michael Zender[1], Florian Witzgall[2], Steffen L. Drees[3], Elisabeth Weidel[1], Christine K. Maurer[1], Susanne Fetzner[3], Wulf Blankenfeldt[2,4], Martin Empting[1], Rolf W. Hartmann[1,5]
[1]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Department Drug Design and Optimization, Saarbrücken, Germany, [2]Helmholtz Centre for Infection Research (HZI), Department Structure and Function of Proteins, Braunschweig, Germany, [3]Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany, [4]Institute for Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany, [5]Pharmaceutical and Medicinal Chemistry, Department of Pharmacy, Saarbrücken, Germany

Pseudomonas aeruginosa is an important human pathogen that causes nearly 10% of all hospital-acquired infections. A potential therapeutic strategy is the development of drugs that target proteins involved in the *pqs* quorum-sensing system. The thioesterase PqsE takes a special position in the quorum-sensing network of *P. aeruginosa*, because in addition to its role in the biosynthesis of the autoinducers HHQ or PQS, it is able to activate the expression of several key virulence factors like pyocyanin independently of the transcription factor PqsR. This regulatory function depends on RhlR.
By screening a fragment library comprising 500 synthetic small molecules with differential scanning fluorimetry (DSF), we identified several compounds that stabilized PqsE towards thermal denaturation. The binding of these compounds to PqsE was confirmed by isothermal titration calorimetry and the mode of interaction was elucidated by X-ray crystallography, revealing that these ligands occupy the same site in the active center as 2-aminobenzoylacetate (2-ABA), the product of the reaction catalyzed by PqsE. Therefore it was speculated that those compounds may inhibit the thioesterase activity of PqsE, which was indeed confirmed in two different *in vitro* assays and with an *in cellulo* assay. However, the PqsE thioesterase inhibitors had no effect on the he PqsE-RhlR mediated response. Our results provide clear evidence that the thioesterase acitivity of PqsE is not required for its regulatory function in the quorum-sensing network of *P. aeruginosa*. Due to its dual function, PqsE is a promising anti-virulence target.

Mechanisms: Signalling, Systems & Synthetic

P196

Metabolic regulation of the Type Three Secretion System (T3SS) in *Pseudomonas aeruginosa*

Yassmin Abdelhamid, Martin Welch
University of Cambridge, Cambridge, UK

Type three secretion system (T3SS) has a well-established role in *Pseudomonas aeruginosa* infections. The pathogen uses the T3SS to inject toxins directly into the recipient's cytoplasm, thereby subverting host cell function leading to tissue damage and death. In other microorganisms, pyruvate kinase activity has been linked with the control of pathogenicity. In *P. aeruginosa*, pyruvate kinase is encoded by two isozymes, PykA and PykF. The main objective of this work was to characterize these isozymes and to understand their role in regulation of T3S. Kinetic parameters of the purified proteins revealed that PykA and PykF have relatively similar Khalf values for the substrate, phosphoenolpyruvate (1.08 mM and 0.77 mM, respectively), whereas the KM values for ADP differed by ca. two-fold (0.05 mM and 0.10 mM, respectively). Both isoenzymes require Mg2+ for optimal activity, although unlike pyruvate kinase from other bacterial species, they do not require K+. No enzyme activity was detected in a *pykA* mutant or in a *pykA-pykF* double mutant, whereas a *pykF* mutant was essentially similar to the wild-type for pyruvate kinase activity. Consistent with this, transcriptional activity assays confirmed that the promoter driving *pykA* expression is much more active than the promoter driving *pykF* expression. However, although a *pykA pykF* double mutant failed to express components of the T3SS compared with the wild type or *pykA* or *pykF* single mutants. In conclusion, although PykA is apparently the dominant isozyme in pyruvate metabolism, it seems that PykA and PykF play a combined role in regulation of the T3SS.

Mechanisms: Signalling, Systems & Synthetic

P197

The pyochelin locus is involved in the conditional essentiality of the ferric uptake regulator Fur in *Pseudomonas aeruginosa*

Martina Pasqua[1], Daniela Visaggio[2], Alessandra Lo Sciuto[1], Shirley Genah[1], Ehud Banin[3], Paolo Visca[2], Francesco Imperi[1]
[1]Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Rome, Italy, [2]Department of Sciences, University Roma Tre, Rome, Italy, [3]Institute for Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat Gan, Israel

In *Pseudomonas aeruginosa* regulation of metabolism and virulence in response to iron availability is mediated by the ferric uptake regulator Fur. Differently from other bacterial species, attempts to obtain *fur* deletion mutants in *P. aeruginosa* failed, and for this reason Fur has been proposed to be an essential protein in this bacterium. By investigating a *P. aeruginosa* PAO1 *fur* conditional mutant, we demonstrated that Fur is not strictly essential for *P. aeruginosa* growth in liquid media and for biofilm formation, as well as for pathogenicity in the *Galleria mellonella* insect model of infection. However, Fur-depleted cells are severely impaired in the ability to grow as colonies on solid media, and this may have hampered former selection of *fur* null mutants. Transposon-mediated random mutagenesis experiments identified the *pch* locus, encoding the pyochelin siderophore, as a major cause of the colony growth defect of the *fur* conditional mutant, and deletion mutagenesis confirmed this evidence. Impaired colony growth of pyochelin-proficient Fur-depleted cells does not appear to be dependent on oxidative stress, as these cells do not accumulate higher levels of reactive oxygen species (ROS), and are not rescued by antioxidant agents or overexpression of ROS-detoxifying enzymes. Notably, ectopic expression of *pch* genes revealed that pyochelin production has no inhibitory effects in a *fur* deletion mutant of a siderophore-null isolate of *Pseudomonas syringae* pv. *tabaci*, suggesting that the toxicity of the *pch* locus in Fur-depleted cells likely involves *P. aeruginosa*-specific pathway(s).

Mechanisms: Signalling, Systems & Synthetic

P198

Acyl carrier protein stabilizes the lipopolysaccharide sugar kinase WaaP in *Pseudomonas aeruginosa*

Naomi Kreamer, Travis Stams, Rajiv Chopra, Tsuyoshi Uehara, Charles Dean
Novartis Institues for BioMedical Research, Emeryville, CA, USA

Unlike many other Gram-negative pathogens the core region of Pseudomonas aeruginosa lipopolysaccharide (LPS) is highly phosphorylated, which is required for assembly and integrity of the outer membrane. The sugar-kinase, WaaP, phosphorylates the inner core heptose and is essential for growth of P. aeruginosa. We solved the crystal structure of P. aeruginosa WaaP purified from Escherichia coli, revealing a WaaP was in complex with E. coli acyl-carrier protein (ACP). Acyl-ACP was bound to WaaP distal to the kinase pocket via charged protein-protein interaction and insertion of a palmitate chain into a C-terminal large hydrophobic tunnel in WaaP. This novel interaction was recapitulated from P. aeruginosa using mass spectrometry of a WaaP-His protein pulldown. The crystal structure was used to design mutations of WaaP to elucidate the role of the ACP complex. We tested WaaP variants for stability and ability to restore growth and the ability to resist in P. aeruginosa WaaP depletion and E. coli waaP deletion strains. Changes at catalytic residues or the kinase HRD motif were stable but unable to support growth, consistent with loss of kinase activity. In contrast, the ACP interaction variants of WaaP suggest that ACP stabilizes the WaaP protein. To our knowledge, this is the first example where acyl-ACP does not provide the acyl substrate in an enzymatic reaction but rather stabilizes an enzyme via the acyl chain of ACP. The acyl chain of ACP bound to WaaP is associated with phospholipid not LPS biosynthesis, postulating a possible regulatory link between LPS and phospholipid biosynthesis.

Mechanisms: Signalling, Systems & Synthetic

P199

2C or not 2C? Regulation of carbon flux through the glyoxylate shunt in the opportunistic pathogen, Pseudomonas aeruginosa.

