Session Title: MONDAY Poster Session 1
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 001
Abstract Topic: Developing a Novel Bioactive Root Canal Sealer with Antibiofilm and Remineralization Properties
Abstract Title: Developing a Novel Bioactive Root Canal Sealer with Antibiofilm and Remineralization Properties
Author Block: B. Baras, S. Wang, M. A. Melo, M. D. Weir, T. W. Oates, H. H. Xu; University of Maryland School of Dentistry, Baltimore, MD.
Abstract Body:
The main objective of root canal therapy is to eradicate root canal microbiota and achieve a tight seal to prevent microbial persistence and reinfection. Due to limited accessibility of instruments and disinfecting agents, root canals with complex anatomy often remain contaminated, leading to treatment failure. Also, characteristics of root canal biofilm renders the removal of root canal microbiota a major challenge. Objectives: (1) develop a novel bioactive root canal sealer with antibiofilm and remineralization properties through the incorporation of dimethylaminohexadecyl methacrylate (DMAHDM) and nanoparticles of amorphous calcium phosphate (NACP); (2) investigate the effects on biofilm inhibition against Enterococcus faecalis, which is often associated with endodontic treatment failure, and sealer paste flow properties compared with an epoxy-resin-based sealer AH Plus™; and (3) investigate the calcium (Ca) and phosphate (P) ion release from the sealers. Methods: A series of two-part chemical-cure endodontic sealers were formulated by mixing triethylene glycol dimethacrylate (TEGDMA) and bisphenylglycidyl dimethacrylate (Bis-GMA) at a 1:1 mass ratio. DMAHDM and NACP were incorporated at 5% and 20% by mass, respectively. Different mass fractions of glass filler were added to adjust the flow of sealers according to the ISO recommendations. For biofilm evaluation, three root canal sealers were included: the first sealer contained no DMAHDM or NACP; the second sealer contained 20% NACP; and the third sealer contained 5% DMAHDM and 20% NACP. All sealers contained 40% glass fillers. Colony-forming units (CFU), live/dead assay, and biofilm polysaccharide production were determined. Ca and P ion releases from the endodontic sealers were measured. Results: Incorporating 20% NACP, 5% DMAHDM and 40% glass yielded flow (28.99±0.69) mm, significantly lower than AH plus™ (37.65±0.56) mm, but still within the range of ISO recommendations. Increasing the glass mass% reduced the sealer's flow, compared to AH plus™ (p<0.05). Adding DMAHDM decreased the biofilm CFU by more than 4 logs, compared to AH plus™ and experimental controls. DMAHDM group also significantly reduced polysaccharide production by biofilms, compared to AH plus™ and experimental controls (p<0.05). The control and DMAHDM groups showed high levels of Ca ion release of (2.757±0.135) and (2.543±0.103), and P ion release of (1.97±0.12) and (1.557±0.0877) mmol/L, respectively, at 28 days. Conclusions: A novel bioactive endodontic sealer was developed with strong anti-biofilm activity and high levels of Ca and P ion release for remineralization, without compromising the flow properties. The novel root canal sealer is promising to inhibit E. faecalis endodontic biofilm, while releasing Ca and P ions to remineralize and strengthen the tooth root structures.
**Abstract**

**Background:** High resistance of pathogenic microorganisms growing in biofilm is a serious complication in the treatment of infectious diseases using conventional antibiotics. A promising solution is to find tools that can prevent biofilm formation, or eradicate the existing biofilm. The emergence of new resistance may be significantly reduced if these instruments do not have a significant antibiotic activity. In recent decades increasing attention is paid to the study of various natural substances, synthetic inorganic and organic compounds, and in last years, also nanomaterials that can modulate the physical-chemical properties of biofilms or control mechanisms closely associated with biofilm formation. Our study is focused on antibiofilm activity of relatively inert gold (AgNPs) and platinum nanoparticles (PtNPs) of different shapes.

**Methods:** Two opportunistic pathogenic strains of *Pseudomonas aeruginosa* ATCC 10145 and ATCC 15442 were used as model microorganisms. Spherical (10 nm) and rod-shaped (52x26 nm) AuNPs and spherical PtNPs (3-5 nm) were used to study antibiotic activity. The growth of biofilm under different concentrations of nanoparticles was performed in pre-sterilized, polystyrene, flat-bottomed, 96-well microtiter plates. Biofilm was quantified by crystal violet assay. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells. Activity of N-acyl homoserine lactones (AHLs) involved in the regulation of biofilm formation was determined using *Agrobacterium tumefaciens* strain harboring a traG::lacZ/traR reporter gene responsive to AHLs.

**Results:** Gold and platinum nanoparticles inhibited the growth and biofilm formation of the studied microorganisms. Minimum inhibitory concentrations (MIC) of both metallic nanoparticles were determined for suspension growth, biofilm formation and eradication of matured biofilm. Considerable species dependence has been demonstrated. For example, the MIC of spherical AuNPs to suppress biofilm formation was 115 mg / l for *P. aeruginosa* ATCC 10145 and 20 mg / l for *P. aeruginosa* ATCC 15442. MIC of rod-shaped AuNPs was up to 3 orders lower to spherical nanoparticles. The level of signaling molecules quorum sensing was often due to the stress induced by nanoparticles increased. **Conclusions:** Significant antibiofilm activity of different types of gold and platinum nanoparticles has been demonstrated. Effective nanoparticles concentration was dependent on the *P. aeruginosa* strain, nanoparticles shape, and biofilm age. Acknowledgement: We thank Dr. Petr Slepička (Department of Solid State Engineering, UCT Prague) and Dr. Zdeněk Sofer (Department of Inorganic Chemistry, UCT Prague) for the preparation of nanoparticles.
Biofilm-associated infections pose a complex problem to the medical community, in that residence within the protection of a biofilm affords pathogens greatly increased tolerances to antibiotics and antimicrobials, as well as protection from the host immune response. The microorganisms in biofilms live in a self-produced matrix of hydrated extracellular polymeric substances (EPS) with polysaccharides often being a major constituent. Due to their importance for the establishment and maintenance of biofilm architecture, a significant amount of research into targeting exopolysaccharides with glycoside hydrolases (GHs) as a means for dispersing biofilms has been performed. Using a whole-genome approach, we identified ten enzymes as putative extracellular GHs from *Klebsiella pneumonia* and four putative GHs from *Escherichia coli*. Each GHs gene was ligated into the pET28a expression vector and different GHs was purified to homogeneity with nitrilotriacetic acid (Ni-NTA) purification. We will further detect whether these GHs can prevent biofilm formation and enhance biofilm sensitivity to antibiotics, especially for Gram-negative bacteria.
Chemical surfactants have a major impact on all our lives since they comprise a major component of many of the everyday products we use. The worldwide use of surfactants has grown enormously over the last few decades; quantities of around 9 million tonnes in 1995 rising to 13 million tonnes in 2008 are reasonable estimates. The major shift in attitude towards surfactants which has occurred in the last few years has been driven by the sustainability agenda. Companies using surfactants in their products are now looking to replace some or all of the chemical surfactants with sustainable biosurfactants produced principally by microorganisms from sustainable feedstocks. Mannosylerythritol lipids (MELs) are biosurfactants produced by a range of fungi. A range of structural variants of MELs can be formed and the proportion of each present in fermentation depends on the yeast strain, the carbon substrate used for growth and the duration of the fermentation. In order to allow assessment of the possible functions of MELs as replacements for conventional olio-chemical based surfactants, small quantities of MEL were produced by controlled fermentation. Fermentations of the yeast *Pseudozyma aphidis* using soybean oil as a carbon substrate yielded up to 50 g MELs/L. The MEL formed by this strain is a mixture of MEL-A, MEL-B and MEL-C. Initially, MEL-A was formed and this was subsequently converted into MEL-B and MEL-C. The MELs produced were tested against different Gram-Negative and Gram-Positive microorganisms on planktonic form and biofilm; showing significant bacteriostatic and bactericidal effect. The results show that MELs are promising antimicrobial molecules for biomedical technological applications and need to be studied in detail at large-scale systems and in conjunction with animal tissue models.
Triggering recurrent infections of the alimentary tract mucosa and colonizing the genitourinary canals of hosts, *Candida albicans* is the most prevalent fungal species of the human microbiota. Given the limited number of available antifungal therapies and the increase in antifungal resistance, the search for new, safe and effective antifungal treatments is required. There have been several attempts to control candida infections using medicinal plants with varying degrees of success and concomitant effects. This study was aimed at elucidating the destruction of candida biofilms by *Senna alata* and *Heliotropium indicum*. Using the biofilm assay technique, the n-Hexane, ethyl acetate and ethanol extracts from *Heliotropium indicum* and *Senna alata* leaves and whole plant respectively were tested against *C. albicans*, *C. tropicalis* and *C. Glabrata* that were isolated from 72 individuals. Studies of *S. alata* against *C. albicans* showed that its n-hexane extract had the highest activity. Against *C. tropicalis*, the highest activity of *S. alata* was recorded by the ethyl acetate extract. The highest activity of *S. alata* against *C. glabrata* was by its ethanol extract. The ethyl acetate extract of *H. indicum* had the highest activity against *C. albicans*. The n-hexane extract of the same plant had its highest activity against *C. tropicalis*. The ethanol extract of *H. indicum* also had its highest activity against *C. glabrata*.

Comparative studies of the two plants revealed that *S. alata* has more potential against candida biofilms than *H. indicum*. The plant *H. indicum* is hepatotoxic therefore internal use of this plant is not advised. **Keywords**: Antimicrobial activity, extract, biofilms, spread plate, assay
The Anti-Inflammatory Drug Mesalamine as Novel Antimicrobial Strategy

J. Dahl, U. Jakob; University of Michigan, Ann Arbor, MI.

According to the Review of Antimicrobial Resistance, 'multi-drug resistant superbugs will cause 10 million deaths per year and $100 trillion lost to the global economy by 2050', prioritizing this even over cancer. Thus, alternative antimicrobial strategies are urgently needed to confront this threat of human health. Recently, our lab has discovered that the universally conserved biopolymer polyphosphate (polyP) serves as one of the major posttranslational response systems to oxidative stress in a wide range of pathogenic bacteria by protecting cells from oxidative stress-mediated protein unfolding. In addition, bacteria lacking the ability to produce polyP are defective in virulence, biofilm formation, and persistence. This makes the bacteria-specific enzyme responsible for the production of polyP, namely polyP kinase (PPK), a potentially powerful antimicrobial drug target. We therefore developed an in vitro PPK assay and screened small molecule libraries for PPK inhibitors. One of our identified hits turned out to be 5-aminosalicylic acid/mesalamine, an anti-inflammatory drug that has been used to treat patients with ulcerative colitis (UC) for over 70 years. We hypothesized that mesalamine contributes to this effect by modulating the polyP content of pathogenic bacteria and potentially sensitizing them to stress, including antibiotics and attack by the immune system. Treatment with a non-lethal dose of mesalamine resulted in a 50-60% reduction in polyP levels in a variety of PPK-containing pathogens, clinical isolates of P. aeruginosa and K. pneumonia, as well as members of the gut microbiome in vivo. In addition, mesalamine treatment caused an increase in sensitivity to the inflammatory oxidants HOCl and HOSCN, resulted in a defect in biofilm formation, reduced formation of ampicillin-resistant persister cells in diverse wild-type strains and clinical isolates, and decreased the ability of wild-type strains to colonize Caenorhabditis elegans. In neither case did mesalamine treatment affect the phenotype of the respective ppk deletion strain, indicating that the observed effects are indeed PPK dependent. These results strongly suggest that microbiota-encoded PPK is indeed a physiologically relevant target of mesalamine in humans and suggest that mesalamine might be ideally suited to serve as alternative treatment option of bacterial infections, e.g. of P. aeruginosa-infected burn wounds.
Abstract UTI accounts for an estimated 25-45% nosocomial infection, out of which 90% are associated with urinary catheter, called Catheter Associated Urinary Tract Infections (CAUTI). The microbial populations within CAUTI frequently develop as biofilm. The present study aimed to study bacterial colonization of indwelling urinary catheters and development of strategies for prevention of biofilm formation in urinary catheters. For this the microbial contamination of indwelling urinary catheters was investigated. Biofilm forming ability of the isolates was determined by Tissue Culture Plate method. Prevention of biofilm formation by *Pseudomonas aeruginosa* was also determined by treating the catheter with some agents. In the study total of 560 urinary catheter samples were collected from different hospital of Amravati city, Maharashtra, India and processed for isolation and identification of pathogens. The percentage of contaminated and non contaminated catheter sample was studied and it was found that approximately 93% catheterized samples were contaminated with different uropathogens. In the study, 22 different uropathogenic species and 2940 strains were isolated from 560 urinary catheter samples. The most prominent uropathogenic bacteria isolated was *Pseudomonas aeruginosa* and 472 strains were isolated. The second most isolated uropathogen was *Candida albicans*. *Escherichia coli* were the third highest count. Out of total isolates 2000 isolates were biofilm producer and 940 isolates were non biofilm producers. It was observed that from total biofilm positive isolates, 604 isolates were strong biofilm former, 972 isolates were moderate biofilm forming and 424 isolates were weak biofilm former. The sterilized newly Foley urinary catheters were used for the coating with different antimicrobial agents to prolong the durability and prevent biofilm formation in the urinary catheter. *Pseudomonas* species was introduced as the culture in bladder. Each day catheter section was studied for the presence of *Pseudomonas* species by the standard procedure. During the study it was found that coated catheter resist more against bacterial attachment then uncoated catheter. In uncoated catheter (control) the biofilm formation by *Pseudomonas aeruginosa* was observed in 3days. The highest inhibition of biofilm was observed with triclosan, cefazidime+ CuNps and Copper nanoparticles. It prolonged the attachment for 24, 21 and 19 days respectively. Amla oil was least effective with activity for just 7 days.
Evaluation of Antifouling Coatings to Reduce Bacterial Biocorrosion of Steel

S. Huang¹, M. Elias², R. Hicks¹; ¹University of Minnesota Duluth, Duluth, MN, ²University of Minnesota, St. Paul, MN.

Background: Microbial colonization of steel surfaces can lead to biocorrosion and be detrimental to the integrity of metal surfaces. Biocorrosion is a serious problem for freshwater and marine industries and government organizations. Biocorrosion of port docks and infrastructure severely impacts the maritime transportation industry worldwide. We tested the effectiveness of several non-toxic, antifouling biochemical coating additives and determined that SsoPox, a quorum quenching (QQ) lactonase enzyme and surfactin were the most effective treatments for reducing the number and area of corrosion tubercles on steel coupons in the laboratory.

Methods: To further test the effectiveness of these coating additives, we started a field study in the Duluth-Superior harbor (DSH) using steel coupons cut from the same material used to construct steel sheet pilings in the harbor. Replicate experimental (surfactin and SsoPox lactonase enzyme in acrylic coating) and control (bare steel and acrylic coating alone) coupons were placed at two sites in the DSH. We also scratched one surface of each experimental coupon to determine if coating additives can provide localized protection where the coating matrix is damaged. Control and experimental coupons were retrieved from each site after 1, 2 and 9 months of exposure. Microbial communities within tubercles that developed on the corroding coupons were sampled and DNA extracted. The extracted DNA was used to describe changes in the overall composition of bacterial communities using 16S rDNA-amplicon Illumina DNA sequencing. Biocorrosion was evaluated by counting the number and coverage of corrosion tubercles on coupons, and by measuring surface roughness using SEM imaging.

Results: On the unscratched surface, surfactin and SsoPox treated coupons showed significant reductions in the number (31% and 50%, respectively) and percent coverage (50% and 62%, respectively) of corrosion tubercles compared to the acrylic-coated control coupons after exposure. On the scratched surface, images showed clear signs of tubercles starting to form on the scratched area. There were more tubercles covering larger areas compared with the unscratched surfaces in all treatments. However, the surfactin and SsoPox treated coupons still showed significant reductions in the number (22% and 52%, respectively) and percent coverage (42% and 67%, respectively) of corrosion tubercles comparing to the acrylic-coated control coupons with scratches.

Conclusions: The SsoPox lactonase enzyme treatment showed superior biocorrosion inhibition and was able to prevent corrosion even when the coating was scratched. We are now evaluating different types of these QQ enzymes in coatings on steel coupons within the Duluth-Superior harbor to develop a reliable, cost-effective, biocorrosion control method to treat steel structures.
Abstract Title: Mannich Base Limits *Candida albicans* Virulence by Inactivating Ras-cAMP-PKA Pathway

**Author Block:**
S. Rajasekharan¹, S. Kannapan Mohanvel², C. Kamalanathan¹, V. Ravichandran¹, A. Kumar Ray³, A. Satish⁴;
¹PRIST University, Thanjavur, INDIA, ²D.G. Vaishnav College, Arumbakkam, INDIA, ³Shandong University, Aoshanwei, CHINA, ⁴Central Institute of Brackishwater Aquaculture, Chennai, INDIA, ⁵Department of Biotechnology, Holy Cross College, Tiruchirappalli 620-020, INDIA.

Mannich bases and its derivatives are regarded as supreme pharmacophores in therapeutics. The study investigates the antimycotic potential of Mannich bases, 1-((1H22 benzimidazol-1-yl) methyl) urea (C1) and 1-((3-hydroxynapthalen-2-yl) methyl) thiourea (C2), against *Candida albicans*. Biofilm and hyphal inhibitory activities of the Mannich bases were tested by crystal violet quantification, XTT, fluorescence imaging cAMP rescue, qRT PCR, and by molecular docking analysis. The compounds inhibited the biofilms of *C. albicans* and restrained the filamentation abilities of the pathogen. Structure-activity relationship studies revealed that the presence of urea or thiourea moiety in the tail section is essential for interacting with adenylate cyclase (AC). The Mannich bases seemed to block Ras-cAMP-PKA pathway by inhibiting second messenger activity required for hyphal induction and biofilm formation. In conclusion, the study warrants point-of-care testing of C1/C2 and provides a starting point for deriving several structurally modified Mannich bases which might plausibly replace the prevailing antimycotic drugs in future.
Antibiofilm Strategies

Evaluation of the Biofilm Disinfection Efficacy of a Novel Biofilm Disruption Technology

M. Myntti¹, A. Hamood², K. Bounds¹;
¹Next Science, LLC, Jacksonville, FL, ²Texas Tech University Health Science Center, Lubbock, TX, ³Texas Tech University, Lubbock, TX.

Background: The NIH estimates that biofilm-based infectious diseases represent up to 80% of all infectious diseases. The presence of biofilms within chronic wounds remains an unrecognized important barrier to healing. Due to the physical barrier created by the EPS layer of a biofilm, the bacteria within the biofilm are resistant to antimicrobial treatment and this prevents chronic wounds from healing. For the medical community, the presence of biofilm in chronic wounds is often unrecognized as a barrier to healing. Resistance to antimicrobial treatment is due to the physical barrier created by the biofilm and the expression of up to 800 new proteins secreted within hours of attachment/clustering. To address the issue of antibiotic resistant organisms and improve chronic wound treatment success, Next Science has developed a biofilm disruption solution (BDS), TorrentX, and a biofilm disrupting gel (BDG), Blast X. These products target the biofilm and the microorganisms that reside within. In this study, we assessed the efficacy of the BDS and BDG in eliminating biofilms produced by either P. aeruginosa or S. aureus in-vitro.

Methods: Several sets of either P. aeruginosa or S. aureus biofilms were developed on 0.5mm cellulose disks for 24 or 48 hours. For each time point, one set of disks was treated with the BDS for either 30 seconds (30s) or 30 minutes (30m) and immediately examined. In another set, the disks were treated as mentioned above and incubated for an additional 24 hours. In the last set, the disks were treated with BDG or washed with BDS then treated with BDG and examined after 24 hours. Saline wash was utilized as a control. After each treatment, the disks were vortexed to dislodge the biofilm and the number of microorganisms per disk (CFU/disk) was determined.

Results: S. aureus: For 24 hour biofilms: Biofilms that were washed with BDS for 30m and immediately analyzed were eliminated. In addition, biofilms that were treated with BDS for either 30s or 30m and analyzed after 24 hours were eliminated. Furthermore, disks treated with either BDG or the combination of BDS/BDG contained no biofilm. For 48 hour biofilms: 30s-washed biofilms were not affected. 30m-washed biofilm that were analyzed either immediately or after 24 hours, were eliminated. Furthermore, disks treated with either BDG or the combination of BDS/BDG contained no biofilm. P. aeruginosa: For 24 hour biofilms: Biofilms washed in BDS for 30s and analyzed after 24 hours were reduced by 5 logs. In addition, 30min-washed biofilm that were analyzed either immediately or after 24 hours, were eliminated. Furthermore, disks treated with either BDG or the combination of BDS/BDG contained no biofilm. For 48 hour biofilms: 30s-washed and immediately analyzed biofilms were reduced by 5.5 logs. In addition, 30min-washed biofilm that were analyzed either immediately or after 24 hours, were eliminated. Furthermore, disks treated with either BDG or the BDG/BDS contained no biofilm.
Antibiofilm Activity of Graphene Quantum Dots via Inhibition of Amyloid Fibrillation

Y. Wang, U. Kadiyala, Z. Qu, P. Elvati, N. Kotov, A. Violi, J. VanEpps; University of Michigan, Ann Arbor, MI.

Background: While biofilms are an essential part of Earth’s ecosystem, they cause numerous technological and health problems. The resilience and sophisticated organization of biofilms is enabled by extracellular matrix (ECM) that creates a protective network of biomolecules around the bacterial community. Current anti-biofilm agents can interfere with ECM production but, based on small molecules, they can be degraded by bacteria and diffuse away, which reduce their efficacy. Here we show that graphene quantum dots (GQDs) can effectively disperse mature Staphylococcus aureus (S. aureus) biofilms by preventing the self-assembly of amyloid fibers - a key stabilizing component of the ECM.

Methods: The effects of GQDs on mature S. aureus biofilms were evaluated by confocal microscope and scanning electron microscope. Amyloid-rich biofilms were grown in peptone-NaCl-glucose (PNG) medium while control amyloid-poor biofilms were grown in tryptic soy broth supplemented with glucose (TSBG) medium for 3 days. Mature biofilms were then exposed to 0, 50, or 500 µg/ml GQDs for 1 day. The effect of GQDs on extracted ECM from the biofilm cultures was analyzed by transmission electron microscopy (TEM) and circular dichroism (CD) spectrometry. We also monitored the effect of GQDs on purified PSMα1 peptides, one of the phenol soluble modulins peptides with capacity to form amyloid-like fibrils in S. aureus by TEM, CD spectrometry, and Fourier transformed infrared spectroscopy (FTIR). Finally, we investigated how GQDs interact with PSMα1 peptides at a molecular level using molecular dynamic (MD) simulation with NAMD code and CHARMM general force field.