Stephen Dolan, Martin Welch
University of Cambridge, Cambridge, UK

Pseudomonas aeruginosa (PA) is the major pathogen associated with chronic and ultimately fatal airway infections in patients with cystic fibrosis. The glyoxylate shunt is an anaplerotic pathway that allows micro-organisms to grow on acetate or fatty acids as a sole carbon source - a feat that most higher-organisms are incapable of. Mutants defective in the glyoxylate shunt enzymes are rapidly cleared from a mouse pulmonary infection model. For this reason, the junction between the glyoxylate shunt and the TCA cycle is one of the most promising targets for the development of novel antimicrobial compounds.
However, the enzymology of the branchpoint between the TCA cycle and glyoxylate shunt in P. aeruginosa differs substantially from that in well-characterized organisms such as E. coli, and little is known about how flux through the shunt is regulated in PA. We have utilised a combination of transcriptomic, proteomic and metabolomic approaches to investigate this metabolic branchpoint in considerable detail. Comparative RNA-Seq analysis and metabolomics of PA grown on glycerol or acetate as sole carbon sources identified relevant pathways and metabolites which impinge upon branchpoint regulation. Factors controlling enzyme expression levels were investigated by using DNA-affinity purification; this has identified several previously uncharacterised transcription factors which bind to the upstream region of the genes encoding various branchpoint enzymes. Finally, the formation of multi-enzyme, mega-complexes was investigated using formaldehyde cross-linking combined with mass spectrometry. Collectively, these approaches are allowing us to develop a multi-scale model accounting for the regulation of flux through the glyoxylate shunt in PA.

Mechanisms: Signalling, Systems & Synthetic

P200

Use of the inter-relation of rhamnolipids and polyhydroxyalkanoates biosynthetic pathways for the construction of a *Pseudomonas aeruginosa* RL-overproducing strain

Uriel Gutiérrez-Gómez, Gloria Soberón-Chávez
National Autonomous University of Mexico - Biomedical Research Institute, University City, Mexico City, Mexico

Pseudomonas aeruginosa is an ubiquitous bacteria capable of producing a large variety of metabolites including rhamnolipids (RL) and polyhydroxyalkanoates (PHA). We attempt to study the metabolic link between RL and PHA biosynthetic pathways to determine a metabolic engineering strategy for increasing RL production.

Chromosomal gene knockout mutations were obtained by using the lambda Red recombinase system. *P. aeruginosa* strains were cultivated in PPGAS medium using glucose as carbon source. RL were quantified using the orcinol assay. PHA were determined by hypochlorite digestion and crotonic acid quantification.

Unlike other *P. aeruginosa* strains, the overexpression of *rhlAB* or *rhlABR* operons on $PA14_{wt}$ strain did not increase RL, but PHA did. Therefore, in order to increase the metabolic flux to RL biosynthesis, PHA biosynthetic pathway was blocked and, in addition, *rhlAB* and *rhlABR* operons were overexpressed. Using *phaG*, *phaC1* and *phaC2* triple mutant the highest titer of RL was obtained which represents a 35 % increase compared to $PA14_{wt}$. Because PA14 $phaG^-$ strain still produces about 50 % PHA of $PA14_{wt}$, we explored the hypothetic function of RhlA enzyme (75 % similar to PhaG transacylase) in PHA production. In PA14 $phaG^-$ $rhlA^-$/pHERD20T-rhlA strain, PHA production was restored.

In conclusion, we obtained a *P. aeruginosa* RL-overproducer strain by blocking PHA biosynthesis and overexpressing *rhlABR* operon under its own promoter. In addition to producing HAA (precursor in RL biosynthesis) RhlA, in the absence of PhaG, can restore PHA production. Therefore, this is evidence that RhlA can produce PHA intermediates such as HAA-CoA or R-hydroxyacyl-CoA.

Mechanisms: Signalling, Systems & Synthetic

P201

EXPRESSION OF VERY LONG O ANTIGEN LIPOPOLYSACCHARIDE IS REPRESSED IN MUCOID *PSEUDOMONAS AERUGINOSA*

Ashley Cross, Joanna Goldberg
Emory University, Atlanta, GA, USA

Numerous phenotypic changes take place as *Pseudomonas aeruginosa* transitions from the environment to causing life-shortening chronic lung infections in cystic fibrosis (CF) patients; mucoidy, due to the overproduction of the exopolysaccharide alginate, is the most conspicuous. In addition, most mucoid isolates do not express lipopolysaccharide (LPS) O antigen. While LPS isolated from non-mucoid strains includes both long and very long (VL) lengths of O antigen, LPS from the rare mucoid strains that do have LPS express less VL O antigen. To investigate this reciprocal regulation, we initiated studies with the laboratory strain PAO1 and its mucoid derivative, PAO1 *mucA22*. Western blot analysis of LPS extracted from these strains showed that the mucoid strain expressed a reduced amount of VL O antigen compared to its non-mucoid parental strain. Furthermore, immunoblot analysis of protein extracts from the mucoid strain revealed that there was less Wzz2 (the VL O antigen chain length regulatory protein) compared to the non-mucoid strain, which was supported by parallel RT-qPCR analysis of *wzz2* transcript levels. In non-mucoid PAO1 *mucA22 algD::*FRT, which has a deletion in *algD*, the first gene in the alginate biosynthesis operon, an increase in VL O antigen, Wzz2, and *wzz2* was observed. Other alginate mutants of PAO1 *mucA22* also had altered O antigen profiles suggesting that alginate expression may be impacting the expression of *wzz2*. Understanding the inverse regulation of alginate and VL O antigen may elucidate mechanisms that are important for the establishment of chronic infections in CF and provide opportunities for intervention.

Mechanisms: Signalling, Systems & Synthetic

P202

Fic-1 in *Pseudomonas fluorescens* 2P24 was induced by quinolone antibiotics and affected DNA replication

Can-Hua Lu[1,2], Gao-Qi Gu[1], Ernesto S. Nakayasu[2], Zhao-Qing Luo[2], Li-Qun Zhang[1]
[1]*Department of Plant Pathology, China Agricultural University, Beijing, China,*
[2]*Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA*

Bacterial Fic proteins (filamentation induced by cAMP) modify target proteins by AMPylation or other chemical modifications. A number of pathogenic bacteria employ Fic-domain-containing effectors to thwart host immune pathways by mechanisms such as AMPylation to promote infection, but the functions of most of the house-keeping Fic proteins in cellular processes are still poorly understood. We previously demonstrated that Fic-1 from the soil bacterium *P. fluorescens* 2P24 affects DNA replication and bacterial growth by AMPylating GyrB, a subunit of the DNA gyrase on Tyr^{111} and ParE, a component of DNA topoisomerase IV on Tyr^{109}. Ectopic expression of Fic-1 led to the induction of the SOS response and the formation of filamentous cells in both *P. fluorescens* and *Escherichia coli*, but the environmental cues responsible for the induction of *fic-1* remain unknown. To explore the environmental conditions capable of triggering *fic-1* expression, we developed a reporter strain which contains a transcriptional fusion of *fic-1* with the β-lactamase gene. Combined with the Phenotype Microarray technology, we identified 6 quinolone antibiotics as inducers for the *fic-1* transcription. These compounds include ciprofloxacin, ofloxacin, and norfloxacin, but not aminocoumarin-like novobiocine. Our results suggest that the expression of Fic-1 is induced by environmental stresses that interfere with DNA replication.

Mechanisms: Signalling, Systems & Synthetic

P203

Genome-wide analysis of the FleQ direct regulon in the biocontrol strain *Pseudomonas fluorescens* F113.

Esther Blanco-Romero, Daniel Garrido-Sanz, Francisco Martínez-Granero, Candela Muriel, David Durán, Rafael Rivilla, Miguel Redondo-Nieto, Marta Martín
Universidad Autónoma de Madrid, Madrid, Spain

Bacterial motility plays a crucial role in competitiveness and rhizosphere colonization. In this regard, FleQ appears to be the master regulator of flagella and exopolysaccharide biosynthesis in pseudomonads, including *Pseudomonas fluorescens* F113, an interesting biocontrol bacteria.

The aim of this work was to identify the genes and operons regulated by FleQ and to analyze the overlap between the FleQ and AmrZ regulons in *P. fluorescens* F113. By using the ChIP-seq method we expected to find the DNA regions where FleQ binds to regulate gene expression. Regulation was subsequently confirmed with RT-qPCR assays in wild-type and *fleQ* mutant backgrounds.

ChIP-seq analysis has shown that FleQ is a global regulator in *P. fluorescens* F113 and 106 FleQ putative binding sites have been identified. Genes presumably regulated by FleQ included, as expected, flagellar and motility-related genes and others involved in adhesion and exopolysaccharide production. Surprisingly, the ChIP-seq analysis also identified iron uptake-related genes. Regulation of these genes by FleQ was verified by RT-qPCR. The results also showed that FleQ shares an important part of its direct regulon with AmrZ, a global regulator implicated in environmental adaption. Although AmrZ also regulates motility and iron uptake, the overlap occurred only with the iron-related genes, since both regulators control a different set of motility-related genes.

In conclusion, in this work FleQ is described as a major regulator involved in *P. fluorescens* F113 environmental adaption by affecting genes attributed to several functions and sharing partially its regulon with AmrZ.

Mechanisms: Signalling, Systems & Synthetic

P204

The Pseudomonas aeruginosa type VI secretion system delivers the Tse7 nuclease in bacterial preys

Panayiota Pissaridou, Alain Filloux
Imperial College, London, South Kensington, UK

Pseudomonas aeruginosa is an opportunistic bacterium which possesses a variety of protein secretion systems including the Type Six Secretion System (T6SS). Three T6SS clusters and several vgrG and hcp gene islands are present in P. aeruginosa genome. The vgrG1b cluster lies next to the H1-T6SS. It is a 7-gene cluster whose composition and structure is highly reminiscent of the Proteus mirabilis hcp-vgrG operon. The sequence of the genes in the vgrG1b cluster is highly conserved in all P. aeruginosa genomes except for two genes that we have called tse7 and tsi7. We show that Tse7 is a nuclease with a Tox-GHH2 domain which is distinct from other T6SS nucleases identified thus far. This domain, whose sequence between P. aeruginosa strains is most variable, lies at the Tse7 C terminus, while the N terminus is a predicted PAAR domain. PAAR domains have been proposed to sit on the tip of VgrG proteins and we showed here that delivery of Tse7 is strictly dependent on VgrG1b. The delivery of the Tse7 nuclease is also dependent on the H1-T6SS cluster, and injection of the nuclease in bacterial preys results in DNA degradation and growth arrest. Our study highlight the broad diversity of effectors which are available to P. aeruginosa while one single T6SS system can deliver toxins with a wide range of activity, notably peptidoglycan hydrolases, ADP-ribosylating toxins, NADP glycohydrolase toxin or nuclease. The striking variability in the tse7-tsi7 sequence between P. aeruginosa strains is suggesting a role in inter-strain competition.

Mechanisms: Signalling, Systems & Synthetic

P205

Genome-wide mapping reveals direct targets of the RNA-binding protein RsmN in carbon metabolism and virulence of *Pseudomonas aeruginosa*

Hazel Silistre[1,2], Manuel Romero[2], Laura Lovelock[2], Victoria Wright[2], Kok-Gan Chan[3], Kar-Wai Hong[3], Paul Williams[2], Miguel Cámara[2], Stephan Heeb[2]
[1]Institut Pasteur, Paris, France, [2]University of Nottingham, Nottingham, UK, [3]University of Malaya, Kuala Lumpur, Malaysia

Pseudomonas aeruginosa (PA) is known for its versatility to switch lifestyles to adapt acute or chronic infection conditions. Whilst motility and type III secretion system (T3SS) of PA are acute virulence traits, biofilm formation and type VI secretion system (T6SS) are chronic virulence traits. The GacS/GacA two-component system, the RetS and LadS sensors and the Rsm system which consists of the RNA-binding proteins RsmA and RsmN (or RsmF) and cognate small regulatory RNAs control virulence through regulation of quorum sensing, motility, biofilm formation, anaerobic growth, and production of secondary metabolites at the post-transcriptional level in PA. This complex system is therefore being targeted for antivirulence therapy.

A previous study revealed that although the RNA-binding pocket was conserved, a structural rearrangement resulted in a different protein folding of RsmN in comparison with RsmA. Utilising protein-RNA co-purification and RNAseq techniques and electrophorotic mobility shift assays, direct targets of RsmN and its binding specificities to target mRNAs were demonstrated in this study. The biological function of RsmN was verified in phenotypic assays using a double deletion mutant of *rsmA* and *rsmN* as a Δ*rsmN* phenotype was masked in the presence of RsmA. RsmN was found to suppress chronic mode of infection by reducing biofilm development and T6SS both directly and indirectly via multiple pathways while promoting acute mode of infection. Furthermore, small non-coding RNA of the carbon catabolite repression (CCR) system, CrcZ, was detected as a common target of RsmA and RsmN suggesting a cross-talk between the Gac/Rsm and the CCR systems.

Mechanisms: Signalling, Systems & Synthetic

P206

Regulation of ribonucleotide synthesis by the *Pseudomonas aeruginosa* two-component system AlgR in response to oxidative stress

Anna Crespo[1], Lucas Pedraz[1], Marc Van Der Hofstadt[2], Gabriel Gomila[2], Eduard Torrents[0]
[1]Institute for Bioengineering of Catalonia (IBEC). Bacterial infections and antimicrobial therapy group., Barcelona, Spain, [2]Institute for Bioengineering of Catalonia (IBEC). Nanoscale Bioelectrical Characterization., Barcelona, Spain

Ribonucleotide reductases (RNR) are key enzymes for the metabolism of all living cells. RNR mediate the synthesis of deoxyribonucleotides, DNA precursors, for DNA synthesis and repair. Three forms of RNR exist, classes I, II and III, each one accounting for a different activation mechanism and allowing biosynthesis to occur under different conditions. While eukaryotic cells use only class Ia RNR, bacteria can harbour any combination of classes, granting them adaptability. The opportunistic pathogen *Pseudomonas aeruginosa* is one of the few known organisms that encodes all three classes, allowing it to adapt to different environments and form biofilms. Here, we study the well-described induction of RNR activity through oxidative stress, whose molecular mechanism has never been elucidated. We examine the action of the AlgZR two-component system, the primary regulator of alginate biosynthesis and the mucoid phenotype, on the RNR activity. Through bioinformatics, we identify the AlgR binding locations in class I and class II RNR promoter regions, which we characterize functionally through EMSA and physically through atomic force microscopy imaging. Gene reporter assays provide evidence that both classes are regulated by AlgR *in vivo* and uncover the mechanism through which the AlgZR system controls the RNR network under different environmental conditions and physiological states. Finally, we demonstrate that AlgZR-mediated regulation is the link that causes induction of ribonucleotide reductase activity through oxidative stress.

Mechanisms: Signalling, Systems & Synthetic

P207

Interactions between PvdA and PvdF, enzymes involved in pyoverdine biosynthesis in *Pseudomonas aeruginosa* PAO1

Priya Philem, Sigurd Wilbanks, Iain L. Lamont
University of Otago, Dunedin, New Zealand

Background: During infections, *Pseudomonas aeruginosa* secretes an iron scavenging compound, pyoverdine. Pyoverdine consists of a peptide group containing the unusual amino acid formylhydroxyornithine attached to a chromophore and an acyl side chain. Formylhydroxyornithine is formed from the conversion of ornithine to hydroxyornithine by PvdA and subsequently to formyl hydroxyornithine by PvdF. The instability of hydroxyornithine indicates substrate channeling between PvdA and PvdF. This research aims at investigating the interaction of PvdA and PvdF.

Methods: A co-purification pull down method was used to determine whether PvdA and PvdF interact. Hexahistidine-tagged PvdA was co-expressed with untagged PvdF, and vice versa. Interaction was tested by looking for the presence of the binding partner following purification of the His-tagged protein by affinity chromatography. Protein identities were confirmed by proteins mass spectrometry.

Results: When the proteins were co-expressed in *E. coli*, PvdF co-purified with Histidine-tagged-PvdA demonstrating protein-protein interactions. PvdA showed little or no co-purification with PvdF that has an N-terminal hexahistidine tag.

Conclusions: These results demonstrate interaction between PvdA and PvdF. The histidine tag at the N-terminal of PvdF may interfere with interaction with PvdA. Use of a bacterial two hybrid system be used to further investigate the PvdA-PvdF interaction.