Results: As the concentration of GQDs increased, porosity of amyloid-rich biofilm increased, and its thickness decreased. Meanwhile the amyloid-poor biofilm showed limited change with addition of GQDs. The GQDs changed the morphology of isolated ECM from the amyloid-rich biofilms. All featured peaks of fibrillation in the CD spectrum were significantly decreased after 2-hour incubation with GQDs. TEM images demonstrated strong inhibition on PSMα1 amyloid-like fiber formation as a function of dose of GQDs. CD and FTIR spectra confirmed the secondary structure change of peptides in presence of GQDs. MD simulation revealed that GQDs, dock near the N-terminus of the peptide to form supramolecular complexes and change the secondary structure of PSM, which disrupts their fibrillation.

Conclusions: The specificity for the GQD effect on PNG-grown biofilm versus the TSBG-biofilm suggests that amyloid is the unique target of GQDs on the ECM. Inhibition of amyloid fibrillation reduces biofilm stabilization. Concomitantly, the resulting free PSM monomers stimulate biofilm dispersion to further enhance the anti-biofilm effect. Disruption of functional amyloid formation in biofilms represents a novel strategy for mitigation of bacterial communities.
Background: Otitis media (OM) is often polymicrobial, with nontypeable Haemophilus influenzae (NTHI) and Moraxella catarrhalis frequently co-cultured from clinical specimens. Bacterial biofilms in the middle ear contribute to the chronicity and recurrence of OM; therefore, strategies to disrupt biofilms are needed. We have focused our vaccine development efforts on the majority subunit of NTHI Type IV pili, PilA. Antibodies against a recombinant, soluble form of PilA (rsPilA) can both disrupt and prevent the formation of NTHI biofilms in vitro. Moreover immunization with rsPilA prevents and resolves NTHI-induced experimental OM. Herein, we show that antibodies against rsPilA also prevent and disrupt polymicrobial biofilms.

Methods: Dual-species biofilms formed by NTHI and M. catarrhalis at temperatures that mimic the nasopharynx (34°C) or the middle ear (37°C) were exposed to antiserum against either rsPilA or OMP P5 of NTHI, another adhesin. Results: NTHI+Mcat biofilm formation was significantly inhibited by antiserum directed against both adhesin proteins and at either temperature. However, only anti-rsPilA disrupted pre-formed NTHI+Mcat biofilms at either temperature, and actively dispersed both NTHI and M. catarrhalis via interspecies quorum signaling. These newly-released NTHI and M. catarrhalis displayed greatly enhanced sensitivity to killing by antibiotics, particularly those that inhibit protein synthesis. Conclusions: Taken together, these results reveal new opportunities for treatment of biofilm-associated diseases via a strategy for treatment of these chronic diseases that combines vaccine-induced antibody-mediated biofilm dispersal with use of traditional antibiotics at a significantly reduced dosage to exploit the newly-dispersed, antibiotic-sensitive phenotype. Combined, our data strongly support the utility of rsPilA both as a preventative and as a therapeutic vaccine antigen for polymicrobial OM due to NTHI and M. catarrhalis. Funded by NIH-R01-DC003915 to LOB.
Biofilms are the root cause of chronic infections that are refractory to conventional antibiotic treatment. A promising strategy to combat biofilm-related infections is to induce biofilms to disperse. Dispersion has been characterized as a process in which cells liberate themselves from matrix-encased biofilms and transition to the free-living state, with thus released cells being rendered susceptible to antimicrobial agents and the immune system. We previously demonstrated that *P. aeruginosa* requires autogenously produced pyruvate and pyruvate fermentative processes as a means of redox balancing to form structured biofilms, with depletion of pyruvate or inactivation of components of the pyruvate fermentation pathway impairing biofilm formation. Given the role of pyruvate in biofilm formation, we reasoned/hypothesized that pyruvate is likewise required for the maintenance of the biofilm structure, with depletion of pyruvate resulting in dispersion. Therefore, *P. aeruginosa* and *S. aureus* biofilms were grown in 24-well plates for up to 5 days and subsequently exposed to pyruvate depleting conditions for 16h using pyruvate dehydrogenase (PDH) in the presence/absence of tobramycin/vancomycin. Thus, treated biofilms were subsequently analyzed by microscopy, crystal violet (CV) stain, turbidity measurements, and viable cell counts. A porcine burn wound model was used to determine the efficacy of pyruvate depletion in reducing *P. aeruginosa* biofilm and planktonic cells in the absence/presence of tobramycin. Treatment efficacy was assessed by viability counts. Here, we demonstrate that depletion of pyruvate using PDH coincides with the disaggregation of biofilms as determined using CV staining, with confocal microscopy demonstrating PDH-treated biofilms to demonstrate voids indicative of dispersion. Likewise, depletion of pyruvate from the growth medium significantly reduced biofilm formation by *S. aureus*, and induced dispersion of established *S. aureus* biofilms. Additionally, pyruvate depleting conditions significantly enhanced the efficacy of tobramycin and vancomycin in killing biofilms by *P. aeruginosa* and *S. aureus*, respectively compared to antibiotic treatment alone. The role of pyruvate was not limited to *in vitro* conditions, as treatment of porcine second-degree burn wound infected with *P. aeruginosa* biofilm cells with PDH not only reduced the bacterial burden but also increased the efficacy of tobramycin by 2-logs in killing biofilm cells compared to treatment with tobramycin alone. Considering that pyruvate depletion not only impairs the formation of biofilms but also induces biofilms to disperse with thus, dispersed cells being more susceptible to antibiotics in vitro and in vivo, our findings indicate pyruvate depletion to be a promising strategy to combat biofilm related infections.
Preparation Stafal® (Bohemia Pharmaceuticals, Brno) is available in the Czech and Slovak Republic under the specific treatment program. It includes polyvalent bacteriophages of the family Myoviridae and genus Kayvirus. It is intended primarily for topical treatment of skin and the subcutaneous tissues infections preventing their potential progression into sepsis. The drug can also be used prophylactically to avoid pyogenic staphylococcal postoperative complications. The prophylactic effect of the Stafal® preparation (concentration $10^4$-$10^8$ PFU/mL) on biofilm formation was tested using the modified Microtiter Plate Method (MTP) in 9 selected strains of *Staphylococcus aureus* including MRSA strains. Furthermore, the impact on 24-hour biofilm was tested after 24 and 48-hour biofilm exposure to preparation of selected concentrations approaching the therapeutic values ($10^6$, $10^7$ and $10^8$ PFU/mL). The persisting biofilm layer was stained with crystal violet and examined spectrophotometrically at 595 nm ($A_{595}$). The effect of the preparation was evaluated by the Friedman ANOVA test. In prophylactic testing, it was shown in most of the strains that the higher the concentration of the preparation the less biofilm was detected. At the highest concentrations the bacteria were eradicated and therefore no biofilm had been formed. One single strain evinced to form stronger biofilm when phage concentration was very low against phage-free control. Testing the effect of the preparation on a mature biofilm demonstrates these results. In the MTP, the variability of measurements was quite high yet it still proved a reduction in $A_{595}$ with increasing concentrations of the phages after 24 and 48 hrs. Moreover, no significant reduction in $A_{595}$ within the SF80 (BF−) was observed even after 48 hrs. These results indicate that biofilm is penetrated and destroyed by the phages. However, due to the variability of measurements in individual strains the effect is not entirely clear, not only because the biofilm layer gets disrupted during the washing step but also due to remnants of dead stained cells. The absorbance ($A_{620}$) of each well was measured at 620 nm Phage preparations are known to have antimicrobial effect on *Staphylococcus aureus* strains and they could be used especially where conventional antibiotic therapy fails, e.g. in the treatment of infections caused by MRSA. This has also been confirmed by our study. In addition, we have proved Staphal®, with its phage endolysins, to be able to disrupt links within the extrapoly saccharide matrix and to destroy bacteria incorporated in biofilm. The preparation acts in vitro on planktonic forms of bacteria but also on bacteria in biofilm, and according to our results it could also be used prophylactically, e.g. in the form of hydrogel-coated catheters. The work was supported by grants 16-29916A and 16-31593A (Ministry of Health of the Czech Republic).
Biocide Treatment against Biofilm Forming Consortiums in Production and Injection Waters from the Petroleum Industry

**Background:** Oil reservoir production and injection systems contain diverse species of microorganisms. Among these, Sulfate Reducing Bacteria (SRB) constitute a main concern because they are responsible of environmental and economic problems like oil souring, microbiologically influenced corrosion (MIC) and pipeline plugging. Formation of biofilms in pipeline systems can create an environment that favors microbial growth and resistance to action of conventional non-oxidizing biocides. For this reason, it needed to explore new technologies for microbial control in the waters associated to oil field. In this study, we compare the potential effect of two different biocides, THPS and glutaraldehyde, to inhibit anaerobic SRB-biofilm. **Methods:** Two different microbial consortiums of SRB isolated from an oil field injection (SRB-3128) and production (SRB-3129) waters were used in the present study. Biofilm formation was tested using carbon steel coupons (SAE-1020), standing in 30 ml of Postgate C medium and adding 3 ml of starting inoculum from each consortium. THPS and glutaraldehyde biocides were added to final concentrations of 25, 50,100 and 200ppm. Most Probable Number (MPN) of bacteria, total soluble protein, carbohydrate concentration and sulphide production were determined to evaluate the effect of biocides in the biofilm formation. **Results and Conclusions:** SRB-2128 formed biofilm with a MPN of $3.87 \times 10^7$ cells per cm² while SRB-3129 formed biofilm with $1.4 \times 10^6$ cells per cm². Glutaraldehyde, at the highest concentration evaluated, have no effect on biofilm viability. Conversely, THPS inhibited biofilm formation in both consortiums having a great impact in microbial cell viability. In conclusion, the present study showed that THPS biocide at 100 ppm can effectively reduce both biofilm formation of two different SRBs consortiums and sulphide production. According to these results, it is important to investigate these conventional biocides and explore other type of biocides using omic approaches in order to understand molecular mechanisms of antimicrobial action.
Session Title: **MONDAY Poster Session 1**
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 019
Abstract Topic: Antibiofilm Strategies
Abstract Title: The Effect of the Joint Biological Agent Decontamination System (JBADS) on Aircraft-Associated Microbiology
Author Block: A. L. Crouch¹, B. W. Stamps², C. A. Drake¹, H. Nunn², J. M. Hollomon¹, B. S. Stevenson², W. J. Crookes-Goodson³;
¹UES, Inc./AFRL, Wright Patterson Air Force Base, OH, ²Oklahoma University, Norman, OK, ³Soft Matter Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, WPAFB, OH.

Microbiological contamination of aircraft can take many forms, including contamination by biothreat agents, pathogens, and corrosion-causing microorganisms. Most approved decontamination methods require surface application of disinfectants and biocides, a labor intensive process. The efficacy of surface treatments is limited, particularly in areas that are difficult to access and/or heavily contaminated. Therefore, there is a requirement for a decontamination system that is rapid, efficacious, and limits exposure of maintenance crews to biological hazards. The Joint Biological Agent Decontamination System (JBADS) was developed specifically for biothreat agent (anthrax) decontamination and relies upon heat and humidity for non-contact, total aircraft decontamination. In this study, JBADS was assessed for its ability to reduce naturally occurring populations of aircraft-associated microorganisms on a decommissioned cargo aircraft. Twenty-nine locations on the aircraft were assessed for levels and types of microorganisms pre- vs. post-decontamination. Three assessment methods were used: adenosine triphosphate (ATP) measurements, cultivation, and amplification and sequencing of 16S/18S small subunit ribosomal DNA. Following exposure of the aircraft to JBADS (170 +/- 5°F; 90 +/- 5% relative humidity; 72h), the microbial community was re-assessed by the same methods. ATP measurements in highly contaminated areas were reduced to baseline and the number of cultivable microorganisms in contaminated areas was reduced 99.99%, with most locations having ≤1 log growth/in². Correspondingly, the number of cultivable, potentially corrosion-causing microorganisms was reduced from 99 to 11. Less quantifiable DNA was recovered after amplification in samples following exposure to JBADS at most sites. There was also a shift in the relative abundance of OTUs in both the bacterial and eukaryotic communities after decontamination. These results demonstrate that deploying JBADS to reduce levels of aircraft-associated microbiota may be a viable option for remediating aircraft following detection of pathogens, biothreat agents, and fungal and bacterial biofilms, or for preventative maintenance against corrosion-causing microorganisms.
**Abstract**

**Title:** Enzyme Capacity Evaluation of Increase the Efficiency of Cleaning Agents in the Removal of Bacterial Biofilm Under Different Conditions

**Author Block:** M. Silveira Derami1, T. Martins Bresciani2, M. Rodrigues Pinto Garcia2, H. Aoyama1, D. da Silva Leite1, M. A. Miranda2; 1University of Campinas, Campinas, BRAZIL, 2Federal Institute of Sao Paulo, Campinas, BRAZIL.

**Background:** The removal of organic material from medical devices is a great challenge in the healthcare system. Such an efficient removal could contribute to reduce the formation of biofilms and therefore diminishing environmental and health costs. The addition of enzymes to detergent formulations has a beneficial effect on the biofilm removal through hydrolysis processes on the organic material. Thus, this work aims to evaluate bacterial biofilm removal using detergent containing proteases at different assay conditions. **Methods:** Bacterial biofilm of *Escherichia coli* ATCC 35218 was grown in microplates and after was treated with 0.9% NaCl (negative control), 0.25% SDS (positive control) and detergent solutions, containing serine proteases (E1 and E2).

**Results:** The serine proteases showed statistically significant results, compared to the negative control. In 0.5h at room temperature, E1 and E2 removed 35% and 50% of biomass and promoted 65% and 80% cell death, respectively. Dilution of the detergent base 5x and 10x removed 50% and 65% of biomass and 80% and 95% of cell death, respectively, for E1. For E2 there was no change in biomass removal but 95% of cell death was observed at the lowest evaluated concentration. Combined proteases (c.p.) E1 and E2 acted synergistically, removing 86.5% of biomass, independently of the exposure time and enzymes concentrations, and promoting cell death of 94.7%, in 2h. There was no statistically significant difference in the biomass removal by the isolated proteases (i.p.) regardless of concentration, time and temperature. However, increasing E1 and E2 concentrations, the biomass removal was enhanced from 82.5% to 89.2% (0.5h) and 87.5% to 92% (2h), at 30°C. At 45°C, it was also observed increase in the biomass removal from 85% (0.5h) to 93% (2h), for 0.5% enzyme concentration and from 90% (0.5h) to 96% (2h), for 5% enzyme concentration. Cell viability was influenced by the temperature, time and synergistic effect of E1 and E2. At 30°C, for 0.5h, cell death increased from 85% (i.p.) to 92% (c.p.). At 45°C, for 0.5h, it was also observed a cell death increase for the joint action of the proteases, with a best performance for 2h, where the results increased from 96% (i.p.) to 99% (c.p.). **Conclusions:** The individual results indicated that E2 had a higher efficiency in the biomass removal and cell death when compared with E1. The higher efficiency of E1 by detergent dilution could be due to the consequent reduction of the inhibitor sodium formate present in the formulation. The serine proteases acted synergistically, and their efficiencies were modulated by changes in the performance conditions.
Background: Apical periodontitis is an inflammatory disease in the apical region of teeth that results from infection by multi-species bacterial biofilm residing in the root canal. Although our recent report showed that Lactobacillus plantarum lipoteichoic acid (Lp.LTA) could inhibit biofilm formation of Streptococcus mutans, its inhibitory effect has not been examined against biofilm formation of multi-species oral pathogens. In this study, we investigated whether Lp.LTA could inhibit biofilm formed by multi-species oral pathogenic bacteria.

Methods: Highly-pure and structurally-intact Lp.LTA was purified from L. plantarum through butanol extraction followed by sequential application of hydrophobic interaction column chromatography and ion-exchange column chromatography. Four representative oral pathogenic bacteria, Actinomyces naeslundii, Lactobacillus salivarius, Streptococcus mutans, and Enterococcus faecalis, were co-cultured to form multi-species biofilm. Single-rooted premolars extracted from orthodontic patients were obtained under the approval of the Institutional Review Board of the Seoul National University Dental Hospital. The roots were sliced to a thickness of 500 μm and sterilized by autoclaving at 121°C for 15 min. The four-species bacteria were cultured in the presence or absence of Lp.LTA on plastic culture plates, glass bottom dishes, or human dentin slices for 72 h at which multi-species biofilm was most well-formed. Furthermore, biofilm pre-formed for 48 h was treated with or without Lp.LTA for 15 min, followed by additional treatment with endodontic medicaments such as calcium hydroxide or chlorhexidine digluconate for 24 h. Confocal microscopy and crystal violet assay were used to determine biofilm formation. Biofilm on human dentin slices were visualized with a scanning electron microscope. Results: Biofilm formation of multi-species bacteria on the culture dish was dose-dependently reduced by Lp.LTA compared to non-treatment control group. Lp.LTA also inhibited multi-species biofilm formation on the dentin slices in a dose-dependent manner. Interestingly, Lp.LTA could disperse even the preformed multi-species biofilm without affecting bacterial survival compared to non-treatment group. Moreover, Lp.LTA potentiated the effectiveness of calcium hydroxide or chlorhexidine digluconate in the removal of preformed muti-species biofilm. Conclusions: These results suggest that Lp.LTA is a potential anti-biofilm agent for treatment or prevention of oral infectious disease including persistent apical periodontitis, which are caused mainly by multi-species bacterial biofilm.
**Session Title:** MONDAY Poster Session 1  
**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 022  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** Modulation of Cross-Kingdom Quorum Sensing by Halophilic Archaea  
**Author Block:** T. P. Thompson¹, S. A. Kelly¹, V. Fuentes², B. F. Gilmore¹;  
¹School of Pharmacy, Queen's University Belfast, Belfast, UNITED KINGDOM; ²Centro de Investigaciones, Madrid, SPAIN.

**Background:** Cell-cell communication through the production of autoinducer molecules has been widely studied in bacteria and found to play a pivotal role in biofilm formation. Biofilms are estimated to be associated with 80% of microbial infections and, contribute widely to an enhanced resistance to antimicrobials (1). An alternative anti-biofilm approach is to interrupt this bacterial communication system. QS within the domain Archaea is understudied compared with bacterial QS systems. The aim of this study was to determine whether archaea are capable of modulating cross-kingdom QS through production of QS and QS inhibitory activities. **Methods:** A combination of culture dependent (crude extracts from archaeal isolates) and culture independent (genomic mining) techniques were employed to investigate the production of compounds capable of induction or inhibition of bacterial QS, by halophilic archaea. Crude extracts were screened for activity using the bioreporter strains Agrobacterium tumefaciens ATCC BAA-2240, Escherichia coli JM109 pSB536, pSB401 and pSB1142, and Chromobacterium violaceum (CV026). Whole genome sequence data from cultured haloarchaea were mined for putative lactonase and synthase sequences, based on homology to N-acyl homoserine lactone from Bacillus thuringiensis (Refseq: WP_060631703.1) and PAS domain S-box protein from Methanosaeta harundinacea (Refseq: WP_014586016.1). Selected genes were cloned using a pTA1228 plasmid and Haloferax volcanii H1424-based expression system (2). **Results:** The presence of compounds capable of eliciting a QS response in bacterial bioreporters has been demonstrated from a range of halophilic archaea isolated from a Triassic Salt Mine, in Kilroot, Northern Ireland. Initial characterisation suggests the presence of N-acyl homoserine lactones (AHL) or AHL-like compounds that are capable of inducing reporter strains. Archael lactonases have been cloned, expressed and characterised for their ability to degrade AHLs using a RP-TLC overlay assay. **Conclusions:** Previous studies have demonstrated that molecules that induce or stimulate QS are equally capable of inhibiting the QS in another species (3). Therefore, activities characterised in this study, may provide valuable insights into archael and cross-kingdom signalling, and may be a source of novel antibiofilm activities. **References:** 1. Brackman G, Coenye T. Quorum sensing inhibitors as anti-biofilm agents. Curr Pharm Des. 2015;21(1):5-11 2. Stroud A, Liddell S, Allers T. Genetic and biochemical identification of a novel single-stranded DNA-binding complex in Haloferax volcanii. Front Microbiol. 2012;3(JUN):1-14 3. McClean KH, Winson MK, Fish L, et al. Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. Microbiology. 1997 Dec;143 (Pt 12):3703-11
Enzymatic and Structural Studies of Highly Stable and Active Quorum Quenching Lactonases

C. Bergonzi, A. Jenks, M. Elias; University of Minnesota, Minneapolis, MN.

Numerous bacteria communicate using small, diffusible signalling molecules to adapt to environmental challenges through biofilm production, motility, iron uptake. A fascinating bacterial communication system is quorum sensing (QS), a system used to regulate gene expression, and thereby coordinate actions in a cell density-dependent manner. Bacteria constantly produce signaling molecules, whose concentration increase proportionally with cell density: when a specific cell density is reached, termed “quorum”, a certain concentration of the signaling molecule is reached and will result in a population-wide changes in behavior. These changes, in bacterial pathogens, relate to the expression of virulence factors and the formation of biofilms. Interfering with bacterial communication is an appealing strategy in our fight against multi-antibiotics resistant strains and bacterial biofilms. We have identified enzymes, called lactonases that can efficiently degrade a certain class of bacterial signaling molecules, and interfere with bacterial communication. These enzymes, isolated from hyperthermophilic organisms, are extremely stable and resistant to harsh chemical treatments. We have engineered these enzymes to increase their stability and efficiency against specific signaling molecules and demonstrated that they effectively inhibit biofilm formation and virulence factors production, including on complex biofilms and for plant pathogens. Our group isolated a new thermophilic lactonase from bacterium Geobacillus caldolyxolyticus. It exhibits a large substrate specificity and shows high catalytic efficiency. We disclose the first structures of a lactonase from this family in complex with both the intact and hydrolyzed lactone substrates. Structural analysis allowed us to decipher the catalytic mechanisms for lactone hydrolysis and will be the foundation of future engineering studies.
Background: Biofilms formed on implanted medical devices and musculoskeletal injuries lead to infectious complications. Cis-2-decenoic acid (C2DA) has been shown to inhibit as well as disperse biofilm for both gram-negative and gram-positive bacteria. Advanced regenerative medicine therapies, such as guided regeneration membranes, can serve as a template for healing traumatically injured tissues as well as for delivery of antimicrobial agents. The goal of this project was to examine loading, release, and efficacy of C2DA loaded into acyl-modified electrospun chitosan membranes.