Mechanisms: Signalling, Systems & Synthetic

P208

Alkylquinolone and virulence factor production of *Pseudomonas aeruginosa* in co-culture with *Mycobacterium abscessus*

Ruth Säring, Franziska S. Birmes, Susanne Fetzner
WWU Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany

Pseudomonas aeruginosa is a ubiquitous opportunistic pathogen regulating its virulence via quorum sensing (QS). Beside *N*-acylhomoserine lactones it uses 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ) as QS-signalling molecules. Through differential expression of the *pqs* operon, which includes *pqsE* coding for the "PQS response protein", they can influence the production of virulence factors such as pyocyanin, pyoverdine and rhamnolipids. *Mycobacterium abscessus* subsp. *abscessus* is an emerging pathogen in cystic fibrosis capable of degrading PQS and HHQ. This ability is mediated by the *aqdRABC* genes which are present in strains of the subspecies *abscessus* but appear to be absent in members of the subspecies *massiliense*.
Co-cultivation of *P. aeruginosa* PAO1 with *M. a. abscessus*, a complemented *aqdBC*-deletion mutant, and a recombinant *M. a. massiliense* harbouring *aqdC* in 30% LB and in synthetic cystic fibrosis medium led to reduced levels of HHQ and PQS. PQS and HHQ concentrations in co-cultures of PAO1 with a *M. a. massiliense* strain or with the *aqdBC*-deletion mutant of *M. a. abscessus* were in the same range as those in PAO1 solo-cultures. Interestingly, there were no detectable differences in production of the virulence factors pyocyanin, pyoverdine or rhamnolipids in the various co-cultures. RT-qPCRs will reveal whether the unaltered expression of virulence factors correlates with unaltered transcript levels of key genes like *pqsE*, *pqsR* and *pqsA*, or give a hint on differential regulation of virulence gene expression by factors other than alkylquinolone-based QS under the co-culture conditions.

Mechanisms: Signalling, Systems & Synthetic

P209

A multisensor hybrid histidine kinase and an orphan response regulator with diguanylate cyclase activity form the two component phosphorelay system CfcR/CfcA in *Pseudomonas putida* KT2440.

Víctor G Tagua, María Isabel Ramos-González
Estación Experimental del Zaidín (EEZ-CSIC). Profesor Albareda, 1. 18008, Granada, Spain

The gene *cfcR* encodes an orphan multidomain response regulator with diguanylate cyclase activity, which is responsible for the synthesis of cyclic diguanosine monophosphate (c-di-GMP). This second messenger is a key player in the regulation of the transition from planktonic to sessile lifestyles. When overexpressed *cfcR* causes a pleiotropic phenotype consisting of increased biofilm formation, cells aggregation and crinkly colony morphology. In a genomic screen for crinkle free colony (cfc) mutants, the gene *cfcA* encoding a multisensor CHASE3/GAF hybrid histidine kinase was identified. The conserved histidine residue in the HisKA domain of CfcA was required for in vivo activation of CfcR.
Autophosphorylation of CfcA was evidenced with purified His-tagged peptides using ^{32}P-ATP and the transfer of the phosphoryl group to internal receiver domains in the C-terminus of CfcA was verified. In addition CfcA transphosphorylated the response regulator receiver domain of CfcR in vitro, confirming that these two elements constitute a two-component phosphorelay system. The role of sensor and receiver domains of CfcA in the activity of this histidine kinase is being investigated using mutants generated by homologous recombination.

The gene *cfcA* is part of a cluster with *cfcB* and *cfcC*, which encode respectively the methyltransferase and methylesterase of a chemosensory pathway. Two chemosensory pathways are present in *Pseudomonas putida* besides of the chemotaxis pathway CheR/CheB, i.e. WspC/WspF, which has been already characterized in order pseudomonads, and CfcB/CfcC. We are currently investigating the connection between the two component system CfcR/CfcA and the species specific chemosensory pathway CfcB/CfcC.

Mechanisms: Signalling, Systems & Synthetic

P210

PQS dioxygenase AqdC from *Mycobacterium abscessus* reduces virulence factor production in *Pseudomonas aeruginosa* PAO1

Franziska S. Birmes, Susanne Fetzner
WWU Münster, Institute for Molecular Microbiology and Biotechnology, Münster, Germany

The opportunistic pathogen *Pseudomonas aeruginosa* is often associated with patients suffering from cystic fibrosis or burn wounds. It regulates its virulence factor production via a complex quorum sensing network incorporating the signal molecules PQS (*Pseudomonas* quinolone signal, 2-heptyl-3-hydroxy-4(1*H*)-quinolone) and HHQ (2-heptyl-4(1*H*)-quinolone) besides *N*-acylhomoserine lactone signals. In order to interfere with quorum sensing and thereby reduce the virulence of *P. aeruginosa*, we searched for enzymes capable of degrading PQS.
AqdC from *Mycobacterium abscessus* - an emerging pathogen in cystic fibrosis - is a cofactorless dioxygenase, catalyzing the cleavage of PQS to *N*-octanoylanthranilate and carbon monoxide with a specific activity of 60 µmol min-1 (mg protein)-1. AqdC shows highest activity towards PQS in comparison to PQS congeners with shorter or longer alkyl chains.
Presence of only 0.33 U purified AqdC (ml culture)-1 reduced the alkylquinolone concentrations in *P. aeruginosa* LB cultures to 10 %, and RT-qPCRs revealed approximately half of the transcripts amounts of *pqsA* and *pqsE* genes coding for proteins involved in alkylquinolone biosynthesis. Moreover, the addition of AqdC led to a reduction of the virulence factors pyocyanin and pyoverdine. These results indicate that the ability of AqdC to degrade PQS leads to interference with quorum sensing in *P. aeruginosa* and possibly attenuates its virulence. Treatment with AqdC in *P. aeruginosa* infection models will give insight into the necessity of PQS for successful infection and the suitability of PQS quorum sensing interference as potential anti-virulence therapy.

Mechanisms: Signalling, Systems & Synthetic

P211

AMRZ IS A GLOBAL ENVIRONMENTAL REGULATOR INVOLVED IN c-di-GMP TURNOVER IN *PSEUDOMONAS FLUORESCENS* F113

Candelas Muriel, Eva Arrebola, Francisco Martinez-Granero, Esther Blanco-Romero, Daniel Garrido-Sanz, Miguel Redondo-Nieto, Rafael Rivilla, Marta Martín
Universidad Autónoma de Madrid, Madrid, Spain

AmrZ is a regulator of gene expression across pseudomonads. *P. fluorescens* F113 is a PGPR which has been considered as a model strain for rhizosphere colonization studies. A ChIP seq analysis of AmrZ in F113 and *P. aeruginosa* revealed that this protein is involved in environmental sensing and adaption.

We have analyzed the swimming motility, biofilm formation and competitive colonization phenotypes of an F113 *amrZ* mutant background. Besides, a transcriptomic analysis (RNA-Seq) has been performed to investigate the role of AmrZ in c-di-GMP turnover. Moreover, we have also analyzed c-diGMP levels using a biosensor based in pCdrA and by LC-MS. Phenotypic analysis in mutants affected in *amrZ* regulated genes has also been performed.

The *amrZ* mutant showed increased swimming motility, reduced biofilm formation and was less competitive for rhizosphere colonization than the wild type. The RNA-Seq analysis revealed that *amrZ* is involved in the regulation of more than 50% of the genes related with c-di-GMP turnover in F113. Consistent with this result, *amrZ* mutant showed very low levels of intracellular c-di-GMP. Finally, the analysis of the motility and biofilm phenotypes of mutants affected in genes regulated by AmrZ and implicated in c-di-GMP turnover showed the role of AmrZ in regulating these traits trough modulation of c-di-GMP levels.

AmrZ is a major determinant of c-di-GMP levels controlling motility, biofilm formation and rhizosphere competitive colonization.

Mechanisms: Signalling, Systems & Synthetic

P212

Regulation of the *Pseudomonas* Quinolone Signal by the TetR Family Regulator, PsrA

Greg Wells, Samantha Palethorpe, Everett Pesci
East Carolina University, Greenville, North Carolina, USA

Pseudomonas aeruginosa is a gram negative, ubiquitous pathogen which is frequently isolated from nosocomial infections and is well known for causing chronic lung infections in cystic fibrosis (CF) patients. *P. aeruginosa* uses three complex signaling mechanisms which are required for virulence, one of which functions through the *Pseudomonas* Quinolone Signal (PQS). A previous study has shown that PsrA, a TetR family regulator, is involved in the regulation of PQS production. It has also been shown that PsrA regulates components of the fatty acid β-oxidation pathway. Expression of *psrA* has been observed in the CF lung and it is suggested that *P. aeruginosa* senses free fatty acids in the lung, which influences the regulation of fatty acid degradation and PQS synthesis. Our data show that PsrA binds to the promoter region and represses expression of the FadE homolog, *PA0506*. PA0506 is predicted to have acyl-CoA dehydrogenase activity which converts acyl-CoA moieties (used for PQS synthesis) into enoyl CoA during fatty acid degradation. We show that PQS production is greatly reduced in a Δ*psrA* mutant and that a double Δ*psrA*Δ*PA0506* mutant has PQS production restored to a wild type level. This suggests that inhibition of *PA0506* by PsrA maintains the supply of acyl-CoA for PQS synthesis. Finally, previous data show that long chain fatty acids can bind to and inhibit PsrA activity. Interestingly, we show that addition of specific fatty acids positively affects expression of *PA0506* while increasing PQS production at the same time.

Mechanisms: Signalling, Systems & Synthetic

P213

The 2-alkyl-4-quinolone response protein PqsE modulates c-di-GMP signalling by inducing antisense transcription

François D'Heygère, Servane Le Guillouzer, Quentin Liot, Éric Déziel
Institut Armand Frappier-INRS, Laval (QC), Canada

The Pseudomonas Quinolone Signal (PQS), a molecule belonging to the family of 4-hydroxy-2-alkyquinolines (HAQs, aka 2-alkyl-4-quinolones, AQs), is a quorum sensing signalling molecule inducing the activity of the transcription factor MvfR (PqsR) in Pseudomonas aeruginosa. While the positive regulation by the MvfR/PQS system of target genes, such as phz genes, is mediated by the activity of PqsE after induction of pqsABCDE by PQS binding to MvfR, the mechanism by which pqsE activate transcription of target genes remains elusive.
To better understand the regulatory function of PqsE, we have undertaken a genome-wide expression study using the RNA-seq method to acquire a comprehensive transcriptomic portrait of genes, including small RNAs, differentially regulated by PqsE activity. Interestingly, detailed data analysis revealed that pqsE expression impacts several antisense transcripts. Among these genes, we observed that PqsE induces antisense transcription at the distal portion of nicD, a gene coding for a diguanylate cyclase involved in c-di-GMP synthesis, previously reported to play a role in the sessile-planktonic lifestyles switch. Our results show that disruption of antisense transcription of nicD enhances biofilm formation, reduces swarming motility, and induces the formation of small colony variants (SCVs), phenotypes all compatible with an increased concentration in c-di-GMP.
These observations support an unexpected relation between the MvfR/PQS quorum sensing system and c-di-GMP, and reveal a new example of antisense transcription in P. aeruginosa gene expression.

Mechanisms: Signalling, Systems & Synthetic

P214

MEMBERS OF THE CmaX-CfrX-CmpX OPERON ARE DIFFERENTLY REGULATED BY AlgU AND SigX IN RESPONSE TO HEAT AND COLD SHOCKS IN PSEUDOMONAS AERUGINOSA

Emeline Bouffartigues[1], Ishac Si Hadj Mohand[1], Damien Tortuel[1], Olivier Maillot[1], Xu Binjie[3], Alexis Bazire[2], Alain Dufour[2], Daniel J. Wozniak[3], Nicole Orange[1], Marc GJ. Feuilloley[1], Pierre Cornelis[1], Sylvie Chevalier[1]
[1]EA4312-Laboratory of Microbiology Signals and Microenvironment (LMSM), University of Rouen Normandy, Normandy University, Evreux F-27000, France, [2]EA3884-Laboratoire de Biotechnologie et Chimie Marines (LBCM), Institut Universitaire Européen de la Mer, Université de Bretagne-Sud, Lorient, France, [3]Departments of Microbial Infection and Immunity, Microbiology, Center for Microbial Interface Biology, Ohio State University, Columbus, OH 43210, USA

P. aeruginosa is a highly adaptable opportunistic pathogen, a phenotype that is mainly related to its large number of transcriptional regulators, including 19 extracytoplasmic function (ECF) sigma factors. The well-known RpoE-like ECF sigma factor AlgU is involved in alginate biosynthesis and cell wall stress response. Interestingly, the newly characterized ECF SigX emerged as a second sigma factor responding to envelope stress, controlling in particular membrane composition remodelling. However the functional interactions that may exist between SigX and AlgU to maintain envelope homeostasis remain to be discovered. To this aim, the regulation of a putative operon located directly upstream of sigX was studied according to temperature variation, a key parameter affecting the membrane state. This predicted operon forms a cluster of three genes, *cmaX-cfrX-cmpX*, among which *cmaX* on one side, and *cfrX* and *cmpX* on the other side, were proposed to belong to AlgU and SigX regulons, respectively. Accordingly, we have identified an internal SigX-dependent promoter region upstream *cfrX*, in addition to the AlgU-dependent promoter region upstream cmaX. We show that expression of cmaX is increased in response to a heat shock through an AlgU-dependent mechanism that does however not involve RpoH. Remarkably, *cfrX* and *cmpX* expression is up-regulated via a SigX-dependent mechanism in response to a cold shock, and it requires also the AlgU-dependent alginate and motility regulator AmrZ, whose expression is partly controlled by SigX in this condition. Taken together, these data give further insights into the highly complex networks that are involved in maintaining membrane homeostasis.

Mechanisms: Signalling, Systems & Synthetic

P215

New insights into the molecular mechanisms regulating SigX activity in *Pseudomonas aeruginosa*

Emeline Bouffartigues, Damien Tortuel, Olivier Maillot, Vincent Dubot, Olivier Lesouhaitier, Nicole Orange, Marc GJ. Feuilloley, Pierre Cornelis, Sylvie Chevalier
Laboratory of Microbiology Signals and Microenvironment (LMSM EA4312), University of Rouen Normandy, Normandy University, Evreux F-27000, France

Commonly reversibly-associated to their inner membrane anti-sigma, extracytoplasmic function (ECF) sigma factors are one of the most conserved regulatory mechanism among the bacterial world allowing signals transduction. In addition to responding to signals perceived outside the cytoplasm and to controlling a pool of genes which products are involved in the envelope metabolism, RpoE-like ECF sigma factors are self-regulated and co-transcribed with genes encoding the components of a transduction system to which they belong. The newly characterized ECF SigX is involved in the envelope stress response, controlling in particular membrane composition remodelling and P. aeruginosa pathogenicity. However the mechanisms regulating its activity are not yet known. In an attempt to characterize this system, we have investigated the involvement of CfrX and CmpX in SigX regulation. We have shown that cfrX and cmpX genes, which are located upstream sigX, belong to the same transcriptional unit and are co-transcribed from a proximal SigX-dependent promoter. This promoter was then used to monitor SigX activity in *cfrX* or *cmpX* mutants. While this promoter activity increased in a *cfrX* mutant, it decreased in a *cmpX* mutant, compared to the wild-type strain, suggesting that CfrX is a negative regulator and CmpX a positive regulator of SigX activity. These results will be discussed with respect to anti-sigma and mechanoreceptor channel functions predicted respectively for CfrX and CmpX.

Mechanisms: Signalling, Systems & Synthetic

P216

Purification and characterization of RhlR; a transcriptional regulator involved in quorum sensing in *Pseudomonas aeruginosa*

Larson Grimm, Martin Welch
University of Cambridge, Cambridge, UK

Pseudomonas aeruginosa is a multi-drug resistant, opportunistic human pathogen. The organism readily forms biofilms and produces an arsenal of potent secreted virulence factors, which facilitate infection of the host. The expression of these virulence factors are largely mediated by a cell-cell communication mechanism called quorum sensing (QS). Over the last two decades, much has been learned about the molecular mechanism(s) of QS in *P. aeruginosa*, and it is now clear that three key signalling sub-systems are involved; the las, PQS, and rhl pathways. Although we now know much about the las and PQS pathways, the rhl pathway has stubbornly refused to submit to biochemical investigation. This is significant because it is now becoming clear that rhl signalling plays a key role in virulence regulation, as well as enabling survival of the pathogen in oxygen-limited environments. The transcriptional regulators involved in las and PQS signalling (LasR and PqsR, respectively) are relatively well-characterized. However, very little is known about the main rhl sub-system receptor, RhlR. This is primarily because RhlR is exceptionally difficult to express; the gene encodes a large number of "rare codons", leading to sub-optimal translation. However, we have found that codon optimization overcomes this problem, allowing large amounts of RhlR to be expressed in the soluble fraction of cells. This finding, opening the way towards detailed structural and biochemical characterization of the protein, is important because we have recently obtained evidence that suggests RhlR not only binds an N-acyl homoserine lactone (BHL) ligand, but also the alkyl quinolone, PQS.