Methods: Chitosan membranes were fabricated by electrospinning using deacylated chitosan (Primex, 71% degree of deacetylation; 312 kDa average molecular weight). Nanofibrous membranes were acylated after spinning by immersing in hexanoic anhydride (HA) and pyridine for one hour. The membranes were washed in deionized water to get rid of excessive unreacted pyridine and HA. After drying, membranes were loaded with either 0 or 500 µg of C2DA in 100% ethanol. An elution study was performed for 14 days by immersing the membranes in 500 µl of sterile phosphate buffered saline (PBS), with daily sampling and media refreshment. Eluate concentrations were determined using high performance liquid chromatography (HPLC). For biofilm inhibition, chitosan membranes were inoculated with $10^5$ CFU of *Staphylococcus aureus* (UAMS-1 strain) and incubated at 37°C for 24 hours. The membranes were removed and placed in a 48 well plate with sterile PBS. The membranes were washed and sonicated for 5 minutes in 500 µL of PBS. Biofilm attached to membranes was determined by using BacTiter Glo™ viability assays, comparing C2DA loaded membranes to non-loaded. Unsonicated membranes were fixed in 3% paraformaldehyde/glutaraldehyde, in phosphate buffer for scanning electron microscopy. Results: The HPLC elution results indicated an initial burst release of C2DA, followed by lower but detectable levels through day 14. The cumulative release showed a release of almost 40% of C2DA, which indicated that the membranes still retained 60% of C2DA. Immediately after loading, C2DA-loaded membranes reduced bacterial attachment by 87% compared to non-loaded controls. After 14 days of elution in PBS, C2DA-loaded membranes showed 40% less bacterial attachment compared to non-loaded membranes. Conclusion: This study showed that acylated chitosan membranes can provide a sustained release of C2DA for biofilm dispersion and inhibition. These membranes may provide clinically useful therapeutic strategies for use in guided regeneration applications to prevent infections. Future work will include optimization of loading with varying concentrations of C2DA and combination with other antimicrobials, as well as further preclinical evaluations of biofilm inhibition, dispersal, and eradication.
Background: Bacterial biofilms present a challenge by forming in numerous domains with complex surface geometries. Bacteria attach to hydrated surfaces and form biofilm by encasing themselves in a protective extracellular matrix, bestowing resistance to antibiotics and removal. Thus, biofilms are a source of recurring infection and contamination on medical devices and water systems. To mitigate this, we developed a flexible electrode-based device that (1) conforms to complex geometries, (2) measures impedance to detect biofilm formation in real-time, and (3) implements an electric field-based treatment. This latter function combines an electric field with an antimicrobial for a synergistic removal of biofilm (Bioelectric Effect, BE). However, this platform is reliant on the generated electric field, the distribution of which changes when the device is cylindrically conformed. In this work, we examined the impact of 3D cylindrical geometries on sensing and treatment efficacy. Methods: Fabrication involved patterning interdigitated electrodes (IDEs) via photolithography onto a flexible polyimide substrate adhered to a 101.6 mm wafer, followed by 200 nm Au/20 nm Cr E-beam deposition and lift-off. Three curvatures were examined, two of which for representing common biofilm-susceptible environments: 1) \( r = 2.25 \text{ mm} \) (an 18 Fr urinary catheter), 2) \( r = 12.7 \text{ mm} \) (a 1 in water pipe), and 3) a planar control. A 3D-printed hemispherical mold was produced for each curvature, to which the devices were conformed affixed on a glass coverslip. PDMS was poured over the sensor/mold and cured. Mold removal produced a channel with the device in the specific configuration. Escherichia coli were seeded (\( \text{OD}_{600} = 0.25 \)), and the impedance percent change (100 Hz, 50 mV signal) was continuously monitored as biofilm formed for 24 hours with Luria Broth (LB) media flow at 3.5 ml/h. 10 μg/ml gentamicin was diluted in the LB with the sensing electric field for the subsequent 24 hour treatment. Treatment efficacy was evaluated using crystal violet (CV) staining to quantify biofilm biomass. Results: Throughout the 24-hour growth period, the impedance decreased an average of 19% in the planar case, 28% for the 2.25 mm, and 30% for the 12.7 mm. The treatment phase impedance increase was similar, measured at an average of 5.5% for the planar, 7.2% for the 2.25 mm, and 9.4% for the 12.7 mm. This was confirmed with biomass measurements. The CV absorbance was 0.043 for the 2.25 mm and 0.034 for the 12.7 mm. Conclusions: Biofilm was detected via a similar decrease in impedance at each curvature. The biofilm decrease with the BE was also similar, quantified via impedance and biomass. This is attributed to the scale of the IDEs and biofilm being much smaller than the radius of curvature. Thus, these interactions occur in an effectively planar setting, ensuring comparable detection and treatment in this range of 3D cylindrical domains.
**Session Title:** MONDAY Poster Session 1  
**Poster Board Number:** 026

Abstract Topic: Antibiofilm Strategies

Abstract Title: *In vivo* Efficacy of a Unique First-In-Class Series of Antibiofilm Antibiotic for Biofilm-Related Wound Infections Caused by *Acinetobacter baumannii* and Methicillin-resistant *Staphylococcus aureus*

**Author Block:** J. C. Rogers, D. L. Williams, B. Kawaguchi, N. B. Taylor, G. Allyn, M. A. Badham, B. R. Peterson, P. R. Sebahar, T. J. Haussener, H. K. Reddy, B. M. Isaacs, P. F. Pasquina, R. E. Looper; University of Utah, Salt Lake City, UT.

**Background:** Biofilms can be one of the most complicating factors in wound healing. In chronic wounds, biofilms have been identified in more than 80% of cases. Current therapeutic measures include intravenous (IV) antibiotics, oral antibiotics and/or topical agents. One or more therapies may need to be administered for months at a time. However, traditional antibiotic therapies focus solely on planktonic bacteria. Yet the biofilm phenotype predominates in natural ecosystems, including human tissues. Despite improving knowledge of biofilms, the clinical problem persists and current therapies specific to biofilm-related infections are limited.

**Methods:** To address this need, a first-in-class series of antibiofilm antibiotic (CZ) was synthesized. During screening of the CZ series, CZ-1-179 displayed broad-spectrum activity against biofilms. A focused approach was taken to assess the in vitro and *in vivo* efficacy of CZ-1-179 vs. *Acinetobacter baumannii* and MRSA (methicillin-resistant *Staphylococcus aureus*) in planktonic and biofilm phenotypes. In an excision pig wound study, wounds were inoculated with planktonic bacteria or well-established biofilms and treated with a topical CZ-1-179 gel, topical standards of care or clinically-relevant IV antibiotics. Closure rates and CFU/g tissue were assessed to determine efficacy of a topical CZ-1-179 gel compared to clinical standards. CZ-1-179 was examined first in vitro, then formulated as the active ingredient in a topical formulation for *in vivo* evaluation. It was hypothesized that when applied topically in an excision pig wound model, CZ-1-179 would have the ability to treat and prevent wound infection caused by *A. baumannii* and MRSA in both phenotypes. For comparison, current standards of care including IV (colistin/imipenem) and topical (SSD) therapy were also tested. It was further hypothesized that wounds inoculated with well-established biofilms would harbor more bacteria than those inoculated with planktonic bacteria and therefore take longer to heal.

**Results:** Data indicated that wounds inoculated with well-established biofilms had ~2 log10 units more bacteria compared to those inoculated with planktonic bacteria (p<0.05). IV antibiotics resolved, however both *A. baumannii* and MRSA were still cultured from the wounds, never fully eradicated, leaving wound beds still colonized with bacteria (~3 x 10^2 CFU/g tissue). In the swine treated with both IV and topical antimicrobials, SSD took 2 days longer to clear bacteria in wounds compared to CZ-1-179.

**Conclusions:** Wound infections caused by planktonic or biofilm inocula in a pig excision wound model were effectively treated and eradicated by CZ-1-179 gel. Taken together, these data indicate that CZ 1-179 may be a promising agent to treat and prevent biofilm wound-related infections, with potential to be used alone or in combination with current standards of care.
**Background:** *Pseudomonas aeruginosa* is a pathogen that has been frequently associated with nosocomial infections difficult to eradicate, due to capacity of biofilm formation. Antibiotics, such as ciprofloxacin (CIP), are usually used in the treatments against this bacterium, however, resistance strategies are increasingly common to this genus. Then, it’s necessary to study new strategies of control in order to replace or potentiate conventional methods. In this context, antimicrobial peptides, extracted from several living organisms, as well as their analogous forms, may represent an alternative for the development of new drugs. Therefore, the objective of this work was to evaluate the influence of the synthetic peptide aurein K (K-au) on the antimicrobial and antibiofilm activity of CIP against *P. aeruginosa* ATCC 9027. **Methods:** The antimicrobial activity (MIC / MBC) of CIP and K-au was determined by microdilution in broth, according to Clinical Laboratory Standard International. And then, it was verified the synergism between K-au and CIP through the checkerboard assay. The kinetics of death of *P. aeruginosa* after treatment with K-au/CIP combination was determined by CFU (Colony Forming Units) counting during 5 hours. The antibiofilm activity was performed against pre-formed biofilm (12 h) of *P. aeruginosa* by treatment of 30 minutes with K-au/CIP, and washed three with saline buffer and kept growing more 18 hours using fresh BHI. The biomass, the number of viable cells and the cellular metabolism of the biofilm, were quantified by the crystal violet assays, CFU counting and XTT reduction, respectively. Results: MIC values of K-au were 31.25 μg.mL⁻¹, while MBC presented as twice MIC, 62.5 μg.mL⁻¹. The interaction between K-au and CIP resulted in an additive effect, establishing a 4-fold decrease for CIP’s MIC when combined with K-au. The results also demonstrated that K-au/CIP elevates the death kinetics of this microorganism. The antimicrobial action of CIP/K-au was, also, significant in all trials, being able to reduce biomass and cell viability when compared to control group. **Conclusion:** The antimicrobial effect of synthetic peptide K-au suggests, besides the therapeutic potential, an adjuvant effect of classical antibiotics, such as ciprofloxacin, in the treatment of diseases caused by *P. aeruginosa*. This could enable the use of lower concentrations of the antibiotic and possibly decrease bacterial resistance rates. **Keywords:** Biofilm, *Pseudomonas aeruginosa*, Ciprofloxacin, Antimicrobial peptide.
**Session Title:** MONDAY Poster Session 1  
**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 028  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** Evaluation of the Effectiveness of Commonly used Hosp. Disinfectants against Mono and Multispecies C. difficile Biofilm  
**Author Block:** T. Rashid¹, F. Haghighi¹, J. Alam², K. W. Garey³; ¹University of Texas School of Public Health, Houston, TX, ²University of Houston, Houston, TX, ³University of Houston, Houston, TX.  
**Background:** Biofilm formation constitute a unique and dynamic ecosystem of microbial communities encased in extracellular matrix for bacterial survival and persistence in various diverse environmental niches. Biofilms harboring multi-drug resistance organism including Clostridium difficile are commonly found in various hospital settings and is an important source for disease transmission and cross infection. This is one of the first studies to look at the efficacy of commonly used hospital disinfectants against single species and multi species C. difficile biofilm.  
**Methods:** C. difficile biofilm for 5 different ribotypes were grown in a 24 well plate in 3 different stages including anaerobic, anaerobic followed by aerobic and aerobic mixed. Multi species biofilm consisted of Staphylococcus aureus, Enterococcus faecalis and Clostridium difficile. The efficacy of working concentration of sodium hypochlorite, hydrogen peroxide, o-phthaldehyde, formaldehyde, quaternary ammonium, hypochlorous acid and chlorine dioxide based disinfectants were tested against 3 and 5 day old C. difficile mono cellular and multicellular biofilm based on label determined contact time. Crystal violet assay, post exposure bacterial count both total and spore count, live/dead assay and microscopy was done to determine efficacy.  
**Results:** Disinfectants were considered to be effective if a 3 log reduction in viable cell count was observed. Among the hospital disinfectants, only sodium hypochlorite based disinfectant was found to be effective to cause a 3 to 4 log reduction in C. difficile count followed by a 1 to 2 log reduction for formaldehyde and o-phthaldehyde based disinfectants. Hypochlorous based disinfectant was found to be effective in absence of organic substrate. Hydrogen peroxide, chlorine dioxide and quaternary ammonium based disinfectants were mostly found to be ineffective. The level of efficacy correlated with the propensity of biofilm formation by C. difficile strains, age of biofilm, biofilm thickness as measured by crystal violet assay and microscopic structure. Prolong contact time for ineffective disinfectants increased the efficacy.  
**Conclusion:** Commonly used hospital disinfectants effective against planktonic C. difficile were rendered ineffective against C. difficile embedded in a biotic organic matrix. This finding has a potential to greatly affect the infection control initiative against C. difficile.
Toggling the Switch: c-di-GMP Regulates Extension/Retraction Dynamics of *Vibrio cholerae* MSHA Pili

F. Yildiz¹, K. A. Floyd¹, J. H. Park¹, C. Lee², N. Biais³, A. Dalia⁴, G. Wong²;
¹University of California, Santa Cruz, Santa Cruz, CA, ²University of California, Los Angeles, Los Angeles, CA, ³University of New York, Brooklyn, Brooklyn, NY, ⁴University of Bloomington, Bloomington, IN.

Type IV pili are dynamic versatile macromolecular nano-machines that serve diverse functions among bacteria. *Vibrio cholerae*, the causative agent of the pandemic diarrheal disease cholera, harbors three distinct type IV pili systems. Of these systems, the mannose-sensitive hemagglutination (MSHA) pilus is vital for surface colonization and biofilm formation. *V. cholerae* biofilms allow not only for environmental persistence, but also heighten infectivity within the human host, therefore understanding biofilm pathways could aide development of novel treatments. The MSHA extension ATPase, MshE, binds to the major biofilm signaling molecule c-di-GMP, however the role of this interaction is not fully elucidated. Therefore, our objective is to analyze and determine how c-di-GMP modulates MSHA pilus biogenesis and function. To visualize MSHA pili, we made a threonine to cysteine substitution (T70C) on the major pilin subunit (MshA) for labeling with thiol-reactive dyes. MSHA production was evaluated by surface pili ELISA and by fluorescence microscopy. The MshA_T70C mutation allowed for functional MSHA, and microscopy analysis revealed for the first time that MSHA are dynamic retractile complexes. To define the dynamics, genes encoding ATPases mshE and pilT were deleted. Loss of pilT resulted in decreased MSHA, but also increased pilus length and no retraction events. Loss of mshE resulted in no observable MSHA. These data indicate that extension/retraction are dependent upon MshE and PilT. Since MshE binds c-di-GMP, we next examined MSHA production under high and low c-di-GMP. Decreasing c-di-GMP through deletion of individual diguanylate cyclases (DGCs) did not alter MSHA production. However further reduction in c-di-GMP through simultaneous deletion of four DGCs, decreased MSHA production and resulted in shorter pili. Over-expression of an IPTG-inducible DGC, showed increased pilus length and bacterial aggregation with increasing IPTG. To determine if c-di-GMP impacts were mediated through MshE, we introduced mshE point mutants with alterations in c-di-GMP binding. Analyses of mutants with reduced (R9A, L10A) and ablated (G11I) c-di-GMP binding showed no MSHA. However, analysis of a triple point mutant (MshE_L10AL54AL58A) attenuated for c-di-GMP binding that still produces MSHA, showed increased pilus length and retraction events. This suggests that these three point mutations induce a constitutively active conformational state in MshE independent of c-di-GMP, and c-di-GMP functions through MshE to drive MSHA extension. Our studies demonstrate for the first time that MSHA pili are dynamic retractile complexes. We also show that cellular c-di-GMP levels alter activity of MshE, where MshE is active at high levels and inactive at low levels. Therefore, c-di-GMP toggles the switch between active and inactive MshE to modulate extension and retraction dynamics of MSHA.
Study of Antibiotic Susceptibility Pattern and Biofilm Formation among Staphylococci Isolated from Tertiary Care Hosp. of Nepal

**Background:** Staphylococci are notorious human pathogens that cause variety of diseases ranging from minor skin infections to chronic systemic infections such as osteomyelitis, endocarditis and biofilm associated infections of indwelling devices. These infections have also the potential to spread both in health care facilities and communities eventually causing minor outbreaks. A multitude of factors including but not limited to antibiotic resistance, biofilm production and formation of antibiotic tolerant persister cells contribute to treatment failures in such staphylococcal infections.

**Methods:** A total of 375 staphylococci isolated from clinical samples received in a tertiary care hospital of Nepal during 2015-2017 were included to study antibiotic susceptibility pattern, biofilm formation and persister cells development. Standard microbiological procedure was used to identify staphylococcal species and CLSI guideline was followed to determine antibiotic susceptibility pattern. Biofilm production was detected using both phenotypic and genotypic methods. In-vitro persister assay is undergoing to study the causal link between persister cells and treatment failures in these isolates.

**Results:** Among 375 Staphylococcal isolates, 161 isolates were identified as *S. aureus* comprising 81% methicillin resistant and 19% methicillin sensitive *S. aureus* and remaining coagulase negative *Staphylococcus* spp. While most of the isolates were resistant to penicillin, these were sensitive towards chloramphenicol, tetracycline and clindamycin. On further verification, PCR detected *ica* genes in 23% of staphylococcal isolates thus revealing the biofilm formation in these samples.

**Conclusions:** Biofilm producing ability of Staphylococci is the major contribution in pathogenesis associated with this organism. To the best of our knowledge, this is the first study in Nepal which will bring new insights into the status of staphylococcal infections eventually helping to minimize the occurrence of such life-threatening diseases in this country.
**Session Title:** MONDAY Poster Session 1  
**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 030  
**Abstract Topic:** Biofilm Antimicrobial Tolerance  
**Abstract Title:** Comparison of the Properties of *Pseudomonas aeruginosa* Biofilm Cells Dispersed with Vapor Nano Bubbles to Cells Derived From Other Dispersal Methods  
**Author Block:** J. Wille¹, E. Teirlinck², F. Van Nieuwerburgh¹, K. Braeckmans², T. Coenye¹;  
¹Laboratory of Pharmaceutical Microbiology, Ghent University, Gent, BELGIUM, ²Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Gent, BELGIUM, ³Laboratory of Pharmaceutical Biotechnology, Ghent University, Gent, BELGIUM.

**Background:** Biofilm infections are challenging to treat due to enhanced antibiotic tolerance and biofilm dispersal is considered as one of the strategies that could be used to increase antibiotic efficacy on biofilms. We recently developed a novel strategy to disrupt biofilms, using laser-generated Vapor Nano Bubbles (VNBs). Previous work showed that VNB disrupted biofilms show increased antibiotic susceptibility compared to undisrupted biofilms. In the present study we compare antibiotic tolerance and the transcriptome of *P. aeruginosa* cells released from a biofilm via VNB-treatment, to the tolerance and the transcriptome of cells released with other dispersal methods.

**Methods:** *P. aeruginosa* PAO1 biofilms were grown in flow-cells at room-temperature for 4 days. Prior to VNB biofilm disruption, gold nanoparticles were added to biofilms. Subsequently, biofilms were exposed to a green (561 nm) pulsing laser. This creates a nano-sized water-vapor bubble around the gold nanoparticle, which disrupts the biofilm. Biofilm dispersal was also induced with 500 µM sodium nitroprusside (SNP) or by a sudden increase of the carbon source concentration (18 mM glutamate). The cells released from the biofilm were collected and their susceptibility to tobramycin (10 µg/ml) and colistin (16 µg/ml) was determined (using time-kill assays). As a control we used cells that were released spontaneously (i.e. without external trigger) and planktonic cells. In addition, RNA was collected from these cells, gene expression profiles were determined using Illumina-based RNA sequencing. **Results:** Dispersed cells (irrespective of the dispersal method) showed reduced killing compared to planktonic cells, when exposed to tobramycin or colistin. While complete eradication (7 log) was obtained for the planktonic culture after 5h incubation with tobramycin, only a 4 log decrease was observed for dispersed cells. Similarly, the planktonic culture (7 log) was completely eradicated after 2 hours treatment with colistin, whereas only a 3 log decrease was obtained for dispersed cells. When comparing spontaneously dispersed cells to cells dispersed by an external trigger, 55 genes showed differential expression. These genes are involved in anaerobic respiration (e.g. *nir*-genes), in alginate production (e.g. *algC*) and β-lactamase regulation *ampD*. 342 genes were found to be differentially expressed in VNB treated cells in comparison to spontaneously dispersed cells and cells dispersed by SNP or glutamate. This includes genes encoding proteins involved in antibiotic resistance (e.g. *mexY*), virulence (e.g. *pvdG*) and quinolone signaling (e.g. *pqsC*, *phnA*). **Conclusions:** Dispersed cells (irrespective of the dispersal method) have an enhanced tolerance to tobramycin and colistin. RNA sequencing revealed that gene expression in VNB-dispersed cells is different from that in cells dispersed with other approaches.
Abstract

**Background**: *Staphylococcus epidermidis* is the leading cause of recalcitrant biofilm-associated infections. Studies have majorly focused on the mechanisms mediating *S. epidermidis* biofilm formation and development. However, the molecular mechanisms that underlie *S. epidermidis* biofilms resistance to physico-chemical disinfection remain unclear. Alternative sigma factor B (σB) and catalase have been implicated in the survival of planktonic forms of bacterial species against stresses. However, the potential role of σB and catalase in *S. epidermidis* biofilms resistance to physico-chemical disinfection is little known. Thus, this study evaluated the effects of heat, sodium chloride (NaCl), sodium hypochlorite (NaOCl) and hydrogen peroxide (H$_2$O$_2$) exposure on σB and catalase activities in *S. epidermidis* biofilm and planktonic cells. **Methods**: *S. epidermidis* isolates were obtained from skin swabs of outpatients at Kisumu County Hospital in western Kenya. Biofilm and planktonic cells were formed from the *S. epidermidis* isolates in polystyrene tubes. Experimentally, 50°C, 0.8 M NaCl, 5 mM NaOCl and 50 μM H$_2$O$_2$ induced significant stress to *S. epidermidis* populations hence were used for tryptic soy broth (TSB) supplementation. The biofilm and planktonic cells were grown in TSB alone (untreated controls) and TSB adjusted to 0.8 M NaCl, 5 mM NaOCl and 50 μM H$_2$O$_2$ for 30 and 60 min. For heat exposure, the biofilm and planktonic cells were exposed to 50°C and 25°C (controls) for 30 and 60 min. Then, σB and catalase activities were quantified by RT-qPCR and a catalase assay involving measuring the height of O$_2$-forming foam respectively for the disinfectants-treated cells and the controls. Statistical comparisons were determined by Student t-test. **Results**: The heat-exposed *S. epidermidis* biofilms had significantly increased σB activity than the planktonic cells ($p=0.0256$). Further, σB activities in the NaCl-treated biofilms and planktonic cells were statistically similar ($p=0.3004$). However, NaCl-treated biofilms had significantly higher σB activity than the untreated controls ($p=0.0203$). The NaOCl-exposed biofilms showed significantly increased σB activity than the planktonic cells ($p=0.0109$). Further, the H$_2$O$_2$-treated biofilms had higher σB activity than the planktonic cells ($p=0.0157$). The σB activities in H$_2$O$_2$-treated planktonic cells and the unexposed controls were statistically similar ($p=0.1358$). The *S. epidermidis* biofilms subjected to the four disinfectants showed enhanced catalase activities than the planktonic cells ($p=0.05$). **Conclusions**: *S. epidermidis* biofilms enhance σB and catalase activities in response to the disinfectants suggesting a protective role of σB and catalase in the biofilms against physico-chemical disinfection. Thus, σB and catalase may be promising targets for the development of effective anti-staphylococcal biofilm eradication approaches.
Session Title: **MONDAY Poster Session 1**

Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 032

Abstract Topic: Biofilm Antimicrobial Tolerance

Abstract Title: *Prevalence of Biofilms & Enterotoxins Produced by Staphylococcus aureus-Inducing Pneumonia in South-South Geopolitical Zone, Nigeria*  

Author: F. O. Enwa, E. I. Ogbomo

Block: Delta State University, Abraka, NIGERIA.