Mechanisms: Signalling, Systems & Synthetic

P217

Metabolic network analysis of *Pseudomonas aeruginosa* persister cells

Anna Blazier[1], Glynis Kolling[1], Alejandro Amezquita[2], Yvan Le-Marc[2], Aline Metris[2], Ewelina Wachnicka[2], Jason Papin[1]
[1]University of Virginia, Charlottesville, Virginia, USA, [2]Unilever, Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire, UK

Persister cells are transient phenotypic variants in a bacterial population that are able to tolerate antimicrobial treatment. These cells have been implicated in the recalcitrant nature of chronic infections and in the resistance to disinfection and preservation systems that can lead to contamination of industrial products. While the tolerant nature of persister cells is classically associated with a reduced metabolic state, the characteristics of persister cell metabolism are not well understood. For instance, it is unclear how persister cells evade treatment through dormancy yet remain primed for some metabolic activity. To study how their metabolism differs from normal cells, we are building a model of persister cell metabolism by integrating high-throughput experimental data, such as transcriptomics and metabolomics data, with a genome-scale metabolic network reconstruction of *Pseudomonas aeruginosa*, PA14. PA14 cells from control or antimicrobial treatment cultures were isolated and data corresponding to gene expression, metabolite profiling, and carbon source utilization collected. With the integration of the high-throughput profiling data into the network reconstruction, the resulting computational model of persister metabolism can be used to investigate (1) what metabolic pathways are necessary for persister cell viability and (2) what metabolic processes promote switching between persister and normal phenotypes. This computational model-driven analysis of persister metabolism enables the identification of metabolic targets that, when inhibited, could eliminate the presence of persister cells.

Mechanisms: Signalling, Systems & Synthetic

P218

Monooxygenases involved in *Pseudomonas aeruginosa* alkyl quinolone biosynthesis

Steffen Lorenz Drees, Christian Schmerling, Simon Ernst, Susanne Fetzner
Institute for Molecular Microbiology and Biotechnology, University of Muenster, Muenster, Germany

Metabolites of the alkyl quinolone (AQ) biosynthetic pathway possess various functionalities required for *Pseudomonas aeruginosa* to establish infections. Besides 2-heptyl-4(1*H*)-quinolone (HHQ) and the *Pseudomonas* quinolone signal (PQS, 2-heptyl-3-hydroxy-4(1*H*)-quinolone), signal molecules in quorum sensing, a variety of related compounds is produced, most of which serving distinct physiological roles. For example, 2-alkyl-4-hydroxyquinoline-*N*-oxides are respiratory inhibitors with antistaphylococcal properties. Alkyl quinolone biosynthesis is mediated by the pqsABCDE operon and pqsH and pqsL.

Both, PqsH and PqsL, are classified as group A flavin monooxygenases, a subgroup of flavoenzymes with a common mechanism, in which substrate binding drives the reduction of the protein-internal flavin by NAD(P)H. Biochemical analyses revealed that both enzymes show deviations from this tenet. In vitro, PqsL exclusively uses reduced flavin as electron donor for hydroxylating its substrate 2-aminobenzoylacetate. Enzyme activity could be supported with the FAD reductase HpaC, which also plays a role in providing the co-substrate in vivo.

Localization analysis demonstrated that PqsH, which catalyses the 3-hydroxylation of HHQ, is attached to the cytoplasmic membrane. Recombinant protein from solubilized membranes had a higher activity than MBP-fusion proteins and purified without attached chaperones, suggesting that membrane association may support folding. Although PqsH can utilize NADH as electron donor, it also appears to be capable of acquiring electrons out of membrane-associated redox processes.

The findings suggest that the specific characteristics of PqsL and PqsH have a regulatory purpose for the equilibrium of AQs. Moreover, PqsH likely has enhanced access to the membrane-internal pool of AQs, which could allow for higher catalytic efficiency.

Mechanisms: Signalling, Systems & Synthetic

P219

Understanding the mechanistic relationship between ribosome modification and environmental response.

Lucia Grenga[1,2], Richard Little[1], Govind Chandra[1], Gerhard Saalbach[1], Jacob Malone[1,2]
[1]John Innes Centre, Norwich, UK, [2]University of East Anglia, Norwich, UK

In order to fully understand how *Pseudomonas* generate precise, effective responses to the environment, we need to first characterize the mechanisms underlying signal transduction at the molecular level. Our research focuses on dissecting the regulatory contribution of the novel RimABK signalling pathway to *Pseudomonas* host interactions. Genetic evidence suggests that RimK represents the output of the system. A member of the ATP-Grasp superfamily; RimK is responsible for the post-translational modification of the ribosomal protein RpsF. This modification is associated with profound effects on the structure and function of the *Pseudomonas* ribosome, and as a consequence, the remodelling of the bacterial proteome.

Our initial characterization of the Rim system raises a number of questions relating to the mechanistic function of RimK. How exactly does RimK ribosomal modification lead to altered proteome composition? Is this a consequence of altered translation, or mRNA recognition by the modified ribosomes, or possibly a combination of both? While advances in quantitative proteomics have enabled us to examine the overall impact of RimK on the *Pseudomonas* proteome, the development of new technologies and analytical tools have since allowed us to probe previously unexplored aspects of ribosomal regulation. Here, I will discuss how these genome-wide methodologies have advanced our understanding of ribosome function and regulation in *Pseudomonas* species.

Mechanisms: Signalling, Systems & Synthetic

P220

Cell wall stress response in *Pseudomonas aeruginosa*: involvement of the ECF sigma factor SigX

Emeline BOUFFARTIGUES, Damien TORTUEL, Rachel DUCHESNE, Ali TAHRIOUI, Maud FLECHARD, Gwendoline GICQUEL, Olivier MAILLOT, Olivier LESOUHAITIER, Nicole ORANGE, Marc FEUILLOLEY, Pierre CORNELIS, Sylvie CHEVALIER
Normandie Université, Université de Rouen Normandie, Centre de Sécurité Sanitaire de Normandie, Laboratoire de Microbiologie Signaux et Micro-environnement, LMSM EA 4312, Rouen, France

The bacterial cell envelope is the first line of defense against environmental threats. Cell envelope stress responses (CESRs) detect cell wall integrity alterations and reprogram gene expression to ensure bacterial survival. Pathogens need CESRs to survive inside the host, where their envelopes face host immune system effectors and antimicrobials. The underlying signal transduction can be mediated by extracytoplasmic function (ECF) σ factors. *P. aeruginosa* displays two CESR ECF σ factors, AlgU and the recently described SigX. Using complementary approaches including OMICs, qRT-PCR, CLSM, anisotropy, FAME and phenotypic analyses, we have shown that i) SigX regulates directly or indirectly more than 300 genes being involved in numerous cellular processes (virulence, motility, adhesion, biofilm formation); ii) membrane fluidity alterations originating from a *sigX* mutation result in strong dysregulations of CbrA/B, Crc and Hfq networks, linking SigX to the metabolic pathways; iii) expression and activity of SigX are increased in response to numerous conditions (high sucrose or tobramycine sub-lethal concentrations, low osmolarity, cold shock); suggesting that SigX responds to envelope perturbations. ECF σ factors activity is usually regulated via sequestration by their cognate anti sigma factors. In *sigX* near vicinity, the *cfrX-cmpX* operonic structure is transcribed at least partly from a SigX-dependent promoter. Based on molecular tools and *in silico* analyses, we have shown that the hypothetical protein CfrX and the predicted mechanosensitive channel CmpX are involved in SigX activity. Future challenges will now put emphasise on the interactions linking SigX, CfrX and CmpX to get further insights into CESR in *P. aeruginosa*.