The emergence of recalcitrant strains of *Staphylococcus aureus* is also alarming & an awareness of the virulence has been shown to help prevent, combat or eradicate *Staphylococcus aureus* infections. This study aimed at evaluating the biofilm forming capacity of *Staphylococcus aureus*, the prevalence of *Staphylococcus aureus*-induced biofilms & enterotoxins as well as the prevalence of *Staphylococcus aureus*-induced pneumonia in the south-south geopolitical zone, Nigeria. A total of 1500 clinical specimens (sputa) were collected from clinically diagnosed pneumonia patients in randomly selected health institutions in the south-south geopolitical zone, Nigeria & cultured using a selective medium for *Staphylococci*. Seventy-nine (79) samples out of 1500 investigated yielded *Staphylococcus aureus*. The 79 clinical isolates were further screened for biofilm formation using crystal violet binding assay and for enterotoxins using Reverse Passive Latex Agglutination (RPLA) method. The results showed that prevalence of *Staphylococcus aureus*-induced pneumonia in the south-south geopolitical zone, Nigeria is low; but the biofilm forming capacity of *Staphylococcus aureus* is high with the highest and lowest mean biofilm thickness (absorbance) of 0.358±0.06 and 0.211±0.07 respectively. Also, the results showed that the most prevalent *Staphylococcus aureus* enterotoxins in the south-south geopolitical zone, Nigeria is Enterotoxin B. These findings are very important in monitoring the virulence and resistance patterns of *Staphylococcus aureus*. Keywords: Biofilm, enterotoxin, *Staph aureus*, inducing, pneumonia.
Biofilm Antimicrobial Tolerance

Applying Reserpine to Drug Development: Can a Modern Strategy be Developed?

D. Parai, S. K. Mukherjee; University of Kalyani, Kolkata, INDIA.

**Background:** The emergence of antibiotic resistance among bacteria has been increased over the last few years due to careless and widespread use of conventional drugs. Moreover, few antibiotics even trigger biofilm formation at sub-inhibitory dosages. This clinical urgency motivates scientists towards alternative drug development against biofilm-associated diseases. Phytochemicals are referred to be one of the popular modern therapeutic approaches for its availability, less toxicity, high chemical group diversity and biochemical specificity. *Pseudomonas aeruginosa* is considered a major clinical concern due to its ability to form biofilms which makes it difficult to be eradicated, leading to chronic nosocomial infections. The goal of this work was to assess the antibiofilm and Antivirulence activity of reserpine, a plant-derived indole-alkaloid, on *P. aeruginosa* PAO1. **Methods:** The anti-biofilm activity of reserpine was evaluated by crystal violet staining, MTT assay, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). Reserpine effects were also assessed by qRT-PCR of quorum sensing (QS)-regulated genes and biochemical quantification of the QS-mediated virulence factors pyocyanin, rhamnolipids, proteases and elastases. Moreover, molecular docking was performed to address possible physical interactions with reserpine as a test ligand. And lastly, a model urinary catheter was used to investigate the potential clinical application of reserpine on a medical device. **Results and Discussion:** Crystal violet and MTT assay revealed the inhibitory antibiofilm effect of reserpine. Both CLSM images and Congo red binding assay suggested the significant loss of EPS after treating with IC$_{50}$ and IC$_{80}$ dosages of reserpine. Reserpine could also restrict the pellicle formation at the air-liquid interface to hinder bacterial attachment and growth. It demonstrated to affect swimming and swarming motilities at sub-MIC. Reserpine has been found to inhibit the secretion of virulence factors significantly at IC$_{50}$ and IC$_{80}$ dosages. The findings of biochemical tests were also corroborated by the transcriptional level expression study of QS-regulated genes. Docking result further demonstrated that reserpine has the potential to outcompete the in vivo AHLs and AQs for LasR and MvfR proteins respectively, by forming complexes which lead to the down-regulation of QS-controlled genes. It could eradicate a good amount of mature biofilm from the model device at various sub-MIC dosages which indicated its possible usage against device-associated biofilm contaminations. **Conclusion:** These findings provide insights into the underlying mode of action of reserpine, which might be useful in the field of new antibacterial drug development and be accounted as a new lead to generate other analogues with improved pharmacological applications and warrants further study.
Background: Biofilms display increased tolerance to antibiotics (eg. tobramycin) compared to exponentially growing planktonic cells. Consequently, infections that are biofilm-based, such as the lung infections caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis, are extremely difficult, if not impossible, to cure with antibiotics. It is possible that the activity of specific regulatory pathways may lead to the antibiotic tolerance phenotype that is characteristic of biofilms. In this work, we asked whether any *P. aeruginosa* transcriptional regulators contribute to the tobramycin tolerance of biofilms formed in 96-well plates.

Methods: Transposon mutants from the PA14 non-redundant transposon mutant library with annotated insertions in most of the non-essential sigma factors, transcription factors and two-component systems were compiled into a library subset that contained approximately 550 mutants. This sub-library was screened twice for mutants with increased biofilm susceptibility to tobramycin. Briefly, the sub-library was used to inoculate 96-well plates containing M63-arginine, and the plates were subsequently incubated statically for 24 hours to allow for biofilm formation. Biofilms were then exposed to 100 µg/mL tobramycin for 24 hours (a sub-bactericidal concentration for most mutants in the library). Following antibiotic treatment, biofilms were incubated with fresh recovery medium for 24 hours so that surviving cells could re-enter the planktonic state. Using a plate replicator, 5 µL of the recovery medium was then transferred to LB agar plates. Operating under the premise that biofilms with less surviving cells would release less planktonic cells into the recovery medium, lack of growth on the LB agar plates was scored as a hit in the screen.

Results: Twenty mutants were identified in the screen as potentially having increased biofilm susceptibility to tobramycin. Mutants with annotated insertions in *amgS* and *PA14_27950* were identified in the screen, and deletion of these genes has been previously linked to increased planktonic tobramycin susceptibility by other groups. We also identified mutants with annotated insertions in genes involved in biofilm formation (eg. *fleQ*, *sutA*, *pvrS*, and *rcsB*). Interestingly, several mutants with insertions in uncharacterized transcriptional regulators were also identified. Validation of the mutants through confirmation of the transposon insertion sites as well as quantitative assessments of biofilm susceptibility to tobramycin are currently underway.

Conclusions: Overall, several mutants with annotated insertions in genes involved in transcriptional regulation were identified as potentially having increased biofilm susceptibility to tobramycin. Future work will confirm the importance of these regulators in *P. aeruginosa* biofilm tolerance to tobramycin and establish their mechanism of action.
Biofilms are highly complex communities, composed of highly structured extracellular polymers and subpopulations of differentiated cells, such as persisters. These contribute to the resistance of bacterial biofilms to antibiotics, creating a significant issue in the treatment of infections and resulting in elevated levels of mortality and morbidity. Here, we use a combinatorial approach to explore the penetration and efflux of a variety of antimicrobial agents into biofilms of the important pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We use a microfluidic system coupled with time-lapse microscopy to probe the activation of transcriptional fusions between cell damage reporters and fluorescent proteins in response to antimicrobial agents. In addition, through use of antimicrobial labels we explore the effect the antimicrobials have on the distribution of exopolysaccharide (EPS) and extracellular DNA (eDNA) within the biofilm. Further, working with partners in the European Association of National Metrology Institutes (EURAMET) we are developing cross platform methods for the label-free localisation of antimicrobial agents within the biofilm. These platforms include 3D OrbiSIMS (secondary ion mass spectrometry), IR s-SNOM (Infrared scattering scanning near-field optical microscopy) and Raman spectroscopy. Here we describe initial findings and demonstrate their potential for use in understanding antimicrobial dynamics.
Abstract

Investigating the Effect of Biosurfactants Rhamnolipid and Surfactin on Inhibition of *Salmonella enterica* sv Typhimurium LT2 Biofilm Formation on Multiple Surfaces

S. Khonsari, M. Marvasi, D. Purchase; Middlesex University, London, UNITED KINGDOM.

Development of biofilms on different surfaces are formed due to various types of bacterial pathogens. A great concern to the environment health and food industry is the Gram negative *Salmonella enterica* serovar Typhimurium with the ability of biofilm formation on surfaces such as plastics. This pathogen has the ability to infect hosts through food poisoning and could lead to life threatening conditions such as gastroenteritis and diarrhoea. The purpose of this study is to determine the role of outer lipopolysaccharide components in formation of biofilms by *Salmonella* on different types of surfaces. Such surfaces includes polystyrene and polypropylene that are commonly used in food and medical industries in presence of chosen biosurfactants. Previous studies have proved that biosurfactants with nontoxic amphiphilic compounds are able to prevent biofilm formation.

Health and safety management processes organizations such as Hazard Analysis and Critical Control Point (HACCP) and management processes e.g. Good Agricultural Practices (GAP) and Good Manufacturing Practice (GMP) have concerns of controlling the *Salmonella* biofilm formation. However, the resistance of this pathogen to sanitizers and disinfectants has persisted a great concern. Thus, effective antimicrobial agent and related management strategy are crucial for inhibiting and controlling the biofilms formed by *Salmonella*. There have been a number of *Salmonella enterica* sv Typhimurium LT2 isogenic mutant strains in different parts of the lipopolysaccharide layer selected for the purpose of this study. To screen biofilm formation and dispersal, the following deletion mutants rfa, rfaL, rfaJ, rfaF, and rfaG were used under the presence of 0.5 μg/ml rhamnolipid and 5 μg/ml surfactin. To quantify biofilm formation *Salmonella* wild type and mutants was examined using 96 multi-well plates made of polypropylene and polystyrene using crystal violet method. The effect of different biosurfactants concentrations on mutants and wild type biofilm formation have been studied. The results have shown that presence of 5 μg/ml surfactin had significant effect on biofilm inhibition on both polypropylene and polystyrene surfaces in mutants 225, 227, 228 and 229 with deletion of genes in O-antigen and core of LPS. Currently the physiological changes effect including temperature and time on biofilm formation of *Salmonella enterica* sv Typhimurium LT2 are being studied. The results obtain from this study will allow to develop an effective strategy that will improve the inhibition and control of biofilm formation in food and medical industry.
Background: Atopic dermatitis (AD) is one of the most common inflammatory skin diseases. It is an idiopathic disease with no known cure which often affects patients throughout their life. Among impaired skin barrier and dysregulated immunity, a hallmark feature of AD is an abnormally high presence of the bacterium Staphylococcus aureus on the skin, particularly when the atopic flares occur. S. aureus is a pathogen capable of secreting several virulence factors, some of which can elicit inflammatory response from the host during infection. The normal skin microbiome is primarily composed of commensal organisms such as Staphylococcus epidermidis and rarely contains S. aureus. S. epidermidis is the predominant member of the skin microbiome and can secrete molecules that disrupt and inhibit S. aureus biofilms. AD skin also exhibits a reduced amount of sphingosine, a naturally occurring antimicrobial lipid. We hypothesize that S. epidermidis and sphingosine regulate the presence of S. aureus on the skin.

Methods: Axenic cultures of S. aureus ALC2085 and/or S. aureus ATCC 6538, and S. epidermidis 1457 were established in brain-heart infusion broth (BHI) for 3 days, after which the biofilms were exposed to BHI containing sphingosine at a physiological level (45 mg/L for AD condition, or 80 mg/L for normal). Dual-species biofilms were cultured and treated in the same conditions, at two ratios to represent the normal microbiome and AD dysbiosis, respectively: 1:100 ratio and 1:1 ratio of S. aureus to S. epidermidis. Viability of the biofilm was determined every day by harvesting the biofilm and plating on 1:2 Plate Count agar for single-species, or Mannitol Salts agar for dual-species. Results: Single-species biofilm of S. aureus ALC2085 was eradicated at normal sphingosine but not at AD concentrations. S. aureus ATCC 6538 and S. epidermidis 1457, which are more prolific biofilm-forming strains, exhibited more resistance at both sphingosine concentrations. In dual species biofilms, S. aureus ALC2085 was eradicated when both species ratio and sphingosine concentration resembled normal conditions. When the species ratio was normal but sphingosine concentration was reduced, S. aureus viability also significantly decreased. Conclusions: The resistance of S. aureus to sphingosine antimicrobial activity and competition from S. epidermidis depends on the strain: ALC2085 is a laboratory strain with decreased expression of several quorum-sensing genes that exhibits a relatively higher susceptibility to sphingosine than that of strain ATCC 6538, a clinical lesion isolate. The susceptible S. aureus strain was shown to retain viability when there was low sphingosine concentration and an even ratio of the two species. This suggests that S. aureus is allowed to colonize the skin unhindered when the skin microbiome and sphingosine are compromised.
Session Title: MONDAY Poster Session 1
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 038
Abstract Topic: Biofilm Antimicrobial Tolerance
Abstract Title: Monitoring Bacterial and Fungal Biofilm Formation and Dispersal in Real-Time Using Cellular Impedance: Diverse Applications Ranging from Drug Screening to Identification of Novel Regulatory Genes
Author Block: B. J. Lamarche1, Y. Abassi1, A. Mira2, M. D. Ferrer2;
Block: 1ACEA Biosciences, San Diego, CA, 2Center for Advanced Research in Public Health, Valencia, SPAIN.
Abstract Body: The colorimetric assays traditionally used for studying biofilms are inefficient/low throughput, are incompatible with orthogonal assays (i.e. samples are destroyed by the analysis process), and only provide endpoint data. Herein we demonstrate how impedance monitoring by xCELLigence® instruments overcomes each of these limitations, enabling a quantitative and continuous evaluation of biofilms via an assay that is both label-free and totally automated. Using this approach the formation and dispersal of biofilms of Gram positive and Gram negative species was monitored both in monoculture and multi-species co-culture formats. Because each well of the xCELLigence microtiter plate provides a complete time course over assay windows spanning anywhere from minutes to weeks, large numbers of conditions were evaluatable in a single plate. The high temporal resolution of this assay consistently elucidated subtle biofilm behaviors that have historically gone undetected when using other methodologies. Finally, we demonstrate how the high sensitivity and automated nature of the xCELLigence assay have led to its use for clinical theranostic purposes, with dental samples being screened in order to identify, on a patient-by-patient basis, the most effective drug.
Real-Time Characterization of Microbiological and Mechanical Properties of Bacillus Licheniformis and Pseudomonas Fluorescens Dual-Species Biofilms

C. Abriat1, F. Daigle2, N. Virgilio1, M. Heuzey1

1Polytechnique Montréal, Montréal, QC, CANADA, 2Université de Montréal, Montréal, QC, CANADA.

Background: In natural habitats, multiple bacterial species often coexist together in biofilms. They interact in synergetic or antagonistic ways and their interspecies interactions can influence the biofilms’ development and properties. The current knowledge on interspecies interactions is still limited despite recent advances that have enabled analysis of complex microbiological communities. Interfacial rheology is a unique technique to quantify a biofilm’s viscoelastic/mechanical properties at the air-liquid interface in real time. To bring a new understanding on how the interactions between bacteria affect a biofilm’s mechanical properties, we investigated a model biofilm composed of two environmental bacteria commonly found in multiple food contamination cases: Bacillus licheniformis and Pseudomonas fluorescens. Methods: To characterize the bacterial interactions within a multispecies biofilm, we have employed an interdisciplinary approach. We have measured the viscoelastic growth profile of the model dual-species biofilm during its formation at the air-liquid interface by interfacial rheology, a chemical engineering technique. Different bacteria ratios were tested to probe the interactions among bacteria. To complement the rheology results, microbiology experiments including biomass determination, bacterial viability counts and microscopic analysis were performed. Results: Interfacial rheology revealed the formation of very strong elastic biofilms by B. licheniformis, which formed red and rough colony biofilms on Congo Red plates, while the pellicle of P. fluorescens was weaker and the biofilm was smoother. Quantitative analysis of the single species biofilms supported the rheological properties with a higher biomass content for B. licheniformis biofilm. The time-resolved viscoelastic properties, colony biofilm morphology, quantitative analysis of the pellicle and microscopic analysis demonstrated that P. fluorescens mainly contributed to the dual-species biofilm properties. Planktonic growth revealed that P. fluorescens grew faster than B. licheniformis, with no antimicrobial competitive mechanisms between the two species. Our results linked the influence of growth kinetics to the time-dependent composition and properties of dual-species biofilm. Conclusion: By combining macroscale quantitative techniques - interfacial rheology, biomass and bacterial counts - with microscopic analysis, we were able to relate the unique viscoelastic changes observed in dual-species biofilms, to bacterial growth. Future work is aimed at incorporating pathogenic bacteria to this model system, and at studying the influence of environmental stimuli to control the formation or eliminate multispecies biofilms.
Long-Term Time-Lapse Atomic Force Microscopy: A Tool to Image the Origin of Mycobacterial Biofilms

H. A. Eskandarian,
École Polytechnique Fédérale de Lausanne, Lausanne, SWITZERLAND.

Phenomena governing biofilm formation range from biochemical to biophysical principles, and beyond. Often, our ability to measure biochemical or biophysical quantities at the single cell and sub-cellular levels is limited in spatio-temporal resolution. Since bacterial cells are at the spatial resolution limit of optical microscopy, new, complementary imaging modalities are required in order to gain novel insight. One promising approach is the atomic force microscope (AFM), a mechanical microscope capable of resolving features with sub-nanometer precision. Besides this stunning resolution, the AFM enables the correlated mapping of mechanical as well as chemical information of the sample. We have developed a combined optical fluorescence and atomic force microscopy setup for long-term time-lapse imaging samples spanning a range from single bacterial cells to nascent biofilms. Our custom built tool has enabled us for the first time to investigate division site selection, the biomechanics of pole growth, as well as to describe the dynamic evolution of the chemical composition of the mycobacterial surface, all of which are principles that were previously uncharacterised. Additionally, we can robustly follow how a micro-colony of bacteria on a surface develops into a biofilm. Our imaging tool offers us the unique potential to describe how organized patterns of biofilm morphology develop. By measuring cell surface stiffness and biochemical composition of a biofilm surface, we envision developing the potential to predict the morphology of a biofilm. Taken together, long-term time-lapse combined optical fluorescence and atomic force microscopy represents a powerful tool offering a breadth of unique measurements that have the potential to culminate in the description of key biophysical and biochemical principles underlying biofilm physiology and morphogenesis. 1. Van Der Werf, K. O., Putman, C. A. J., De Grooth, B. G. & Greve, J. Adhesion force imaging in air and liquid by adhesion mode atomic force microscopy. Appl. Phys. Lett. 65,1195-1197 (1994). 2. Pfreundschuh, M., Alsteens, D., Hilbert, M., Steinmetz, M. O. & Müller, D. J. Localizing chemical groups while imaging single native proteins by high-resolution atomic force microscopy. Nano Lett. 14,2957-2964 (2014). 3. Eskandarian, H. A. et al. Division site selection linked to inherited cell surface wave troughs in mycobacteria. Nat. Microbiol. 2,17094 (2017).
Filamentation of a *Vibrio cholerae* O139 Strain Reveals a Novel Biofilm Morphology and a Unique Ecological Advantage within Oceanic Communities

**Author:** B. R. Wucher¹, A. Persat², C. D. Nadell¹;

¹Dartmouth College, Hanover, NH, ²École Polytechnique Fédérale de Lausanne, Lausanne, SWITZERLAND.

*Vibrio cholerae* is an aquatic bacterium and the causative agent of the intestinal disease cholera. While much is known about the ability of *V. cholerae* to cause infection, little is understood about the organism’s behavior in its natural reservoirs: open ocean and salt water estuaries. When in these environments, *V. cholerae* competes for space and resources on the chitinous exoskeletons of crustaceans and plankton. This creates an interesting challenge where resource availability is directly tied to finite colonizable space. Over time certain strains of pandemic *V. cholerae* have overtaken each other as the dominant disease-causing strains in these spaces. These displacement events suggest key advantages that certain strains have over others in aquatic and/or pathogenic environments. While searching for phenotypes that could account for these advantages we identified a strain from the serotype O139 (CVD 112) that rapidly filaments under simulated oceanic conditions. Generally, filamentation is associated with an RpoS-mediated stress response. However, CVD 112 has no apparent growth or fitness defects compared to the common lab strain N16961. The biofilm phenotype of this strain is starkly different from that typically seen among common laboratory models of *V. cholerae*. Instead of forming attached microcolonies, the filaments of CVD 112 will aggregate into mobile clusters that can weakly adhere to surfaces. This appears to be a new type of biofilm morphology that is independent of the traditional matrix components, where we see an aggregate of cells held together by the entangling of these filaments. The increased length of these filamented cells has a significant effect on the strain’s ability to colonize static surfaces. We show that this phenotype dramatically increases the colonization of chitin particles under flow, but decreases long-term competitive ability against other strains of *V. cholerae* that produce biofilm matrix more aggressively. As a result, a filamentous strain can competitively displace non-filamenting strains under conditions of rapid resource turnover but is itself displaced on any chitin particle that is sufficiently long-lived. These results highlight a novel ecological function for bacterial filamentation and shed light on the population dynamics of pathogenic *V. cholerae* in the environment.
Adhesive Behavior and Detachment Mechanisms of Bacterial Amyloid Nanofibers

Amyloid nanofibers possess high mechanical rigidity and strength, and those employed in biofilms and bioengineered adhesives have proven capable of adhering strongly to abiotic surfaces. Although bacterial amyloids such as curli nanofibers have been harnessed for use in engineered materials, such as strong underwater adhesives, the adhesive performance of individual nanofibers and dependence of this performance on physical properties has not been investigated. To determine the detachment mechanisms of single amyloid fibers from surfaces, we carried out coarse-grained molecular dynamics simulations that examine the role of nanofiber cohesive energy, rigidity, and adhesive energy on the work of adhesion. Taking a generic model inspired from the curli nanofiber subunit CsgA, we discover that the amyloid nanofibers adhering to a surface can undergo three different peeling processes when pulled at a constant rate normal to the surface. Computational phase diagrams built from parametric studies indicate that nanofibers with low cohesive energy relative to adhesive energy and bending rigidity will break before complete detachment. Strong nanofibers with high cohesive energy detach via peeling smoothly away from the substrate. At intermediate ratios, hinge formation occurs and the work of peeling the nanofiber is twice the adhesion energy due the additional energy required to bend the nanofiber during desorption. Varying the geometry of amyloid subunits revealed that the work of peeling decreases for thicker nanofibers, suggesting that the tape-like monomeric structure of amyloids may facilitate better adhesive performance. We also found that shorter amyloid subunits facilitate smoother peeling of nanofibers, shifting phase diagrams towards hinge and peeling cases. Our results demonstrate how the dimensions and adhesive and cohesive properties of the amyloid nanofibers can be optimized to resist mechanical peeling.
Interfacial Response of Films of Mucoid and Non-mucoid *Pseudomonas aeruginosa* Isolates

**Author:** S. Balmuri, N. Waters, T. Niepa; University of Pittsburgh, Pittsburgh, PA.

Fluid interfaces are energy-rich environments known to influence the self-assembly of small molecules or microparticles, and the formation of thin films by biological entities. The formation of bacterial films at fluid interfaces appears to be a dynamics process, in which cells coping with the existing interfacial energies respond to the entrapment by forming a matrix appropriate to their survival. We hypothesize that cells subjected to interfacial stress adapt and exhibit phenotypic changes essential to their survival.

In this study, we are investigating the response to interfacial confinement of two clinical strains of *Pseudomonas aeruginosa* isolated from the airways of cystic fibrosis patients. To evaluate what phenotypic changes at fluid interfaces provide growth advantage, we are comparing a mucoid (PASL) and non-mucoid strain (PANT) of *P. aeruginosa* secreting various level of polysaccharides. We are further investigating the mechanical properties of films formed by these cells to understand how they adapt and restructure environments with high interfacial energies. Our result demonstrate that the intrinsic properties of cells confer viscoelastic behavior facilitating their adaptation as characterized by the apparent moduli of the elastic films observed using pendant drop elastometry and particle tracking. Further characterization of whole genome and the transcriptomes of cells under interfacial confinements will provide new insights on the biological implications of interfacial films.
Objective: As polyester polyurethanes are degraded by microorganisms they release aliphatic and aromatic diols, carboxylates, intact polyurethane blocks, and other coating additives into the environment. These foreign chemical species are of significant concern considering the increased use of polyurethanes in everyday life and the environmental impact of microplastics. The objective of this work was to identify if two phylogenetically dissimilar non-motile yeasts isolated from the same environmental consortia used similar mechanisms to degrade biodegradable polyester-only coatings to a polyester polyurethane coating with similar composition.

Methods: We isolated and identified two non-motile yeast strains using Impranil® clearing assays from microbial consortia found inside of in-service aircraft. We used confocal, phase contrast, and infrared microscopies and headspace gas analysis to compare the amount of polymer coating loss and the degradation that occurred near cell masses over 8 days at a relative humidity > 95% on biodegradable polyester coatings (polyethylene succinate (PES) and polyethylene adipate (PEA)) and on a polyester polyether polyurethane coating (Irogran®). We also compared the changes in cell density of the biofilms and planktonic cultures with qPCR and optical density measurements, respectively. Results: We isolated and identified polyester degrading strains of Papiliotrema laurentii and Naganisha albida. Both yeasts created zones of clearing on Impranil®DLN-containing agar plates over 48 hours. The fungi also degraded and metabolized PES, PEA, and Irogran® (a polyester polyether polyurethane) coatings without additional carbon sources. The biodegradation and metabolism of PES coatings by P. laurentii produced the highest output of CO2 over 7 days while N. albida generated the highest CO2 production from PEA coatings. P. laurentii biofilms grew on PES coatings but did not grow significantly on PEA and Irogran® coatings. P. laurentii degraded the polyester polyether polyurethane Irogran® resulting in the settling of the cells into the coating while N. albida did not settle into coatings and minimal surface degradation was observed. Conclusions: These two fungi actively degraded PES coatings as biofilms but only P. laurentii biofilms degraded PEA and Irogran® coatings. Only P. laurentii biofilms increased in cell density on PES coatings with negligible cell growth observed on PEA and Irogran® coatings as a result of degradation. The movement of these fungi on the surface of the different polymers suggests that these fungi interacted with each polymer differently and this movement was not necessarily based on the coating composition. All coatings that were degraded resulted in the settling of the cells into the coating suggesting that degradation generally resulted in the failure of the coating surface near cells masses.
Session Title: MONDAY Poster Session 1
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 045
Abstract Topic: Biofilm Metabolism
Abstract Title: Non-ribosomal Peptide Antibiotics Production in Bacillus subtilis is Governed by the Genetic Distances between Biofilms and their Potential Competitors
Author Block: H. Maan; Weizmann institute of science, Rehovot, ISRAEL.
Abstract Body: Ecosystems flourish with microbial life; in such environments, bacteria establish complex communities called as biofilms. Bacteria within biofilms produce various antibiotics that play a crucial role in antagonizing a competitor invasion and thus allow a given bacterial species to protect its ecological niche. We have previously showed that during interspecies interactions B. subtilis uses surfactin, a non-ribosomal peptides (NRP) to eliminate competing Bacilli (Rosenberg et al., 2016). We now ask what molecular mechanism regulate the arsenal of non-ribosomal peptide antibiotics during interspecies interactions, and whether these mechanisms differ between antibiotics. We focused on three non-ribosomal peptide antibiotics bacillaene, bacilysin and plipastatin, encoded by the operons pks, bac and pps. These NRPs are produced together with surfactin in B. subtilis biofilms. Using transcriptional fusions of the promoters for each antibiotic, we tested the expression level of these clusters as a function of the genetic distance from neighboring colonies. Surprisingly, our results showed that bacilysin and plipastatin are expressed in moderate and low levels within non competing biofilms. However, in the presence of competitors there was a strong induction in expression of bacilysin and plipastatin which was directly correlated to the genetic distance. This induction did not require direct contact and could be complemented by the extracellular milieu of the competing species. In contrast, the majority of the B. subtilis biofilm population expressed the pks operon for bacillaene under our conditions, with moderate influence for the identity of potential competitors. We now aim to understand how does B. subtilis probes the genetic distance to distinguish its competitors from potential co-operators. Coupling antibiotic production with measuring genetic distance from neighbor colonies might be useful for B. subtilis to form successful multispecies biofilms composed of compatible community members.
Aminoglycoside Tolerance of Free-floating Bacterial Biofilms Formed in Synovial Fluid can be Reversed by Exogenous Metabolic Stimuli

J. M. Gilbertie1, B. P. Conlon2, T. P. Schaer3, L. V. Schnabel1;
1North Carolina State University, Raleigh, NC, 2University of North Carolina, Chapel Hill, NC, 3University of Pennsylvania, Raleigh, NC.

Infectious, arthritis is described as bacterial infection of a joint that causes inflammatory cell infiltrate and cytokine production leading to arthritic changes including cartilage damage. Bacteria grown in synovial fluid (SynF), the main component of the joint, form macroscopic free-floating aggregates with similarities to biofilms that display severe antimicrobial tolerance. In addition, bacteria growing in SynF in vitro exhibit altered growth kinetics, decreased production of ATP and lower cellular respiration. Due to the altered metabolism of SynF bacterial aggregates, we hypothesized that exogenously supplied metabolites from the native joint environment could increase cellular respiration and metabolism which would potentiate killing by aminoglycosides. In order to test this hypothesis, S. aureus was grown in equine synovial fluid overnight. Several metabolites were exogenously supplied to the infected SynF for 2 hours with or without the addition of aminoglycosides. We found that the aminosugars found within the native synovial environment, such as glucosamine, increased bacterial growth (p<0.002), ATP production (p<0.0001) and cellular respiration (p<0.0004). Moreover, the addition of aminosugars increased aminoglycoside efficacy (p<0.001) compared to the untreated control. As most antimicrobials act on actively growing bacteria, we concluded that the antimicrobial tolerance of SynF bacterial aggregates could be due to decreased cellular processes and energy generation by the electron transport chain. We further validated that speculation by exogenously increasing the metabolism of these aggregates by synovial-specific metabolic stimuli. Future investigation on the use of aminosugars as adjuvants to aminoglycoside treatment in vivo could lead to a promising therapeutic for patients suffering from infections related to the synovial space such as periprosthetic joint infection.
Background: Dental plaque biofilms are structurally organised, complex microbial communities containing >100 species which form on teeth. Accumulation of plaque bacteria depends on environmental factors such as oxygen level, which is reduced by the early colonizing members of dental plaque enabling strict anaerobes to survive as plaque matures. Previous *in-vitro* models aimed to capture the highest species richness by using anaerobic conditions, but this does not represent real life in the oral cavity. The aim of this study was to develop an *in-vitro* microcosm dental plaque biofilm model using a new model system, and evaluate its efficiency under 5% CO2 and anaerobic conditions. **Methods:** A fed batch culture approach utilising 24 well-plates, with vertically suspended hydroxyapatite discs on a custom-made lid (Eastman Device), was developed to grow microcosm dental plaque biofilms. Pooled human saliva was inoculated in modified brain heart infusion. Microtiter plates were incubated either in air enriched with 5% CO2 or in anaerobic conditions at 37° C. Biofilms were grown undisturbed for 7 days, with a single media change at day 4, to allow baseline plaque formation. Subsequently, biofilms were matured by pulsing with 5% sucrose in the respective growth medium 3 times daily for a further 5 days. The pH of the spent media was recorded after every media change, and samples were collected at day 4 and 7 for baseline, and days 8 to 12 for mature plaque. Biofilms were analysed by enumeration of total aerobic-anaerobic bacteria, and 16S rRNA sequencing was used to characterise the biofilm community. **Results:** In both conditions, the number of total anaerobes was higher than the total aerobes at all time points, and viable counts plateaued after 9 days reaching around 9.0 log_{10}CFU at day 12. The pH of the spent medium during baseline plaque formation was above 6.5 for both conditions, and Stephan curve-like pH recovery patterns were obtained following overnight incubation throughout maturation. Community analysis of the baseline biofilms revealed increased alpha diversity measures under anaerobic conditions (Observed OTU: ~100 and Shannon: 2.5) compared to CO2 grown biofilms (Observed OTU: ~75 and Shannon: 2.0), which were dominated by *Lactobacillus* spp. (40-50%). However, with maturation, these differences in richness and evenness disappeared, and dental plaque biofilms were dominated by *Streptococcus* and *Veillonella* (40-60%) under both conditions. **Conclusions:** The Eastman Device combined with the described experimental approach generated compositionally and physiologically relevant, and mature dental plaque biofilms *in-vitro*. Also, community profile of 5% CO2 grown biofilms proved its capability to successfully mimic natural progression from aerobic to anaerobic conditions, closely resembling the real-life situation.
Biofilm aggregates of bacteria are thought to be able to align their phenotypic behavior with size, density, and growth state of the ensemble. This is achieved by a cell-cell regulatory system termed quorum sensing. In the generic quorum sensor a positive feedback in the production of signal molecules defines the conditions at which the collective behavior switches on. In spite of its conceptual simplicity, a proper measure of biofilm colony “size” has been lacking. We establish that the cell density multiplied by a geometric factor constitutes an appropriate size measure. The geometric factor is the square of the radius for a spherical colony, for a disk-shaped biofilm the geometric factor is the horizontal dimension multiplied by the height, and the square of the height of the biofilm if there is significant flow above the biofilm. Remarkably simple factorized expressions for the size are presented. Mol. BioSyst., 2014, 10, 103-9
Title: Modeling the Structure and Kinetics of Biofilms Formed by Nontypeable *Haemophilus influenzae* (NTHI) *In vitro*

Author: J. Das¹, E. Mokrzan¹, V. Lakhani¹, A. Snedden¹, J. R. Brown¹, J. Jurcisek¹, W. Ray¹, S. Mandal², T. Dey², L. O. Bakaletz²;
¹The Research Institute at the Nationwide Childrens Hospital and the Ohio State University, Columbus, OH, ²The Ohio State University, Columbus, OH.

Abstract Body:

Biofilms formed in the middle ear by nontypeable *Haemophilus influenzae* (NTHI) are central to the chronicity, recurrence, and refractive nature of otitis media and other mucosal infections. However, mechanisms that underlie the emergence of specific NTHI biofilm structures are unclear. We combined computational analysis tools and *in silico* modeling rooted in statistical physics, and computational geometry with confocal imaging of NTHI biofilms formed *in vitro* during static culture in order to identify mechanisms that give rise to distinguishing morphological features under these culture conditions. Our analysis of confocal images of biofilms formed by NTHI strain 86-028NP using pair correlations of local bacterial densities within sequential planes parallel to the substrate showed the presence of fractal structures of short length scales (≤10 μm). We also calculated volume (v), surface area (A), and Euler characteristic (χ) for each of these individual clusters. The Euler characteristic, revealing the number of ‘tunnels’ in the cluster, was calculated using a simplex counting method. In addition, we calculated the smallest opening in individual tunnels using a tool based on computational geometry. Since each of these geometric features potentially regulates specific functions that help NTHI to survive within the biofilm, we studied whether biofilms formed by different mutants of NTHI differ in terms of the above geometric features, and if specific geometric features arise due to optimization of multiple functions related to survival and dispersal of bacterial cells within the biofilm. We developed an agent-based *in silico* model to describe biofilm formation *in vitro* by NTHI or its mutants. The *in silico* modeling revealed that extracellular DNA (eDNA) and type IV pilus (Tfp) expression played important roles in giving rise to the fractal structures and allowed us to predict a substantial reduction of fractal structures for an isogenic mutant (ΔcomE) that was significantly compromised in its ability to release eDNA into the biofilm matrix and had impaired Tfp function. This prediction was confirmed by analysis of confocal images of biofilms formed *in vitro* by the ΔcomE mutant. The fractal structures potentially generate niches for NTHI survival in the hostile middle ear microenvironment by dramatically increasing the contact area of the biofilm with the surrounding environment, facilitating nutrient exchange to resident bacteria, and by generating spatial positive feedback to quorum signaling. However, fractal structures also increase the exposure of NTHI to antibiotics. *Our in silico* model can be used in combination with laboratory or animal modeling studies to further define the mechanisms that underlie NTHI biofilm development during mucosal infections caused by NTHI and thereby guide the rational design of benchwork and preclinical studies.
Background: Many Alphaproteobacteria utilize a polysaccharide-rich polar adhesin to mediate attachment to biotic and abiotic surfaces. *Caulobacter crescentus* is a freshwater oligotrophic species that can adhere permanently to surfaces via its polar adhesin, also called a holdfast. Holdfast-mediated attachment is required for biofilm formation on submerged surfaces, which accumulate biopolymers through a process known as conditioning. Holdfast further mediate the formation of rosettes, clusters of cells attached at their poles by their holdfast. Methods and Results: We report that when grown statically in unshaken liquid medium, *Caulobacter* cells form a biofilm pellicle at the air-liquid interface. In natural environments, this interface comprises the neuston layer, which contains a diverse array of aerobic microbes. Light microscopy revealed that cells in this pellicle are in large arrays of dense rosettes often containing greater than 50 cells per rosette. Given that holdfast is required for rosette formation, we examined whether holdfast is important for pellicle formation. We found that holdfast biosynthesis is required for accumulation of cells at the air-liquid interface. Importantly, synthesis of a major capsular polysaccharide cluster is not required for this mode of biofilm formation. We measured oxygen concentrations as a function of distance from the liquid surface. In both wild-type cultures with a pellicle, and evenly dispersed holdfast null cultures, oxygen exhibited a steep concentration gradient. Within 1 mm of the air surface oxygen drops by 50%, and by 2 mm oxygen is reduced by >99%. No gradient is observed in static broth lacking cells where oxygen concentrations are high and evenly distributed. Conclusions: The holdfast structure is known to mediate surface attachment and biofilm formation at liquid-substrate boundaries. Here we demonstrate that this polysaccharide-rich adhesin is also required for biofilm formation at the air-liquid interface. Accumulation of cells in the neuston layer of static aquatic environments confers access to higher concentrations of oxygen. Similar to submerged surfaces, this micro-environment is known to be enriched in biopolymers. We conclude that the holdfast adhesin is critical for *Caulobacter* to access nutrient-rich micro-environments in oligotrophic settings. We are currently applying a large-scale Tn sequencing approach to systematically characterize genes required for biofilm formation at the air-liquid interface.
Biofilms play an important role in the pathogenesis of Group A Streptococcus (GAS), a gram-positive pathogen responsible for a wide range of mild to severe infections with a global mortality reaching half a million a year. Although most GAS serotypes are able to form biofilms, there is large heterogeneity between individual strains in biofilm formation ability, as measured by standard crystal violet assays. Moreover, no direct correlation between biofilm formation and GAS infectivity has been identified. It is generally accepted that biofilm formation includes the initial adhesion of bacterial cells to a surface and microcolony formation, followed by biofilm maturation, characterized by extensive production of EPS matrix that links together proliferating cells and provides scaffold for three-dimensional biofilm structure. Our studies show however, that for several GAS strains, microcolony formation is not a crucial step in biofilm formation in static conditions, and biofilm can be effectively formed from late exponential or even early stationary planktonic culture, most likely by sedimentation and fixation of non-dividing GAS chains into biofilms. We confirmed that microcolony-independent biofilm formation is similar in morphology and 3-D structure to those initiated by actively dividing planktonic bacteria. We conclude, that some GAS strains can form biofilms by an alternate, non-canonical, mechanism that does not require transition from microcolony formation to biofilm maturation, and which may obscure biofilm phenotypes that arise via the classical biofilm maturation processes.
Background: After bacteria deposit on surfaces they twitch over surfaces using type IV pili (T4P). Pili emanating from bacterial surface could elongate up to several µm and their diameters are in nm scale. Bacterial twitching occurs through cycles of polymerization and de-polymerization of T4P. Polymerization causes pili to elongate and subsequently attach into surfaces. Depolymerization makes pili to retract and detach from surfaces. Pili retraction produces propulsion forces on the bacterium, which will then be pulled in the direction of the vector sum of the pili forces. T4P elongation, retraction, attachment and detachment produce a jerky movement of bacteria on surfaces.

Methods: In this work, a three-dimensional modelling approach of Computational Fluid Dynamics (CFD) coupled with Discrete Element Method (DEM) is proposed to study bacteria twitching in shear flows. This is an extension of the mechanistic agent-based model reported in Jayathilake et al. (2017). The new model has been implemented on the CFD-DEM package, SediFoam (Sun and Xiao, 2016). Each bacterium is modelled as a group of spherical particles and the T4P attached to bacteria are modelled as dynamic Hookean springs which can elongate, retract, attach and detach. The time scale of twitching is much larger than the time scale of fluid flows and hence the dynamics of twitching and fluid flows are separated. The twitching dynamics are updated at a larger time step based on quasi-equilibrium fluid flows.

Results: The CFD-DEM model is validated for rod-shaped bacteria moving in shear flows without any motility of bacteria. When rod-shaped bacteria move in shear flows they freely orbit (Jeffery orbit) and the transit of the orbit is compared with the theoretical results to validate the model. Next, the model is employed to study bacteria twitching on rough surfaces in the presence of surrounding fluid. The effects of bacterial shape, flow rate, surface topography on twitching motility are studied.

Background: Heterotopic ossification (HO) refers to ectopic bone formation, typically in residual limbs following trauma and injury. In the battlefield, wounded warriors who suffer an IED blast are at risk of having wound sites that are contaminated with bacteria, in particular in the biofilm phenotype. In natural ecosystems, 99.9% of bacteria preferentially dwell in biofilms. Thus, in traumatic injuries, soldiers or civilian patients are likely to be contaminated with well-established biofilms as opposed to planktonic bacteria. To assess the potential contribution of well-established biofilms on HO formation, a sheep model was established wherein biofilms were grown on glass beads (silica, to replicate grains of sand) and placed in apposition to the femur following a simulated IED blast. It was hypothesized that sheep inoculated with well-established biofilms in a battlefield-relevant sheep model would increase the degree of HO compared to those that were not inoculated. Methods: Animal work was performed at the University of Utah following local IACUC and external ACURO approvals. To simulate an IED blast, an air impact device (AID) device was used. The AID discharged high-powered bursts of air to the lateral, mid shaft region of the femur to inflict deep tissue trauma. To inoculate sheep, biofilms were grown on the surface of silica beads for 72 hrs and surgically placed onto the periosteum. Radiography was performed post-op to confirm location of the beads. Twenty-four wks post-op sheep were euthanized, femurs were dissected and processed for histological analysis. As controls, data were compared to sheep groups that were not inoculated with biofilms. Results: In a battlefield-relevant model of blast-related trauma, sheep that were inoculated with biofilms showed increased levels of HO formation, endosteal wall thickening and sequestra formation. The majority of sheep did not require antibiotic intervention and survived to the endpoint. HO was identified as a hybrid of trabecular and osteon remodeled bone with hypermineralization (Figure 1). Sheep that were not inoculated with well-established biofilms had lower rates of HO formation, or none at all. Conclusion: HO is a complicated process that affects wounded warriors. Data indicated that the presence of well-established biofilms may affect its formation and subsequent therapeutic strategies. Work is currently ongoing to expand data outcomes and future work will be performed to assess antibiofilm strategies that could be used following blast-related trauma to reduce risk of biofilm contamination and its effect on HO formation. Figure 1: (A) BSE images creating an overhead view showing ectopic bone possibly due to the inoculated biofilm by way of Si beads (B) Micrograph adjacent to the biofilm Si beads. (C) Micrograph of the ectopic bone growth showing remodeling bone by way of osteoblasts.
Session Title: MONDAY Poster Session 1

Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 055

Abstract Topic: Biofilm: From Nature to Models

Abstract Title: Influence of P. aeruginosa Quorum Sensing on the Spatial Arrangement of Dual-species Biofilms

Author Block: P. W. Woods, C. N. Marques; Binghamton University, Binghamton, NY.

Background: Biofilms, the major mode of bacterial growth, are composed of several bacterial species, while free living and colonizing a host. Previous work indicates that when replicating an in vivo model where both S. aureus and P. aeruginosa are present P. aeruginosa brings the S. aureus concentration down to undetectable levels. While within biofilms, bacterial cells can be found in various spatial arrangements, and little is known about the physical bacterial location while these bacteria are in dual-species biofilms. Previous work has demonstrated that quorum sensing in P. aeruginosa affects the growth of these dual species biofilms, with specific genes such as lasI contributing to removal of S. aureus. In this work, we investigated the S. aureus and P. aeruginosa bacterial localization during colonization of a polystyrene surface, and infection of bronchial epithelial cells.

Methods: To achieve this, we used an in vitro model that better represents the course of infection within the lungs of patients with Cystic Fibrosis. This model consisted of introducing S. aureus and P. aeruginosa under staggered conditions at a ratio of 250:1 (S. aureus:P. aeruginosa), where the latter was introduced only after S. aureus biofilms were established. CFTR bronchial epithelial cells (containing the ΔF508 mutation) were used in parallel with unseeded plates to observe the differences between biofilms formed in the presence and absence of human host cells. We also investigated the impact of quorum sensing (QS) impairment of P. aeruginosa (ΔlasI, ΔrhlI, ΔlasI/rhlI) on the development of dual-species biofilms with S. aureus. Biofilms were imaged via laser scanning confocal microscopy and analyzed using ImageJ.

Results: When dual species biofilms are grown using our staggered culture method there is an increase in total biomass compared to single species biofilms. This increase in biomass is similar to the combined biomass of the individual species when cultured as single species. An impairment of QS in P. aeruginosa resulted in an increase of S. aureus biomass, being greatest when co-cultured with the ΔlasI/rhlI. Biofilm development on CFTR bronchial epithelial cells led to an overall increase of S. aureus independent of QS impairment of P. aeruginosa. Bacterial localization of dual-species biofilms exhibited multiple spatial arrangements including layering, dual species clustering, and separate microcolonies.