Mechanisms: Signalling, Systems & Synthetic

P221

cAMP and Vfr control Exolysin expression and cytotoxicity of PA7-like *Pseudomonas aeruginosa*

Alice Berry[1], Julian Trouillon[1], Kook Han[2], Mylène Robert-Genthon[1], Michel Ragno[1], Stephen Lory[2], Ina Attrée[1], Sylvie Elsen[1]
[1]Biology of Cancer and Infection, U1036 INSERM, CEA, University of Grenoble Alpes, ERL5261 CNRS, Grenoble, France, [2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA

Pseudomonas aeruginosa PA7 clonal outliers lack the Type 3 Secretion System (T3SS) and employ T5SS ExlB-ExlA for host cell membrane disruption and virulence. Here we investigated the regulation of ExlB-ExlA expression.
Bioinformatics analysis of the region upstream of the *exlBA* transcriptional start site identified a putative binding site for the Virulence Factor Regulator, Vfr referred to as the Vfr Binding Site, VBS. The specific binding of Vfr to VBS in the *exlBA* promoter was assessed by EMSA. The effect of *vfr* deletion and chromosomal mutation of VBS on *exlB* and *exlA* expression was investigated by RT-qPCR and β-galactosidase activity using a *lacZ* reporter construct. The impact of Vfr on ExlA secretion and bacterial toxicity towards epithelial cells was investigated by western-blot and LDH release assay.
In our studies, we demonstrated that Vfr binds directly to *exlBA* promoter with high affinity and this interaction requires cAMP molecule. We provided evidence that deletion of *vfr* or mutation of chromosomal VBS reduced *exlBA* expression and that Vfr controls ExlA and Type 4 Pili synthesis, both being required for cytotoxicity. The main provider of cAMP, both in laboratory culture conditions or upon host cell infection, is the adenylate cyclase, CyaB. Finally, we discovered that the *vfr* gene in the reference strain PA7 is mutated, explaining strain's reduced cytotoxicity.
In conclusion, we established that CyaB-cAMP/Vfr pathway, known to regulate acute virulence factors in classical *P. aeruginosa* strains, controls virulence of PA7-like strains through direct transcriptional regulation of *exlBA* encoding the pore-forming toxin, Exolysin.

Mechanisms: Signalling, Systems & Synthetic

P222

A new role for the P. aeruginosa twin arginine translocation system in twitching motility

Maxime Gimenez[1,2], Govind Chandra[3], Pascale Infossi[4,2], Sébastien Lhospice[5,2], Marie-Thérèse Giudici-Orticoni[4,2], Tâm Mignot[5,2], Sophie Bleves[1,2], Bérengère Ize[1,2]
[1]IMM-LISM-CNRS, Marseille, France, [2]Aix Marseille Université, Marseille, France, [3]Molecular Microbiology departement, JIC, Norwich, UK, [4]IMM-BIP-CNRS, Marseille, France, [5]IMM-LCB-CNRS, Marseille, France

The Tat pathway is a molecular machine that exports folded proteins from the cytoplasm to the periplasm of bacteria. Tat is widespread in bacteria and Tat substrates are involved in an array of bacterial processes including virulence (Berks, 2015). During the first part of this work we identified 35 Tat-dependent substrates in Pseudomonas aeruginosa using a systematic genome-wide in silico analysis and genetic screen. Notably, several identified Tat substrates appear likely to contribute to P. aeruginosa physiology and virulence. Indeed, we and others have shown that tat mutants have defects in various cellular processes including respiratory energy metabolism, iron and phosphate nutrition and motility (Ochsner et al., 2002; Ball et al., 2016).
We next focused on understanding the involvement of the Tat pathway in twitching motility mediated by type IV pili (TFP) and essential for P. aeruginosa virulence. Interestingly, we showed that while TFP were still present and functional in the tat mutant, the cells were much slower indicating a clear Tat-dependent twitching defect. To understand the molecular link between the absence of the Tat system and twitching defect we constructed and screened a bank of 35 mutants where each Tat-dependent substrate had been individually deleted. Strikingly, we showed that the absence of a single Tat substrate is responsible for the twitching defect of the mutant.
Altogether our results indicate a key role for the P. aeruginosa Tat system in a range of cellular processes and highlight an unexpected link between an export system and twitching motility.

Mechanisms: Signalling, Systems & Synthetic

P223

Insights into the functional diversity of type VI secretion effectors

Kaitlyn LaCourse, Brook Peterson, Hemantha Kulasekara, Matthew Radey, Joseph Mougous
University of Washington, Seattle, WA, USA

The type VI secretion system (T6SS) of Gram-negative bacteria is a contact-dependent protein translocation apparatus that resembles an inverted bacteriophage puncturing device. T6SSs are widely distributed among Proteobacteria and Bacteroidetes and function to deliver effector proteins directly into competing bacterial cells. Advances within the past decade have illuminated the substantial contribution to fitness that the T6SS affords bacteria in various physiological settings and the wealth of antibacterial activities found within T6SS effectors has broadened our understanding of the complexity of microbial warfare. However, the extent of T6S effector activities that remain uncharacterized is an outstanding question. We are interested in addressing this question by defining the evolutionary forces that maintain the T6S effector diversity within individual organisms. *Pseudomonas aeruginosa* is an established model for studying the T6SS. This bacterium encodes multiple T6SSs, each of which utilize an exclusive panel of antibacterial substrates. Using an integrated approach, we have uncovered the role of T6S under varying environments. We dissect the sophisticated methods that bacteria employ to maximize fitness in the unpredictable nature of interbacterial encounter.

Mechanisms: Signalling, Systems & Synthetic

P225

Pervasive targeting of nascent transcripts by the RNA chaperone Hfq in *Pseudomonas aeruginosa*

Tracy Kambara, Kathryn Ramsey, Simon Dove
Boston Children's Hospital, Harvard Medical School, Boston, USA

The RNA-binding protein Hfq is an important post-transcriptional regulator in bacteria, influencing many cellular processes including growth, virulence, and catabolism. Chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-Seq) in *Pseudomonas aeruginosa* revealed that Hfq associates with hundreds of genes. Virtually all of these associations are abrogated in cells treated with the RNA polymerase inhibitor rifampicin, indicating they occur when Hfq binds transcripts as they are being made. Among the genes that are associated with Hfq are several known targets of the translational repressor and regulator of carbon catabolism, Crc. We find that like Hfq, Crc associates with its targets co-transcriptionally. Additionally, we find that Crc promotes the association of Hfq with nascent transcripts. Combining ChIP with transcription inhibition may be a broadly useful approach for studying the co-transcriptional activities of RNA-binding proteins in bacteria. Our findings with Hfq and Crc suggest that functioning co-transcriptionally may be an efficient and perhaps common way for post-transcriptional regulators to exert their regulatory effects.

Mechanisms: Signalling, Systems & Synthetic

P226

Regulatory control of the Pseudomonas aeruginosa hibernation promoting factor.

Tatsuya Akiyama, Kerry S. Williamson, Michael J. Franklin
Montana State University, Bozeman, USA

Bacterial biofilms contain subpopulations of cells that are dormant and highly tolerant to antibiotics. While dormant, the cells must maintain the integrity of macromolecules required for resuscitation from dormancy. Previously, we showed that hibernation promoting factor (HPF) is essential for preservation of ribosomal integrity during starvation, and for recovery of P. aeruginosa from dormancy. In this study, we mapped the regulatory response of hpf expression. The hpf gene is downstream of rpoN, and is predicted to be part of the nitrogen-stress, rpoN operon. However, bioinformatics analysis and 5'-RACE predicted an RpoD or RpoS-like promoter consensus sequence upstream of hpf. We generated yfp-reporter constructs that contained or lacked the rpoN promoter (P_{rpoN}). The greatest hpf expression occurred when P_{rpoN} was included in the construct (P_{rpoN}-rpoN-hpf-yfp). However, when P_{rpoN} was deleted, hpf was still expressed at high levels. Deletion of the predicted P_{hpf} or its promoter UP-element reduced hpf expression close to background levels. To characterize P_{hpf} transcriptional control, expression was assayed in rpoS, dksA2, or relA/spoT regulatory mutants. The results indicated that hpf expression is not eliminated in the stringent response mutants, but that expression is modulated in these strains. Finally, we demonstrated that the 5' untranslated region of the hpf mRNA is important for hpf expression. These results demonstrate that hpf is expressed as part of the rpoN operon, but that it also has its own promoter. RNA folding of the hpf 5'-UTR plays a role in hpf expression, and in regulation of P. aeruginosa survival during dormancy.