Conclusions: Our research further demonstrates that QS and growth surface influence the formation of dual species biofilms, and their spatial localization within a microcolony during biofilm development.
Abstract

**Background:** Emergence and spread of carbapenemase (blaOXA) genes in multi-drug resistant Acinetobacter baumannii (MDR-AB) forming biofilm complicated treatment of the patients infected with this microorganism particularly in intensive care units (ICUs). **Objectives:** The current study aimed to determine the prevalence of molecular class-D OXA carbapenemase in biofilm and non-biofilm forming strains of MDR-AB. **Methods:** A total of 65 strains of MDR-AB were isolated from the patients hospitalized in the ICU of two hospitals in Kerman, Iran. The isolates were identified by conventional microbiological tests as well as API 20NE assay. Antibiotic susceptibility was carried out by disk diffusion method; minimum inhibitory concentration (MIC) of carbapenems was measured by E-test. The presence of blaOXA genes among the isolates were studied by duplex-polymerase chain reaction and application of appropriate primers. Biofilm formation was detected by microtiter plate method. **Results:** The isolates were highly resistant to ciprofloxacin, levofloxacin, piperacillin, nalidixic acid and third generation cephalosporins such as tigecycline (7%; n = 5) and colistin (13%; n = 8). Among the isolates, 77% (n = 50) exhibited high MIC (265 μg/mL) for imipenem. Both the blaOXA-51 and blaOXA-23 like genes coexisted in all the isolates; while, blaOXA-24/40 like gene was only detected in 29 imipenem-resistant strains (P ≤ 0.05). The blaOXA-58 like gene was not detected among the isolated strains. Quantification of biofilm introduced 23 isolates (including blaOXA-24/40 strains) with efficient attachment to microtiter plate; while, those isolates without blaOXA-24/40, or imipenem sensitive strains formed weak or no biofilm. **Conclusions:** Coexistence of the blaOXA-51, blaOXA-23 and blaOXA-24/40 like genes, along with formation of strong biofilm, in MDR-AB strains particularly with indiscriminate use of imipenem, complicated treatment of the patients infected with these bacteria in the hospitals under study.
Inactivation of *Streptococcus mutans* Genes *lytST*, *dltAD* and *gtfB* Impairs its Pathogenicity *In vivo*

**Background:** *Streptococcus mutans* orchestrates the build-up of biofilms that cause dental caries via production of an extracellular matrix rich in exopolysaccharides (EPS). This matrix also contains extracellular DNA (eDNA) and lipoteichoic acids (LTA) that interact with EPS. *S. mutans* can also cause systemic infections upon reaching the bloodstream. EPS is a virulence marker for caries, but the involvement of genes linked to eDNA and LTA metabolism in the pathogenicity of *S. mutans* is unclear. A previous *in vitro* study with parental *S. mutans* UA159 and its strains carrying single deletions to modulate eDNA (Δ*lytS* and Δ*lytT*), LTA (Δ*dltA* and Δ*dltD*) and insoluble EPS (Δ*gtfB*) demonstrated that the deleted genes contributed to increasing the amount of eDNA and LTA in the matrix. eDNA and LTA increased the amount of soluble and insoluble EPS, indicating that these biofilms could be more cariogenic. Therefore, this work evaluated how *lytST*, *dltAD*, and *gtfB* genes affected the development and severity of carious lesions (rodent model of dental caries) and virulence in a systemic infection model (*Galleria mellonella* larvae) to clarify their contribution to the pathogenicity of *S. mutans*. **Methods:** The parental strain UA159 and its deletions strains Δ*lytS*, Δ*dltD*, and Δ*gtfB* were inoculated in SPF Wistar rats, which were fed a cariogenic diet. After 5 weeks, total cultivable microbial and *S. mutans* populations and caries lesions were evaluated. The six strains were injected *intra*-hemocoel *G. mellonella*, and the larvae survival was recorded over time. As the larval defense against infection includes a burst of oxidative stress, biofilms formed by all strains were challenged with H$_2$O$_2$. **Results:** The microbial population recovered from rats showed that the proportion of *S. mutans* in the total microbiota was higher for UA159 versus deletion strains (15-fold for Δ*gtfB*, 3-fold for Δ*lytS*, and 6-fold for Δ*dltD*). For smooth surfaces, UA159 yielded ≥50% more lesions than the deletion strains on enamel; while it caused the highest amount of caries lesions in dentin (≥80% more cavities). For sulcal surfaces faces, UA159 yielded ≥30% more lesions than the deletion strains on enamel; while UA159 also caused a higher amount of caries lesions in dentin (being ≥60% more severe than the deletion strains). The survival of *G. mellonella* was significantly lower in larvae infected with UA159 versus all deletion strains. Also, Δ*gtfB* killed more larvae when compared to the other deletion strains. The higher survival of larvae infected with Δ*lytS*, Δ*lytT*, Δ*dltA*, and Δ*dltD* may be related to the strains lower tolerance to oxidative stress after exposure to H$_2$O$_2$. **Conclusions:** The inactivation of *lytST*, *dltAD* and *gtfB* impaired *S. mutans* cariogenicity and virulence in systemic infection. Hence, strategies to modulate these genes and its products could affect *S. mutans* pathogenicity.
Session Title: MONDAY Poster Session 1
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 059

Abstract Topic: Biofilms and Infection
Abstract Title: Association of *P. gingivalis* and *P. intermedia* Provoke High Levels of Bone Resorption in a Murine Experimental Periodontitis Model

Block: Institute of Biomedical Sciences - University of São Paulo, São Paulo - SP, BRAZIL.

**Background:** Periodontitis is a biofilm-induced oral disease that affects the supporting tissues of the teeth. It has a multifactorial etiology and it is caused by a dysbiotic microbiota where *Porphyromonas gingivalis* is considered a keystone pathogen. We showed before that the presence of *Prevotella intermedia* 17 enhances the biomass of a dual biofilm with *P. gingivalis* ATCC33277. We also have shown that the association with *P. intermedia* benefits the pathogen *P. gingivalis* in the evolving biofilm. The present study aims to investigate the effect of oral infection of the association of *P. gingivalis* with *P. intermedia* on colonization and alveolar bone loss in a murine experimental periodontitis model.

**Methods:** All animal experiments were approved by the institutional animal committee (CEUA-ICB/USP protocol n° 26/2016). In this study, 32 SPF C57BL/6 mice at 5 weeks old were randomly distributed among four groups [Control (C), *P. gingivalis* (PG), *P. intermedia* (PI), and *P. gingivalis* plus *P. intermedia* (PGPI)] (n=8). According to the groups, oral inoculations (1x10^8 cfu of *P. gingivalis* ATCC33277 and/or *P. intermedia* 17) were performed six times in a schedule of two days of inoculation followed by a day of interval, with the aid of a gavage needle. In control group, mice were inoculated with vehicle only. Mice were euthanized at six weeks after the last bacterial inoculation in a CO₂ chamber under anesthesia. Biofilms were collected from the oral cavity and the quantification of *P. gingivalis* or *P. intermedia* was performed by qPCR using species-specific primers for waaA genes. Alveolar bone loss was determined by Microtomography by measuring the distance between the alveolar bone crest and the cemento-enamel junction at three sites of maxillary molars. Sample distributions were checked using ANOVA and nonparametric Kruskal-Wallis test followed by post-tests to determine the statistical significance (Statgraphics, Centurion XVI). A p value of less than 0.05 was considered to be significant.

**Results:** Both strains were able to colonize the oral cavity of mice, according to qPCR. The pattern of colonization was similar for both species and the number of copies of *P. gingivalis* or *P. intermedia* was higher in groups inoculated with the respective pathogens when all the groups were compared. Additionally, no differences were found for *P. gingivalis* in PG and PGPI groups or for *P. intermedia* in PI and PGPI groups. In respect to periodontitis, the alveolar bone loss was higher when the animals were inoculated with the association of *P. gingivalis* and *P. intermedia* than when they were inoculated with only one of the species.

**Conclusion:** The association of *P. gingivalis* and *P. intermedia* in a murine periodontitis model led to a greater loss of alveolar bone, suggesting the role of *P. intermedia* as an accessory pathogen of *P. gingivalis* in a dysbiotic biofilm.

**Acknowledgements:** Fapesp 2013/15977-7; Fapesp 2015/18273-9
**Abstract**

**Rationale:** The development of sensitive imaging techniques for diagnosing and monitoring the treatment of biofilm-associated infections is currently an unmet clinical need. Current clinical imaging modalities are non-specific and fail to differentiate infection from other pathologies such as cancer or sterile inflammation. Here, we report on a modified cell penetrating peptide for its ability to target biofilms of *P. aeruginosa*. **Methods:** The peptide (4Iph)(f)-LP-NSNH-I-KQ-GL (HN17) was synthesized by solid state methods and labeled with FITC. Planktonic PAO1 cells (ATCC type stain) diluted to 1x10^6 CFU/mL were incubated with serial 2-fold dilutions of HN17-FITC ranging from 1 nM to 20 µM. Cell labeling was analyzed by flow cytometry and bacterial killing was assessed using a standard broth microdilution technique. Biofilms of mCherry-expressing PAO1 cells were grown under flow conditions for 24 hr. HN17-FITC (2 µM) or FITC dye was incubated with the biofilms for 1 hr, washed three times, and imaged by confocal laser scanning microscopy (CLSM). Human A549 epithelial cells were infected with mCherry-expressing PAO1 at an MOI of 50:1 for 2 hr followed by HN17-FITC (2 µM) incubation for 1 hr. The infected cells were washed and imaging by CLSM to examine probe distribution. **Results:** HN17-FITC was synthesized at a purity of >90%. HN17-FITC labeled planktonic *P. aeruginosa* cells in a dose-dependent manner and exhibited no killing of *P. aeruginosa* over the concentration range tested (1-20 µM). CLSM analysis of 24 hr flow-grown *P. aeruginosa* biofilms incubated with 2 µM peptide for 1 hr showed that FITC-HN17 labeled biofilm-embedded cells as well as bacterial cells not associated with biofilms. The peptide appeared localized throughout the bacterial cytoplasm in contrast to the membrane-specific dye FM4-64. FITC alone only weakly labeled biofilms. CLSM revealed aggregates of mCherry-expressing PA01 attached to epithelial cells and a HN17-FITC staining pattern that strongly colocalized with these bacterial aggregates. In contrast, the probe only weakly associated with the epithelial cells. **Conclusions:** HN17 is a promising probe for targeting *P. aeruginosa* for potential diagnostic imaging and therapeutic delivery and monitoring. Additional specificity studies are warranted including examining its targeting to biofilms of other bacterial pathogens and in vivo studies in animal models of infection.
**Discovery of Human Bile-mediated Regulation of Curli Fimbriae by In vitro Modeling of Typhoid Chronic Gallbladder Infection**

**Author:** J. F. Gonzalez¹, L. Tucker¹, A. Wetzel², J. Fitch², P. White², J. S. Gunn¹; ¹The Ohio State University, Columbus, OH, ²Nationwide Children's Hospital, Columbus, OH.

**Abstract Body:**

**Background:** Typhoid fever is caused primarily by *Salmonella enterica* serovar Typhi (S. Typhi). Approximately 3-5% of individuals infected with S. Typhi become chronic carriers with the gallbladder (GB) as the site of persistence. These asymptomatic carriers represent the only known reservoir for further spread of disease. We have demonstrated that *Salmonella* can attach and form biofilms (BF) on gallstones (GS) in a mouse model and in humans. Curli, a protein polymer, is a major component of the EPS in *Salmonella* BF. **Methods:** The GB environment was simulated by growing BFs on cholesterol in the presence of human bile (HB) and the transcriptional profile was determined by RNA-seq. Results were validated through RT-PCR, confocal laser scanning microscopy (CLSM) with Congo Red staining, and Western blot. Lux reporter strains were used for expression analysis. Mutants were generated through the Lambda Red method. **Results:** A total of 1063 genes were differentially expressed in the simulated GB environment. The most highly up-regulated genes corresponded to the curli fimbriae operon with the top gene being *csgA* (FC=82.93). This result was validated through RT-PCR (FC=24.38). CLSM showed a significantly higher amount of curli within HB BFs, concentrating mainly in the upper stratum of the BF. Similarly, higher amounts of curli were detected in HB BFs through Western blot. Neither mouse or ox bile mediated enhanced curli expression. Curli genes are arranged in two operons with independent promoters: one containing the structural components CsgA and CsgB (*csgBA*) and the second the important BF regulator CsgD and other structural proteins (*csgDEFG*). Using a Lux reporter strain, we demonstrate that the *csgDEFG* operon is highly expressed in both minimal media (MM) and in HB. The *csgBA* operon is expressed at significant levels throughout BF development only in HB, while levels in MM were significantly lower, peaking at day one and quickly declining. To elucidate the regulatory mechanisms behind these data, selected regulatory gene mutants were examined. A RpoS deletion had no impact on curli expression. Interestingly, a *csgD* mutant showed an elimination of the *csgBA* operon in HB, but not the *csgD(EFG)* operon. The expression of the *csgBA* operon was diminished but not abolished in MM. **Conclusions:** A significant amount of *Salmonella* genes are differentially expressed in a BF in the GB environment. Among these, curli fimbriae appear to be especially important for BF development and maintenance with the transcriptional regulator CsgD playing a key role. Future experiments will be focused on the mechanism of HB-mediated curli regulation.
A Novel Model of Methicillin-Resistant *Staphylococcus aureus* (msra) Foreign Body Osteomyelitis Produced Non-surgically in the Rat

**Author**  
K. E. Greenwood-Quaintance, M. J. Karau, S. M. Schmidt-Malan, R. Patel; Mayo Clinic, Rochester, MN.

**Background:** MRSA implant-associated infections, such as prosthetic joint infection (PJI), are difficult to treat. Rat foreign body MRSA osteomyelitis models have been commonly used as treatment models for new antimicrobial agents. Traditional models have been established by surgically exposing the bone, drilling a hole to the medullary cavity to infect the site and place a foreign body, filling the resultant hole, and closing the fascia and skin with sutures. The surgery is both time and cost consuming. Here, our aim was to develop an equivalent model, using a non-surgical approach, to produce MRSA foreign body osteomyelitis in the rat.

**Methods:** Twenty-five rats were anesthetized with ketamine/xylazine/acepromazine, and the left knee joint shaved and prepped with chlorhexidine scrub. The knee joint was bent to a 45 degree angle to expose the top of the tibial process. A 21 gauge, 1 inch needle connected to a syringe was placed into the medullary cavity of the tibia and a combination of 10 µl of 50 µg/ml arachidonic acid (sclerosing agent) and 50 µl of a 10⁸ colony forming unit (cfu)/ml suspension of MRSA (IDRL-6169, a PJI-associated clinical isolate) were injected. After disconnecting the syringe, the foreign body - a 14 mm 316L stainless steel surgical suture wire (Ethicon, Cincinnati, OH) - was placed into the needle with sterile forceps and pushed through the needle into the medullary cavity using a 1.5 inch 27 gauge needle, while the original needle was being gradually pulled outward. The needles were then removed, the leg straightened, and pressure placed on the knee joint with sterile gauze. After 1, 2, 3, 4, and 7 weeks, 5 rats each were euthanized and the tibiae removed and frozen. In addition, another group of 5 rats underwent a traditional surgical model using the same sclerosing agent, isolate, and inoculum; after 7 weeks, these rats were euthanized and the tibiae removed and frozen. Bones were cryopulverized and wires and bones separated for quantitative cultures. Recovered MRSA was enumerated and reported as log₁₀ cfu/g of bone or log₁₀ cfu/wire. **Results:** The mean quantities of MRSA in bone at 1, 2, 3, 4, and 7 weeks were 5.82, 5.48, 5.22, 5.97, and 4.57 log₁₀ cfu/g bone, respectively. The mean quantities of MRSA on the wire at 1, 2, 3, 4, and 7 weeks were 4.00, 2.59, 1.80, 0.58, and 0.99 log₁₀ cfu/wire, respectively. At 7 weeks, MRSA quantities in bone and wire were similar to those found using the traditional surgical rat model, which yielded a mean log₁₀ cfu/g bone of 5.28 and mean log₁₀ cfu/wire of 1.92. **Conclusions:** The novel non-surgical rat model of MRSA foreign body osteomyelitis is a promising model with comparable MRSA quantities as a traditional surgical rat model. The described novel model involves considerably less time and cost than the surgical model.

Abstract Body:

**Background:** Mycobacterium chimaera is a slow-growing nontuberculous mycobacterial (NTM) species that is widespread in the environment. It has been recently identified as the causative agent of the current global outbreak of NTM infections in patients that underwent cardiac surgeries. Aerosolized M. chimaera from contaminated heater cooler devices (HCDs) used during cardiac surgeries to regulate patient body temperature, has been implicated in these postoperative infections. Failure to eradicate M. chimaera from HCDs, suggest an urgent need for more efficient disinfection strategies. Since M. chimaera can reemerge after an intense disinfection protocol, the hypothesis is to characterize M. chimaera growth and biofilm formation on medical devices. This study will characterize M. chimaera biofilms on various medical device materials under stationary and continuous flow conditions and will aid in the development of effective HCDs decontamination guidelines.

**Methods:** Standardized M. chimaera strain (DSM 44623) is inoculated to an OD$_{595}$ of 0.05 into polystyrene plates, titanium and stainless-steel coupons and is grown for 6 weeks at 30°C under stationary conditions. Any growth will be quantified using crystal violet staining. M. chimaera biofilm formation under low fluid shear condition resembling air-liquid interface will be studied using Drip flow reactors (DFR). Biofilm on metal coupons will be established by operating the DFR under the batch mode for a week and any biofilms identified will be further incubated for 6 weeks with the continuous flow of sterile tap water. Every week, coupon associated biofilm formation will be quantified by direct plate count and expressed as CFU/mm$^2$. 3D-Laser scanning Confocal microscope (CLSM) and Scanning Electron Microscope (SEM) will be used to study the development, thickness and surface morphology of these biofilms.

**Results:** M. chimaera forms robust biofilms on various medical device materials. Biofilms grown in DFR, exhibit differences in its attachment and development when compared to biofilms grown under stationary condition. CLSM studies show that these biofilms attach and develop rapidly on both titanium and stainless-steel surfaces within a week of incubation. SEM reveals that these cells adhere to polystyrene as early as a week of incubation and from week 3 onwards, these cells are completely enclosed in a secretion of an EPS.

**Conclusions:** This study provides important insights into both early and later stages of M. chimaera biofilm formation on various medical device surfaces. These results demonstrate that M. chimaera cells adhere to polystyrene surfaces within a week of incubation. Further, DFR studies suggest that the method of growth will affect M. chimaera biofilm forming properties. Findings from this study will potentially aid to eradicate biofilm formation on medical device materials.
TolB, the Periplasmic Protein that Controls Flagellar Biosynthesis and the Biofilm Formation in Uropathogenic *Escherichia coli*

**Background:** Uropathogenic *Escherichia coli* (UPEC) adheres to and invades host cells in the bladder, then this bacterium forms biofilm-like polymicrobial structures termed intracellular bacterial communities (IBCs) that protect UPEC from antimicrobial agents and the host immune systems. We looked for genes that contribute to IBCs formation using transposon mutagenesis method. Recently, we found that the *tolB* mutant shows defective IBCs phenotype. Here, we studied on the mechanism how the *tolB* gene product contributes to IBCs formation.

**Methods:** Internalization and adhesion of bacteria to bladder epithelial cells were evaluated by counting the bacterial cell numbers in gentamicin assay. IBCs of UPEC carrying a GFP expression plasmid were observed on confocal microscopy. Flagella were stained with Victoria Blue and tannic acid. Bacterial motility was evaluated on a soft agar. Fimbriae activity and expression were evaluated by hemagglutination assays and quantitative PCR analyses, respectively.

**Results:** A number of the *tolB* mutant cells internalized into bladder epithelial cells was 5-fold less than that of the wild-type cells, and IBCs in the mutant were smaller than those in the wild-type. We observed no significant difference in type I fimbriae production and activity between both strains. However, the *tolB* mutant produced defective flagella, then motility of the mutant was less than that of the wild-type while there was no difference in expression of *fliC* and *flhD* which encode a flagellin protein and its regulatory protein, respectively. We also found that deletion of *fliC* or *motA* which encodes a motor protein for flagellar rotation decreases internalization of bacteria and IBCs formation into bladder epithelial cells. However, rates of bacteria adhered to bladder epithelial cells in *fliC*, *motA* and *tolB* mutants were similar to the wild-type.

**Conclusions:** These results indicate that motility of UPEC contributes to internalization and IBCs formation into bladder epithelial cells without affecting adhesion efficiency. Decreased IBCs formation and internalization in the *tolB* mutant is due to defect of flagellar assembly, therefore the mutant loses its motility. Our results also suggest that TolB and flagellum could be potential drug targets for treating IBCs infection caused by UPEC.
Function of Staphylococcus aureus Biofilm - Studied In vitro, in Guinea Pigs and in a Patient

T. R. Thomsen¹, L. Larsen², Y. Xu¹, H. Schønheyder³;
¹Aalborg University and Danish Technological Institute, Aalborg, DENMARK, ²Aalborg University, Aalborg, DENMARK, ³Aalborg University Hospital, Aalborg, DENMARK.

**Background:** Staphylococcus aureus is a major cause of community- and hospital-acquired infections worldwide. S. aureus has a remarkable ability to adapt to a biofilm mode of growth in response to the host environment, and this is crucial for its leading role in device-related infections. The staphylococcal transcriptome was studied in vivo and the joint fluid metabolome in a prosthetic joint infection using deep RNA sequencing and nuclear magnetic resonance spectroscopy, respectively. We compared our findings with the genome, transcriptome and metabolome of the S. aureus joint fluid isolate grown in vitro and in a guinea pig infection model. **Result:** From the transcriptome analysis we found increased expression of siderophore synthesis genes and multiple known virulence genes in vivo. The regulatory pattern of catabolic pathway genes indicated that the bacterial infection in vivo was sustained on amino acids, glycans and nucleosides. Upregulation of fermentation genes and the presence of ethanol in joint fluid indicated severe oxygen limitation in vivo. The gene expression profiles showed adaptation to the hypoxic and acidic environment during infection development in the guinea pig infection model. **Conclusion:** Understanding the function and pathogenesis of bacteria in vivo, both in mono- and multiple species biofilms is an important next step for optimized diagnosis and treatment.
Disruption of Glutathione Metabolism Alters Virulence Phenotypes of *Pseudomonas aeruginosa*

**Background:** *Pseudomonas aeruginosa* causes significant human infection and is often antibiotic resistant. Glutathione (GSH) removes reactive oxygen species (ROS) and NO, compounds generated by the host immune system, by acting as an electron acceptor. GSH is also important for maintaining the redox status inside the cell. Our lab previously demonstrated that a *P. aeruginosa* mutant (*gshA*) which completely lacks GSH is defective for biofilm formation, pyocyanin production, and swarming motility, and is more sensitive to antibiotics and oxidative stressors. In this study, *P. aeruginosa* mutants involved in GSH cycling (glutathione reductase; *gor*) and processing (glutathione S-transferase; *gst*) were evaluated for the aforementioned virulence phenotypes, their competitive ability with *Staphylococcus aureus*, and virulence in the *Galleria mellonella* infection model.