Mechanisms: Signalling, Systems & Synthetic

P226

A thermosensory diguanylate cyclase that mediates temperature-dependent bacterial biofilm development

Trevor E. Randall[1], Henrik Almblad[1], Jacquelyn D. Rich[1], Katherine Leblanc[1], Laura K. Jennings[3], Larissa A. Singletary[3], Weerayuth Kittichotirat[4], Geoffrey L. Winsor[5], Fiona S. L. Brinkman[5], Tara M. L. Winstone[6], Nathan M. Good[3], Roger E. Bumgarner[4], Michael F. Hynes[1], Boo Shan Tseng[7], Manu Singh[8], Ann Karen Brassinga[8], Matthew R. Parsek[3], oe J. Harrison[1]

[1]Department of Biological Sciences, University of Calgary, Calgary, Canada, [2]Cumming School of Medicine, University of Calgary, Calgary, Canada, [3]Department of Microbiology, University of Washington, Seattle, USA, [4]Department of Genome Sciences, University of Washington, Seattle, USA, [5]Simon Fraser University, Vancouver, Canada, [6]Department of Chemistry, University of Calgary, Calgary, Canada, [7]7School of Life Sciences, University of Nevada, Las Vegas, USA, [8]8Department of Microbiology, University of Manitoba, Winnipeg, USA

Many bacteria utilize the intracellular second messenger cyclic diguanylate (c-di-GMP) to control virulence, motility, and extracellular polymer production. Although a growing body of work has elucidated the biochemistry of c-di-GMP synthesis, degradation and effector function, very little is known about how external stimuli are perceived by c-di-GMP regulatory networks. Here we report the discovery of c-di-GMP signaling proteins that function as biological thermostats. The archetype of these proteins is the thermosensing diguanylate cyclase (TdcA) from the opportunistic pathogen Pseudomonas aeruginosa. TdcA synthesizes c-di-GMP and controls biofilm formation in response to body temperature. Site-directed mutation and domain-swapping analyses indicate that heat-sensing is mediated by a cofactorless Per-Arnt-SIM (PAS) domain, which is a previously undescribed function for this widespread family of protein domains. Purified recombinant TdcA displays "thermostatted" enzyme kinetics: it is thermally activated above a threshold temperature (27 °C), and displays a reaction rate that linearly increases more than 50-fold over a 15 °C range. These heat-dependent enzyme kinetics, which cannot be approximated by known biophysical models describing Q_{10} temperature coefficients, enable rapid physiological change over narrow temperature ranges. Using intravital imaging, we demonstrate that tdcA$^+$ P. aeruginosa suppresses early innate immunity in the murine lung, and that immune evasion depends on the c-di-GMP-regulated extracellular polysaccharides PEL and PSL. TdcA orthologues are widespread in Proteobacteria, and putative heat-sensing PAS domains are linked with hundreds of predicted diguanylate cyclases and c-di-GMP-specific phosphodiesterases in the PFAM database. Our data suggest that heat-sensing is a widespread function of c-di-GMP networks, and that this function enables bacteria to build biofilms in habitats with desirable temperatures - including the mammalian body.

Mechanisms: Signalling, Systems & Synthetic

P227

Structural and functional insights into the periplasmic detector domain of the GacS histidine kinase controlling biofilm formation in Pseudomonas aeruginosa

Ahmad Ali-Ahmad, Firas Fadel, Corinne Sebban-Kreuzer, Moly Ba, Gauthier Dangla Pélissier, Olivier Bornet, Françoise Guerlesquin, Yves Bourne, Christophe Bordi, Florence Vincent
CNRS, Aix Marseille Univ AFMB, Marseille, France

Pseudomonas aeruginosa is an opportunistic pathogenic bacterium responsible for both acute and chronic infections and has developed resistance mechanisms due to its ability to promote biofilm formation and evade host adaptive immune responses. Here, we investigate the functional role of the periplasmic detector domain (GacSPD) from the membrane-bound GacS histidine kinase, which is a major actor for biofilm formation and coordination of bacterial lifestyles. A GacS variant devoid of the periplasmic detector domain is severely defective in biofilm formation. Functional assays indicate that this effect is accompanied by concomitant changes in the expression of the two RsmY/Z small RNAs that control activation of GacA-regulated genes. The solution NMR structure of $GacS_{PD}$ reveals a distinct PDC/PAS a/b fold characterized by a three-stranded b-sheet flanked by α-helices and an atypical major loop. Point mutations in a putative ligand binding pocket lined by positively-charged residues originating primarily from the major loop impaired biofilm formation. These results demonstrate the functional role of $GacS_{PD}$, evidence critical residues involved in GacS/GacA signal transduction system that regulates biofilm formation, and document the evolutionary diversity of the PDC/PAS domain fold in bacteria.

Mechanisms: Signalling, Systems & Synthetic

P228

Evolution of LES Prophage Infection Dynamics in Pseudomonas aeruginosa Hosts

Maryam Mohamad, Kirsty Thomas, Chloe James
Environment and Life Sciences, University of Salford, Manchester, UK

The Liverpool Epidemic Strain (LES) of Pseudomonas aeruginosa is a key opportunistic bacterium causing transmissible infections of the cystic fibrosis (CF) lung and has been established to carry distinctive prophages within its genome. Several well-known partnerships between prophage and their bacterial hosts have been characterised, however, very little is known about other phage-host systems. This study evaluated the dynamics of temperate LES Φ4 and P. aeruginosa hosts by exploring the infection profiles with two strains (PAO1 and PA14) and examined whether prophage and host evolve towards a more stable relationship when co-evolved in Artificial Sputum Medium. Analysis of Covariance confirmed that the LES Φ4 infection pathway is influenced by host background ($P < 0.001$); lysis occurred much more rapidly in PAO1 compared to PA14. Furthermore, an evolved lysogen was observed to have become less stable and produced significantly higher active phage particles compared to the un-evolved lysogen ($P < 0.001$). These data suggest that host factors influence the LES Φ4 infection process and that maintenance of productive, lytic activity may be important for the persistence of LES populations in the CF lung environment.

Mechanisms: Signalling, Systems & Synthetic

P229

Natural Pseudomonas fluorescens Isolates from Water Systems Influence the Survival of Campylobacter jejuni Strains 11168 and 81-176 in Aerobic Conditions

Paz Aranega-Bou, Richard Birtles, Chloe James
Environment and Life Sciences, University of Salford, Manchester, UK

Campylobacter jejuni is the main bacterial cause of human gastroenteritis worldwide and a zoonotic pathogen with extensive animal and environmental reservoirs in spite of its fastidious nature. Chickens are the main vehicle for human infection by Campylobacter and their drinking water has been identified as a possible source of contamination. Research suggests that natural microbial communities might promote the survival of Campylobacter in such environmental reservoirs. P. fluorescens is a ubiquitous environmental bacterial species and a well-documented inhabitant of drinking water systems DWDS. This study isolated P. fluorescens from DWDS and screened a range of P. fluorescens isolates and characterised strains for their ability to enhance the survival of C. jejuni strains 11168 and 81-176 in aerobic conditions. Co-culture assays indicated that certain P. fluorescens strains (Pf0-1, ATCC 17400 and WCS 365) and natural water isolates (P1-2) enhanced C. jejuni survival with an increase in viable cell recovery ranging from 0.8 LOG to 4.5 LOG depending on the strain after 24 hours of aerobic incubation. Conversely, other strains and isolates showed no interactive effect (Pf05) or even reduced Campylobacter survival (F113 and P3-4) compared to the mono-culture control. These data suggest that natural populations of P. fluorescens may influence the survival of C. jejuni in chicken farm water systems, and may have implications for transmission to subsequent flocks.

Mechanisms: Signalling, Systems & Synthetic

P230

An inter-domain linker residue acts as a gatekeeper of the mechano-transcriptional regulator DmpR

Henrik Seibt, Uwe Sauer, Victoria Shingler
Umeå University, Umeå, Sweden

DmpR is the master transcriptional regulator of genes involved in (methyl)phenol catabolism of *Pseudomonas putida* CF600. DmpR belongs to the specialised class of bacterial mechano-transcriptional activators that employ ATP-catalysis to engage and remodel σ^{54}-RNA polymerase to allow initiation from the σ^{54}-promoters they control. Biochemical and genetic analysis has previously established that I) binding of a phenolic effectors by DmpR is a prerequisite to allow ATP-binding to trigger a dimer-to-hexamer switch to its active transcriptional promoting conformation, and II) a structured inter-domain linker between the effector-binding and the ATP catalytic domains is involved in coupling these processes. Here we will present evidence from crystallography and mutational analysis, *in vivo* and *in vitro* transcription assays and ATPase activity data, that a tyrosine residue of the inter-domain linker (Y233) occupies the ATP binding pocket and thereby serves to constrain ATP hydrolysis. Using a Y233A substitution derivative, we found that the gatekeeper role of Y233 on the ATPase activity of DmpR has an adverse effect on sensitivity to effector-binding and transcription in intact cells. In the light of our findings, we propose a model in which effector binding relocates Y233 to synchronise effector-binding with multimerisation and thereby to provide appropriate sensitivity of the transcriptional response. Given that Y233 is present in many ligand-responsive bEBPs, the model is likely to be pertinent for regulation of numerous signal-responsive processes controlled by members of this family of proteins.

MICROBIOLOGY
SOCIETY

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.