**Methods:** Biomass production was determined using a crystal violet assay. Chloroform:HCl extraction followed by spectrophotometry was used to quantify pyocyanin production. Motility was assayed by spot plating on semisolid agar and allowing cells to migrate from the point of inoculation for 24 hours. Oxidant sensitivity was determined using disk-diffusion assays. For competition assays, overnight cultures were diluted 1:20 in fresh media and grown to mid-log phase. Strains were mixed at equivalent cell numbers in fresh media and grown for 24 hours before plating. Virulence assays were performed using the *G. mellonella* model. All data were analyzed using a one-way ANOVA followed by posthoc comparisons using the Tukey test.

**Results:** The *gor* and *gst* mutants produce significantly more robust biofilms than wild-type, contrasting the phenotype of the *gshA* mutant. The *gor* and *gst* mutants also swarmed farther when compared to wild-type, again contrasting the *gshA* mutant. In terms of pyocyanin production, we found that all strains were significantly defective when compared to wild type. *gor* and *gshA* transposon mutants were significantly more sensitive to oxidative stressors paraquat, diamide, cumene hydroperoxide, and HO2 when compared to wild type. Interestingly, when the *gshA* and *gst* transposon mutants were grown in coculture with *S. aureus*, they were able to compete with *S. aureus* better than the wild-type strain did. Finally, we found that the *gshA* transposon mutant was significantly less virulent in the *G. mellonella* infection model than wild-type.

**Conclusions:** Disruption of GSH metabolism in *P. aeruginosa* contributes to altered biofilm formation and virulence phenotypes. Continued study of GSH and its contribution to virulence in *P. aeruginosa* has the potential for development of alternative antimicrobial targets to improve outcomes in antibiotic resistant infections.
Vibrio Cholerae Combines Individual and Collective Sensing to Trigger Biofilm Dispersal

Bacteria can generate benefits for themselves and their kin by living in multicellular, matrix-enclosed communities, termed biofilms. The advantages of the biofilm mode of life include increased stress resistance and access to concentrated nutrient sources. However, there are also costs associated with biofilm growth, including the metabolic burden of biofilm matrix production, increased resource competition, and limited mobility inside the community. The decision-making strategies used by bacteria to weigh the costs between remaining in a biofilm or actively dispersing are largely unclear, even though the dispersal transition is a central aspect of the biofilm life cycle and critical for infection transmission. Using a combination of genetic and novel single-cell imaging approaches, we show that Vibrio cholerae integrates dual sensory inputs to control the dispersal response: cells use the general stress response, which can be induced via starvation, and they also integrate information about the local cell density and molecular transport conditions in the environment via the quorum sensing apparatus. By combining information from individual (stress response) and collective (quorum sensing) avenues of sensory input, biofilm-dwelling bacteria can make robust decisions to disperse from large biofilms under distress, while preventing premature dispersal when biofilm populations are small. These insights into triggers and regulators of biofilm dispersal are a key step towards actively inducing biofilm dispersal for technological and medical applications, and for environmental control of biofilm populations.
Pseudomonas aeruginosa forms multicellular aggregates or biofilms using both exopolysaccharides (EPS) and the matrix protein CdrA. We showed for the first time that *P. aeruginosa* can use CdrA to build biofilms that do not require known matrix EPS. We found that CdrA is able to self-interact, which likely permits the formation of such aggregates. This represents a novel function for CdrA. However, we found that these CdrA-containing, EPS-devoid matrices were susceptible to both exogenous and self-produced proteases. Possession of such a proteolytically sensitive matrix could be detrimental to biofilm aggregate stability as *P. aeruginosa* produces its own slew of extracellular proteases and also is found in environments that are rich in exogenous proteases. Interestingly, we found that the *P. aeruginosa* EPS Psl protects CdrA from proteolytic cleavage. Additionally, we determined that the self-produced protease elastase (LasB) degrades unprotected CdrA. We are now investigating the possibility that LasB may provide a non-specific mechanism for modulating bacterial aggregate growth and disassembly. For example, under unfavorable biofilm conditions, the interaction between CdrA and Psl may be destabilized, permitting LasB to cleave CdrA and further promote disaggregation. However, when CdrA and Psl interact, the bacteria are prevented from digesting their own matrix while still being able to produce proteases that are important for virulence and/or survival. In this way, being able to form a biofilm matrix with unique compositions as well as the ability to adapt in response to external changes may improve bacterial survival. Thus, we envision that Psl-CdrA interactions can contribute to biofilm integrity and suggest an advantage for utilizing both proteins and EPS in the matrix. The results led us to propose a model in which CdrA-CdrA interactions can enhance cell-cell packing in an aggregate that is resistant to physical shear, while Psl-CdrA interactions enhance aggregate integrity in the presence of self-produced and exogenous proteases.
Attachment is a vital step for microbes to establish associations with both biotic and abiotic surfaces. Stable attachment of *Caulobacter* cells to surfaces requires an adhesive holdfast comprised of both protein and polysaccharide localized at the tip of the polar stalk, but the exact composition of the holdfast is unknown. While many bacteria produce extracellular polysaccharide adhesins, the anchoring mechanisms of these polysaccharides are not well understood or in most cases completely uncharacterized. The holdfast is anchored to the pole of the bacterial cell or stalk via the holdfast anchor complex, which is composed of HfaA, HfaB and HfaD. Loss of any of the anchor proteins results in holdfast-shedding and both decreased adherence and biofilm formation. Our current work is focused on determining the structure of the anchor complex and the role each Hfa protein plays within this complex. The stalk of *C. crescentus* is 120 nm in diameter and ideal for electron cryotomography. Initial examination of the stalk tip, identified an electron-dense area associated with the periplasmic side of the outer membrane. To determine if this structure was the holdfast anchor, we used a combination of electron cryotomography, light and fluorescence microscopy and genetics. To increase the number of stalk tips available for imaging, we used a *C. crescentus* strain that sheds stalks to purify and concentrate stalks. We examined a variety of holdfast biosynthesis, secretion and anchor mutant to pinpoint which proteins were part of this structure. Holdfast biosynthesis and secretion is primarily encoded by two main loci *hfs*EFGH and *hfs*DABC. A glycosyl transferase mutant, ∆*hfsG*, did not make holdfast but still maintained localized HfaBmCherry and the electron dense stalk-tip structure. A holdfast secretion mutant, ∆*hfsDAB*, has previously been shown to result in delocalization of the holdfast anchor, which was confirmed using HfaBmCherry. The *hfsDAB* mutant produced neither holdfast nor the electron dense structure. In addition, an anchor mutant, ∆*hfaB*, in which the holdfast secretion protein HfsD is still localized to the stalk tip, lost the electron dense structure and sheds holdfast. Finally, the electron dense structure was still present in a ∆*hfaA* or ∆*hfaD* single or double mutant, in which HfaBmCherry still localizes to the stalk tip. We hypothesize that the electron dense structure is primarily a complex of HfaB or HfaB and the holdfast secretion complex together.
The bacterium, *Pantoea stewartii* subsp. *stewartii* (*Pnss*), is the causal agent of Stewart’s wilt in sweet corn. This disease is characterized by water-soaked lesion formation, and wilting of seedling leaves due to exopolysaccharide (EPS)-mediated biofilm formation in the water conducting (xylem) tissue of the plant. The water-soaked lesions contain significant reactive oxygen species (ROS), including H$_2$O$_2$ and superoxides. The transcription factor OxyR is essential in regulating protection against H$_2$O$_2$ and, in addition, EPS production is regulated by OxyR in *Pnss*. Interestingly, we discovered a conserved binding site for OxyR upstream of an operon containing genes encoding components of the Regulator of Capsular Synthesis (Res) Phosphorelay pathway, a well-known regulator of EPS, along with two genes encoding RTX homologs. RTX proteins can have cytotoxic effects on target host cells and the *Pnss* RTX2 is required for water-soaked lesion formation in planta. However, *Δrtx2* produces less EPS, indicating a possible link between activation of the Res pathway, RTX2 and sensing of ROS via OxyR. RTX2 is a large (249.8 kDa) protein containing 5 putative Ca$^{2+}$-binding domains similar to serralin, from *Serratia marcescens* and a hemolysin-like protein from *Desulfovibrio vulgaris*. It also has 5 predicted transmembrane domains in the C-terminus, and large repetitive adhesin motifs homologous to hemagglutinins and to the BAP subfamily of RTX proteins implicated in promoting bacterial-host adhesion during biofilm formation. *Δrtx2* is deficient in adhesion to surfaces. Therefore, in addition to modulating water soaked lesion formation, we hypothesize that RTX2 influences the physiochemical properties of the cell envelope that relate to surface to adhesion and is linked to the transition between the apoplastic and xylem phases of Stewart wilt.
Grapping Hooks Involved in Biofilm Development

The Nontypeable *Haemophilus influenzae* Phasevarion Influences Adherence to Human Airway Epithelial Cells: The First Step in Biofilm Formation

**Abstract**

**Background:** Nontypeable *Haemophilus influenzae* (NTHi) asymptomatically colonizes the human nasopharynx. However, prior or simultaneous upper respiratory tract viral infection permits NTHi to exploit modified expression of host cell receptors, ascend the Eustachian tube, and access the middle ear to induce otitis media (OM). NTHi has a novel genetic system, termed the phasevarion (phase variable regulon). The NTHi phasevarion operates as a reversible ON/OFF switch that regulates expression of multiple genes simultaneously. The phasevarion regulator, ModA, a DNA methyltransferase, methylates a specific sequence on the chromosome; however, variability in the *modA* allele determines the methylation target and therefore the genes expressed. A majority of OM isolates contain one of five *modA* alleles (*modA*2, 4, 5, 9, and 10). In NTHi the phasevarion controls pathogenesis, aids in immunoevasion, and contributes to antimicrobial resistance; however, its role in adherence and in the formation of mucosal biofilms has not yet been fully elucidated. **Methods:** We assayed NTHi strains: 723 (*modA*2), C486 (*modA*4), 477 (*modA*5), and 1209 (*modA*9) locked ON and locked OFF variants for relative adherence to polarized human airway epithelial cells (HAEs) after 1, 3 or 6 hr. Locked strains contain *modA* alleles that cannot phase vary. Relative percent adherence was calculated based on the ratio of CFU adherent NTHi to the inoculum. If ModA strain variants exhibited significance in adherence at earlier time points, we examined those locked ON and OFF variants after 24 hr incubation on HAE cells via confocal microscopy for mucosal biofilms. **Results:** Whereas there was no difference in adherence between locked ON or OFF variants of NTHi 723 (*modA*2) or C486 (*modA*4) after 3 hr, there was significance between locked ON and OFF variants for strain 477 (*modA*5). Strain 477 (*modA*5) was also significantly different between locked ON and OFF variants after 6 hr incubation. **Conclusions:** This work suggested that expression of one or more NTHi adhesive proteins was regulated by the phasevarion. An understanding of NTHi virulence determinant expression, including adhesive proteins as suggested herein, as an outcome of phasevarion status can guide and confirm targets for vaccine development against respiratory tract diseases due to NTHi. Support: NIH/NIDCD R01DC015688
Host suppression of Quorum Sensing During Catheter Associated Urinary Tract Infections

S. J. Cole¹, C. L. Hall¹, M. Schniederbernd², J. M. Farrow III³, J. R. Goodson¹, E. C. Pesci³, B. I. Kazmierczak³, V. T. Lee¹; ¹University of Maryland College Park, College Park, MD, ²Yale University School of Medicine, New Haven, CT, ³The Brody School of Medicine at East Carolina University, Greenville, NC.

Chronic bacterial infections on medical devices, including catheter-associated urinary tract infections (CAUTI), are associated with bacterial biofilm communities that are refractory to antibiotic therapy and resistant to host immunity. Previously, we have shown that Pseudomonas aeruginosa can cause CAUTI by forming a device-associated biofilm that is independent of known biofilm exopolysaccharides. Here, we show by RNA-seq that host urine alters the transcriptome of P. aeruginosa by suppressing quorum sensing regulated genes. P. aeruginosa produces acyl homoserine lactones (AHLs) in the presence of urea, but cannot perceive AHLs. Urea inhibits perception by preventing the uptake of AHL, suggesting that P. aeruginosa has a pathway to import these quorum sensing molecules into the bacterial cytoplasm. Quorum sensing-regulated processes in clinical CAUTI isolates are also inhibited by urea. These data show that urea in urine is a natural anti-quorum sensing mechanism in mammals.
Chronic infections are often biofilm-associated and can contain multiple species of microorganisms. These pathogenic microorganisms, which include bacteria, fungi, viruses, or combinations thereof, are often well-adapted in the host environment due to evolutionary selection of phenotypes which counteract the host immune system and alter pathogenicity of the microorganism. Interestingly some of these host-induced adaptations involve decreased antimicrobial strategies that typically enable microbes to outcompete other organisms in the environment. For example, *Pseudomonas aeruginosa* isolates from cystic fibrosis patients often exhibit impaired pyocyanin production. Recent studies have shown bi-directional cross-feeding networks leading to metabolic interdependencies and hence decreased antimicrobial strategies among otherwise competing microorganisms. They also exhibit increased virulence against the host and better resistance from host immune cells and antibiotic insults. In that direction, our lab is trying to understand putative cooperative interactions between microbes mediated by an anti-oxidant pigment, pyomelanin. More specifically we are interested in exploring possible cooperation between cystic-fibrosis lung infection-derived clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We hypothesize that pyomelanin, which is a secreted anti-oxidant polymer, can be used as a community resource against reactive oxygen species (ROS) by microbes responsible for chronic infection in lungs of cystic fibrosis patients. Using both pyomelanin-producing cystic fibrosis isolates as well as lab-generated pyomelanin producing *Pseudomonas aeruginosa* strains, we have sought to recapitulate pyomelanin-mediated protection against oxidative stress in both mono- and co-culture with *Staphylococcus aureus*. Interestingly, we have observed that the presence of *Staphylococcus aureus* can induce increased pyomelanin production, which supports the hypothesis that this secreted molecule is involved in interspecies interactions.
Background: Biofilms play a critical role in the colonization, persistence and pathogenesis of many human pathogens. Biofilms are substantially more resistant to host defenses compared to planktonic counterparts, and can alter immune cell recognition and response. Multiple mucosal-associated pathogens have evolved a mechanism of rapid adaptation, termed the phasevarion (phase-variable regulon). Regulation by the phasevarion occurs via phase variation of a DNA methyltransferase, Mod, which leads to the coordinated regulation of numerous genes throughout the bacterial genome via epigenetic mechanisms. The phasevarion of nontypeable *Haemophilus influenzae* (NTHI) significantly affects the severity of experimental otitis media and regulates several disease-related processes, which include in vivo biofilm formation. Methods: The role of the NTHI phasevarion on mucosal biofilm formation and immune response was assessed using a chinchilla model of otitis media. Chinchillas were challenged with NTHI strain 723 populations that either expressed ModA2 (ON) or did not express ModA2 (OFF), and were unable to phase vary or change status. Biofilm formation and immune cell infiltration within the middle ear were assessed at 2, 5 and 14 days after challenge. Flow cytometry, transcriptomic analysis and immunohistochemistry were performed to define the composition and distribution of cells within the middle ear mucosal biofilms and fluids. Results: ModA2 status, and subsequent changes in bacterial gene expression, significantly impacted immune responses. The expression of pro-inflammatory cytokines was greater in immune cells from ears challenged with the ModA2 OFF population compared to those challenged with the ModA2 ON population. In contrast, challenge with the ModA2 ON population produced significantly larger mucosal biofilms, which correlated with greater neutrophil infiltration and increased expression of neutrophil elastase and myeloperoxidase, indicators of neutrophil extracellular trap (NET) production. Conclusions: These results identify a role for the phasevarion in the regulation of in vivo biofilm formation. Furthermore, mucosal biofilm formation appears to be tightly linked to innate immune responses directed towards each specific NTHI subpopulation. Biofilm formation increases bacterial recalcitrance to host defense mechanisms, but our results suggested that biofilm composition may also direct host immune responses. Regulation by the phasevarion impacts not only bacterial phenotypes, such as biofilm formation, but also appears to influence host responses. As such, it is critical to understand the implication of this bacterial regulatory mechanism on all aspects of host-microbe interactions, and to define how the interplay between bacterial and host regulation contribute to the chronicity of biofilm diseases. Support: NIH/NIDCD R21DC016709 (KLB)
**Background:** *Pseudomonas aeruginosa* is a leading cause of life-threatening nosocomial infections. Many virulence factors produced by *P. aeruginosa* including biofilm formation are controlled by the cell-to-cell communication process called quorum sensing (QS). QS depends on the synthesis, release, and group-wide response to extracellular signaling molecules called autoinducers. *P. aeruginosa* possesses two canonical LuxI/R-type QS systems: LasI/R and RhlI/R that produce and detect 3OC12-homoserine lactone and C4-homoserine lactone, respectively. Typically, mutations in QS luxI-type (i.e., lasI, rhlI) and partner luxR-type (i.e., lasR, rhlR) genes confer identical phenotypes because each component of the pair needs the other to function. Previously, we discovered that RhlR directs both RhlI-dependent and RhlI-independent regulons. We found that the Δ*rhlR* and Δ*rhlI* mutants have radically different biofilm phenotypes whereas the Δ*rhlI* mutant displays full virulence in animals whereas the Δ*rhlR* mutant is attenuated. The Δ*rhlI* mutant cell-free culture fluids contain an activity that stimulates RhlR-dependent gene expression. We proposed a model in which RhlR responds to an alternative ligand, in addition to its canonical autoinducer.

**Methods:** We used Tn5 random-mutagenesis screen, biofilm analyses, reporter assays, genetics and biochemistry, and animal infection studies in this project. **Results:** In this study, we discover that the *pqsE* gene is required for the biosynthesis of the alternative ligand that activates the QS receptor RhlR in the absence of its canonical autoinducer C4-HSL. *PqsE* is a thioesterase and residues in the active site are required for alternative ligand synthesis. The *PqsE*-produced ligand drives RhlR-dependent virulence gene expression in animal models of infection, and indeed, *PqsE* is required and RhlI is dispensable for virulence. We show that the *PqsE*-derived alternative ligand is sufficient to activate RhlR as a transcription factor. Furthermore, we identify residues in the ligand binding domain of RhlR that are required for its response to the alternative ligand. **Conclusions:** We conclude that the enzyme *PqsE* is required for the synthesis of the alternative ligand that stimulates RhlR and promotes virulence gene expression and biofilm development. Thus, *PqsE* can be targeted for therapeutic intervention. Furthermore, *PqsE* and RhlR function as an autoinducer synthase-receptor pair that activates the expression of genes specifying group behaviors in *P. aeruginosa*. We speculate that the ability of RhlR to detect multiple signals endows *P. aeruginosa* with the plasticity to diversify its QS outputs, while also being especially economical because it does not necessitate the evolution of a new transcription factor for every small molecule stimulus that is detected.
Anthranilate Acts as a Threshold for \textit{Pseudomonas aeruginosa} to Form Biofilm

\textbf{Background:} \textit{Pseudomonas aeruginosa}, an opportunistic human pathogen is of great interest because of their remarkable metabolic versatility and ability to colonize a variety of habitats. \textit{P. aeruginosa} produces various metabolites, such as phenazines, pyocyanin, quinolones, acyl-homoserine lactones, anthranilate, and so on, and most of them are secreted and accumulated during growth. One of them, anthranilate is an important intermediate for the synthesis of tryptophan and \textit{Pseudomonas} quinolone signal (PQS), and metabolized by anthranilate dioxygenase complex (\textit{antABC} gene products) via TCA cycle. Recently, anthranilate has been reported to be an inducer of the \textit{antABC} expression and cause biofilm dispersal in various bacteria by deteriorating the biofilm structure.

\textbf{Methods:} We measured the production and accumulation levels of anthranilate during long-term culture of \textit{P. aeruginosa}. We also measured the expression level of \textit{antABC} that encodes the anthranilate-degrading enzymes throughout the growth of \textit{P. aeruginosa}. To figure out the relation between the anthranilate and biofilm formation, we traced how the level of anthranilate changes as \textit{P. aeruginosa} grows and at what point the biofilm forms. \textbf{Results:} The production and secretion of anthranilate remain very low until \textit{P. aeruginosa} reaches stationary phase, but it begins to secrete at stationary phase and accumulate to a high level at late stationary phase. Interestingly, the level of anthranilate rapidly decreased again when the stationary phase persisted longer. This dramatic change in anthranilate level was driven by \textit{antABC} function, because the high-level accumulation of anthranilate lasted in \textit{antABC} mutant. The biofilm analyses in static- and flow cell-systems demonstrated that the accumulation of anthranilate destabilized the biofilm structure and the biofilm began to form only after the anthranilate level was lowered by \textit{antABC}. \textbf{Conclusions:} In \textit{P. aeruginosa}, the biofilm begins to form after the removal of anthranilate that was accumulated at stationary phase, and thus the anthranilate peak appearing at the stationary phase is a hurdle that \textit{P. aeruginosa} must surpass to form biofilm.
Bacteria must constantly assess their internal conditions and external environment and change their behavior accordingly. The use of nucleotides as second messenger signals is one strategy bacteria use to accomplish this end. Signaling via ribonucleotides such as c-di-GMP, ppGpp, and cAMP is ubiquitous in bacteria and contributes to diverse phenotypes including biofilm formation. However, other promising ribonucleotide signals remain unexplored. One such promising nucleotide is adenosine 3', 5'-bisphosphate (pAp), which is a ubiquitous by-product of sulfur assimilation and acetyl-coA utilization during lipid biosynthesis. Excess pAp has been shown to be cytotoxic in *E. coli*. Since the maintenance of proper amounts of messenger ribonucleotides is important for cell function, its toxicity suggests that pAp may have a potential signaling role. The aim of this study is to determine how excess pAp signals for cell death. We hypothesize that pAp interacts with proteins to signal for cell death. Using a differential radial capillary action of ligand assay (DRaCALA), 3,866 proteins were assessed for pAp binding. Several proteins were identified, including CysH, HisI, UshA, DeoB, and MreB. Future studies will determine whether these protein targets mediate pAp signaling for cell death.
Session Title: MONDAY Poster Session 1
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 083
Abstract Topic: Regulation of Biofilm Development
Abstract Title: Effect of Nano-Silver, Nano-Copper, Deconex Andbenzalkonium Chloride on Biofilm Formation Andexpression of Transcription Regulatory Quorumsensing Gene (Rh1R) In Drug-Resistance Pseudomonas aeruginosa Burn Isolates
Author Block: M. - Shakibaie; Kerman University of Medical Sciences, Kerman, IRAN, ISLAMIC REPUBLIC OF.

Background: Biofilm forming drug-resistant Pseudomonas aeruginosa are responsible for major death in burn center of different hospitals across the globe. Objective: The aims of this study were to evaluate the effect of nano-silver (Ag), nano-copper (Cu), and two hospital disinfectants deconex and benzalkonium chloride on biofilm formation and expression of transcription regulatory quorum sensing gene rh1R in P. aeruginosa burn isolates. Methods: 28 multidrug-resistant P. aeruginosa (MDRPA) strains were isolated from patients hospitalized in the burn center of a referral hospital in Kerman, Iran. Sizes and purities of nanoparticles were checked by TEM and X-ray diffraction (XRD) analysis. The Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of the nanoparticles (NPs), deconex and benzalkonium chloride were determined by broth microdilution method. Antibiofilm activities of these compounds were measured by microtiter assay. Polymerase chain reaction (PCR) was used for detection of qacED1, cepA, copA and rhlR genes. Quantification of rhlR gene expression in presence and absence of the above compounds was carried out by relative quantitative real-time PCR (qRT-PCR).

Results: Benzalkonium chloride had a potent antimicrobial activity and inhibited growth of all the isolates at MIC 0.06 mg/mL, while nano-Ag was effective at MIC 20 mg/mL. Furthermore, 28.5% of the isolates showed strong, 25% moderate, 14% weak and 32% demonstrated no biofilm activity. Ag NPs exerted highest antibiofilm activity, follow by deconex and benzalkonium chloride. The qacED1 was absent in this study, whereas 17.8% and 60.8% of the isolates were positive for cepA and copA genes. Benzalkonium chloride, Ag NPs and deconex increased the expression of rhlR gene 64, 2 and 7 folds, respectively. Conclusion: Our results suggest that, there is direct relationship between decrease in antibiofilm activity and increase in expression of the rhlR gene in the presence of benzalkonium chloride. Absence of qacED1 gene may be contributed in sensitivity of the isolates to the above agents.
Regulation of Biofilm Development

Enhanced Control of Plant Wilt Disease by a Xylose-Inducible DegQ Gene Engineered into B. Velezensis Strain SQR9XYQ

Z. Xu, J. Xie, H. Zhang, D. Wang, Q. Shen, R. Zhang; Nanjing Agriculture University, Nanjing, CHINA.

*Bacillus velezensis* SQR9 (former *B. amyloliquefaciens* SQR9) is a plant-growth-promoting rhizobacterium (PGPR) that promotes plant growth and health. The colonization of PGPR strains along plant roots is prerequisite for them to execute their specific functions. However, one problem of microbial introduction in practice is that the applied PGPR strains do not always successfully colonize the rhizosphere. In *Bacillus* spp., two-component signal transduction system (TCS) DegS/U regulates flagellar motility, biofilm formation and antibiotic production. Phosphorylation of DegU by DegS is positively affected by DegQ protein. In this study, we constructed a xylose-inducible *degQ* genetically engineered strain SQR9XYQ to improve the biocontrol activity. The results from *in vitro*, *root in situ*, greenhouse experiments and RT-qPCR studies demonstrate that (i) the phosphorylation of DegU in SQR9XYQ can be gradually activated by xylose, which is a component of both cucumber and tomato root exudates, and (ii) biofilm formation, antibiotic expression, colonization activity and biocontrol efficiency were improved in SQR9XYQ compared to the wild-type strain SQR9. These results suggest that colonization trait is important for biocontrol strains to maintenance of plant health.
Recent studies have shown that PA3133 (sawR), a probable transcription factor of Pseudomonas aeruginosa, is over-expressed in response to Surface Acoustic Waves (SAW). SAW is known to disrupt P. aeruginosa biofilm formation and antibiotic resistance. We attempt to uncover the regulatory role of sawR in the bioacoustic response of P. aeruginosa. A flow cell system was utilized to demonstrate that the biofilm of the sawR overexpressing strain is highly sensitive to antibiotic treatment and forms less biomass when compared to the wild type (WT) strain, mirroring the effects of SAW exposure on the WT. To examine the genetic effects of sawR, gene expression levels of the sawR overexpressing strain were compared to that of the WT using a microarray. sawR showed a significant impact on gene expression pattern, where several virulence-associated genes were down-regulated in the sawR overexpressing strain, while specific metabolic genes were up-regulated. hmgA is a metabolic gene that is down-regulated in the sawR overexpressing strain. It is known that in the absence of hmgA, strains hyper-produce a brown pigment called pyomelanin, which is also produced when sawR is overexpressed. The decreased expression of multiple virulence factors in the sawR overexpressing strain led us to examine whether sawR, when overexpressed, can reduce virulence. We used a HeLa cytotoxicity assay in which cytotoxic ability of the sawR overexpressing strain was compared to the WT and found that it decreased by approximately 50%. Our data suggests that sawR plays a central role in mediating the response to SAW and key phenotypes such as biofilm formation, antibiotic resistance and pigment production.
The soil bacterium *Bacillus subtilis* is a model for biofilm studies due to its robust colony phenotype and tractable genetics. Interestingly, while matrix production is essential for biofilms, only a fraction of cells in the population actively produce it. Other cells differentiate into diverse fates based on unique patterns of gene expression. One fate for which *B. subtilis* is well-studied is genetic competence, a transient state during which cells are receptive to extracellular DNA (eDNA) uptake for transformation. However, little is known about competent cells within the context of biofilms. Based on physiological and genetic evidence, we hypothesize that competence is exclusive from matrix production; first, the longitudinal chains formed by cells during biofilms blocks the competence machinery for DNA uptake, which localizes to cell poles; second, the master competence regulator ComK is predicted to repress a key biofilm activator gene *sinI*. The goal of this research is to elucidate the mechanisms of differentiation, as well as provide a physiological basis for competence/matrix exclusivity. We show using a dual-labeled fluorescent reporter strain for competence and matrix differentiation that competent cells arise infrequently (<1%) during biofilm development. Overexpression of ComK in cultures reduced both expression of matrix genes and the frequency of matrix producers in a population, as evidenced by beta-galactosidase and gfp reporter assays, respectively. Finally, transformation assays revealed that both matrix production and longitudinal cell chaining inhibit transformation by eDNA. We conclude that biofilm matrix production is incompatible with competence in single cells, and that competent cells expressing ComK inhibit matrix production. Differentiation into the competent state during biofilm formation is likely stochastic or governed by unknown signals. This research lends insight into the rich functional heterogeneity of microbial populations.
Background: Bacteria of the P. fluorescens group adopt diverse morphological phenotypes on the surface of Populus roots that range from micro-colonies to highly-structured and dense biofilms. Biofilm formation in bacteria is under the control of the secondary signaling messenger cyclic diguanylate monophosphate (c-di-GMP), which is a central regulator of bacterial transition from motile to biofilm life-styles. C-di-GMP signaling is likely involved in the control of biofilm formation at roots although the specific sensor and effector proteins that connect rhizosphere cues to changes in cellular functions remain uncharacterized. In the cell, c-di-GMP is synthetized by enzymes called diguanylate cyclases (DGCs), degraded by phospho-diesterases (PDEs), and bound by multiple effector proteins that regulate specific cellular functions. The complex interplay between all the c-di-GMP-associated proteins involved in this signaling pathways is arguably the main hindrance to our understanding of the role of c-di-GMP signaling in biofilm formation in Pseudomonas. The goal of this study is to identify and validate key protein complexes involved in the c-di-GMP signaling pathway to provide a mechanistic understanding of the signal transduction processes governing the formation of biofilm at plant roots. Methods: Most proteins exert their activities through physical interactions with other proteins to form functional complexes. We performed genome-wide yeast two hybrid screens to construct a high-confidence protein-protein interaction (PPI) network centered on c-di-GMP signaling in P. fluorescens. Functional study of identified protein complexes was achieved by systematic CRISPRi-based knockdown of genes encoding interacting pairs and phenotypic analysis for motility, ROS stress, biofilm formation and biofilm structure. Results: Our PPI network revealed highly interconnected core of c-di-GMP binding proteins that are themselves connected to external groups of proteins involved in other cellular processes, including cell signaling, cell adhesion, transport of various nutrients, transcriptional regulation and other DNA transactions. These groups could represent the effector and sensor proteins that exchange information with the c-di-GMP core pathway. We found that despite the high functional redundancy of the c-di-GMP regulatory network in P. fluorescens, many gene knockdowns exhibited measurable biofilm-related phenotypes. The PPI network provides testable functional hypothesis about the biological role of the c-di-GMP binding proteins and their partners, highlighting for example their involvement in cell division and DNA damage repair. Conclusion: PPI networks centered on the c-di-GMP signaling pathways represent a powerful approach for deciphering at molecular level the cell processes regulated by c-di-GMP core during biofilm formation.
**Abstract Title:** Multiple Functions of Biosurfactant to Biofilm Formation by *Pseudomonas aeruginosa* PAO1

**Abstract Body:**

*Pseudomonas aeruginosa* is a Gram-negative bacterium that is an opportunistic pathogen and is often used as a model organism to study biofilm formation. It was recently highlighted by the World Health Organization as an urgent target for research and development of new antibiotics to suppress infection. It is extremely important to prevent biofilms from forming on medical implants such as catheters and heart pumps because biofilm bacteria are often much more resistant to antibiotics than their free-living planktonic counterparts and thus much harder to eradicate. Biofilms may be removed through a combination of physical shear and chemical treatment with surfactants, the main active ingredient in many personal care products. Microorganisms also secrete surfactants, which are called biosurfactants, and used to enhance surface motility and as a bio-weapon against competitors. Biosurfactants have similar properties to conventional chemically-synthesized surfactants but may possess additional novel functions derived from their chemistry and tend to be more eco-friendly and biodegradable than chemically synthesized compounds. We test the effect of a biosurfactant at two points in the biofilm lifecycle on *P. aeruginosa* PAO1: 1) at the early stage of reversible surface attachment; and 2) at the later stage on a mature. We find that our surfactant suppresses the irreversible attachment of PAO1 to clean glass surfaces in a dose dependent manner with long-lasting effects. The surface attachment suppression does not appear to be correlated with the formation of an absorbed biosurfactant monolayer on the glass. In addition to surface attachment inhibition, infusion of our biosurfactant in a microchannel containing a mature PAO1 biofilm causes catastrophic disruption and detach of the biofilm. This suggests that our biosurfactant not only weakens the connection between bacteria and surface but also breaks the internal crosslinks of the extracellular matrix in a PAO1 biofilm. Interestingly, we find that our biosurfactant does not kill the bacteria or slow their growth. Furthermore, the biosurfactant seems to inhibit pyocyanin production, which is the main virulence factor of *P. aeruginosa*. We believe that our biosurfactant represents a promising combination of anti-biofilm properties that act to suppress biofilm formation at different stages of the biofilm lifecycle without killing the bacteria.
Background: The switch from a planktonic to a sessile life style strongly affects light availability and thus is a crucial behavioral decision for photosynthetic microorganisms like cyanobacteria. Mechanisms involved in cyanobacterial biofilm development were, until recently, overlooked in spite of the environmental prevalence and industrial problems associated with these microbial assemblages. We revealed that the planktonic nature of the cyanobacterium *Synechococcus elongatus* is a result of a self-suppression mechanism, which depends on the deposition of a factor to the extracellular milieu. This substance governs expression of small secreted biofilm-promoting proteins. Inactivation of a gene encoding a homolog of ATPases of type II protein secretion and type IV pilus assembly complexes (hereafter T2SE), impairs the inhibitory process leading to biofilm formation. Additionally, the biofilm-forming mutant lacks pili, thus, these cell appendages are dispensable for biofilm development in this cyanobacterium, in contrast to their biofilm-promoting function in type IV pili-producing heterotrophic bacteria.

Methods: To uncover additional components of the self-suppression mechanism, biofilms formed by a barcoded transposon cyanobacterial library were analyzed by next-generation DNA-sequencing and mutants enriched in the biofilms were identified. Newly identified proteins served for immunoprecipitation followed by mass-spectrometry (MS) to identify cellular targets of interaction. The exo-proteome (proteins in culture fluids) was analyzed by MS and the presence of cell-pili was examined by TEM. Results and Conclusions: Screening of a barcoded transposon library revealed that mutants of the RNA chaperone, Hfq, and of Se0862, were enriched in the biofilm. The latter, a highly conserved cyanobacteri al protein, lacks domains that hint at its function. Directed inactivation of either one of the genes encoding these components resulted in biofilm-formation, validating their requirement for the self-suppression mechanism. Furthermore, both mutants are characterized by a modified exo-proteome and unlike the wild-type, lack cell pilis. Co-immunoprecipitation using either Hfq, T2SE or Se0862 as bait indicated formation of a tripartite complex (Hfq-T2SE-Se0862). NMR-analysis indicated structural homology of Se0862 to chaperones of type III secretion systems of pathogenic bacteria. Thus far, such secretion complexes have not been identified in cyanobacteria. Altogether, we identified new components that are essential for cyanobacterial biofilm self-suppression and uncovered formerly unknown regulation of protein secretion and pilus assembly complexes. We suggest that these systems allow cyanobacteria in native habitats to control their mode of growth in response to environmental cues.
Abstract Body:

**Background:** The terminal stage of biofilm development is dispersion. However, the mechanics of biofilm dispersion remains poorly characterized. The work presented here focuses on the mechanisms involved in *Pseudomonas aeruginosa* PA14 biofilm dispersion as induced by the cell-cell communication molecule, cis-2-decenoic acid (cis-DA). Previously, our lab identified a DNA-binding response regulator, designated DspR, required for the cis-DA dispersion response and the DspR binding sites on the *P. aeruginosa* genome. In the current work we hypothesized that proteins encoded by the genes downstream from the identified DspR binding sites have functions necessary for dispersion. In this study, 36 of the 91 identified DspR target genes were investigated for their role in dispersion. These DspR targets were chosen because the predicted functions of the target genes’ protein products are likely necessary for the release of bacteria from the biofilm. To evaluate the DspR target genes for their role in dispersion, biofilms of transposon (Tn) mutants for each target gene were evaluated for the formation of central voids within microcolonies. Central voids are a known indicator of dispersion and mutants with biofilms that formed significantly less voids compared to PA14 wild type biofilms were considered necessary for dispersion. **Methods:** The DspR target Tn mutants and PA14 wild type biofilms were grown in a semi-batch culture system in 24-well plates in 5-fold diluted LB. Following a 6-day incubation, bright field microscopy was used to observe whether the biofilm microcolonies possessed a central void. In addition, the height and width of the microcolonies were measured to determine if microcolony size influenced the dispersion ability of the Tn mutants. Data from the DspR target Tn mutant biofilms were collected in triplicate and compared to PA14 wildtype biofilms by Student’s T-Test. **Results:** The percent of microcolonies showing central void formation was significantly less in 5 of the DspR target Tn mutants compared to PA14 wildtype (p-value < 0.05). Putative functions of these 5 DspR target gene products cover a range of potential functions including metal transport, Type 4 fimbriae assembly, peptidoglycan hydrolysis, lysine metabolism, and sugar binding. No positive correlation was observed between DspR target Tn mutants microcolonies’ height, width, and size ratio (width/height) and dispersion. **Conclusions:** The decrease in the number of microcolonies having central voids, observed for the 5 DspR target Tn mutants, demonstrated these DspR target genes were involved dispersion. The diversity of putative protein functions of the DspR target genes indicates that a wide-range of biological processes are involved in the release of *P. aeruginosa* cells from biofilms.
**Session Title:** MONDAY Poster Session 1  
**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 091  
**Abstract Topic:** Regulation of Biofilm Development  
**Abstract Title:** Epigenetic Regulation Alters Biofilm Architecture and Composition in Multiple Clin. Isolates of Nontypeable *Haemophilus influenzae*  
**Author Block:** K. L. Brockman¹, P. N. Azzari¹, M. T. Branstool², J. M. Atack², B. L. Schulz², F. E. Jen², M. P. Jennings², L. O. Bakaletz²;  
¹Research Institute at Nationwide Children's Hospital, Columbus, OH, ²Griffith University, Gold Coast, AUSTRALIA, ³The University of Queensland, St Lucia, AUSTRALIA.  

**Background:** Biofilms play a critical role in the colonization, persistence and pathogenesis of many human pathogens. Multiple mucosal-associated pathogens have evolved a mechanism of rapid adaptation, termed the phasevarion (phase-variable regulon). This differential regulation occurs via phase variation of a DNA methyltransferase, Mod, which leads to the coordinated regulation of numerous genes throughout the bacterial genome via epigenetic mechanisms. The phasevarion of nontypeable *Haemophilus influenzae* (NTHI) significantly affects the severity of experimental otitis media and regulates several disease related processes. However, the role of the NTHI phasevarion in biofilm formation is unclear.  

**Methods:** Biofilm formation by five NTHI clinical strains that represent the most prevalent modA alleles was assessed. Microenvironmental temperatures and pHs were chosen to mimic those of the nasopharynx (37°C, pH 7), healthy middle ear (37°C, pH 7) and chronic otitis media (37°C, pH 9). For NTHI strain 723, the composition and distribution of extracellular DNA and associated bacterial DNA binding proteins was also assessed due to their known importance in the structural integrity of biofilms.  

**Results:** We found that unique phasevarions, present in multiple NTHI clinical isolates, regulate *in vitro* biofilm formation under disease-specific microenvironmental conditions. The impact of phasevarion regulation was greatest under alkaline conditions that mimic those known to occur in the middle ear during chronic disease. Under alkaline conditions, NTHI that express the ModA2 methyltransferase formed biofilms with significantly greater biomass and less distinct architecture than those formed by a population that did not express ModA2. The biofilms formed by a ModA2-expressing strain also contained less eDNA and significantly less extracellular HU, a DNABII DNA-binding protein critical for biofilm structural stability and for bacterial pathogenesis and persistence in multiple experimental models of disease.  

**Conclusions:** These results identify a role for the phasevarion in regulation of biofilm formation, a process integral to the chronic and recurrent nature of many bacterial infections. Phasevarions regulate a wide range of biological processes in bacteria and add an extra level of complexity to understanding bacterial responses to changing conditions and microenvironments. It is therefore critical to understand the implication of this remarkable regulatory mechanism on all aspects of bacterial biology, including biofilm formation, as well as their impact on disease. Support: NIH/NIDCD R01DC015688
Surface attachment is a crucial step in the initiation of biofilm formation. While the roles of exopolysaccharides, eDNA, and cell surface appendages (such as the flagellum and pili) on attachment have been investigated, little is known about the contributions of other bacterial surface proteins, such as those in the outer membrane. One outer membrane protein of interest is the major non-specific porin OprF of *Pseudomonas aeruginosa*. OprF is required for epithelial cell adhesion during colonization of the cystic fibrosis lung and accumulates in cells during early bacterial attachment *in vitro*. Here we examine the role of OprF in surface attachment and biofilm formation under static and flow conditions. We observed half as much biofilm biomass in OprF mutants than in wildtype when grown for 24 hours under static conditions with tryptic soy broth (TSB). However, there was no difference between wildtype and ΔoprF when the biofilms were grown under similar conditions with lysogeny broth (LB). Furthermore, while wildtype strains produced characteristic mature mushroom structures in both TSB and LB after four days of growth under flow conditions, ΔoprF produced fewer mushroom structures when grown in TSB, and exhibited diffuse aggregates in LB. Interestingly, we observed that after 24 hours of growth under flow, ΔoprF had fewer surface-attached cells than wildtype. Altogether, our results suggest that OprF may be involved in initial surface attachment to abiotic surfaces and that this effect is nutrient-dependent.
**Abstract**

**Background:** Small proteins characterized by a double-glycine (GG) secretion motif, typical of secreted bacterial antibiotics, are encoded by the genomes of diverse cyanobacteria, but their functions have not been investigated to date. **Results and Conclusions:** Using a biofilm-forming mutant of *Synechococcus elongatus* PCC 7942 and a mutational approach, we demonstrate the involvement of four small secreted proteins and their GG-secretion motifs in biofilm development. These proteins are denoted EbfG1-4 (enable biofilm formation with a GG-motif). Furthermore, the conserved cysteine of the peptidase domain of the Synpcc7942_1133 gene product (dubbed PteB for peptidase transporter essential for biofilm) is crucial for biofilm development and is required for efficient secretion of the GG-motif containing proteins. Transcriptional profiling of ebfG1-4 indicated elevated transcript levels in the biofilm-forming mutant compared to wild type (WT). However, these transcripts decreased, acutely but transiently, when the mutant was cultured in extracellular fluids from a WT culture, and biofilm formation was inhibited. We propose that WT cells secrete inhibitor(s) that suppress transcription of ebfG1-4, whereas secretion of the inhibitor(s) is impaired in the biofilm-forming mutant, leading to synthesis and secretion of EbfG1-4 and supporting the formation of biofilms.
Acinetobacter baumannii is a multi-drug resistant nosocomial pathogen known for causing wound related and respiratory infections. It is currently on the WHO’s list of critical pathogens due to its broadly drug resistant nature and the constant appearance of pan-resistant isolates. A majority of the infections caused by this organism are biofilm associated, however there is limited existing literature regarding the mechanisms used by this organism to engage in this multicellular lifestyle. As such, we set out to explore those factors influencing this behavior using an 11,000+ isolate transposon mutant library of A. baumannii strain ABS075. Our screen demonstrated a hit rate of 13.5% of clones with some level of change to their biofilm forming capacity (either increased or decreased). Of these, the majority demonstrated enhanced biofilm formation, with disrupted factors including proteases, phosphatases, hydrolases, pili components, and transcriptional regulators. Conversely, a quarter of our mutants displayed less robust biofilm formation, with elements involved in capsule formation, acyl-CoA dehydrogenase activity, and putative bfm genes proving important. A wide-range of mutants have been characterized in more detail using real-time tracking technology to explore further their influence on the biofilm formation process. Collectively, our work provides a deeper understanding of pathways important to the formation of biofilm in this important human pathogen. We suggest that this could lead to the discovery of pathways to specifically target this problematic pathogen in its biofilm growing state, which would be highly unique and have major potential for the treatment of human infection.
**Background:** Biofilm formation is a dynamic process that leads to mature communities over time. As they mature, they begin to display characteristics including three-dimensional structure formation, water channel development and antibiotic tolerance profiles. Despite a general knowledge of biofilm community formation and the resultant limitations of antibiotic therapy, there is a paucity of data describing specific plume heights, surface coverage and general rate of maturation over extended periods of growth. Furthermore, little is published on the effect that broth media might have on the degree of biofilm maturation. In this study, three strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were assessed for degree of maturation (e.g., surface coverage and plume height) over time in two media types. **Methods:** CDC biofilm reactors were used to grow three stains of MRSA (USA300, USA400 and a clinical isolate) on stainless steel coupons in modified brain heart infusion (BHI) broth or tryptic soy broth (TSB). Biofilms were grown for up to 192 hrs. Each coupon was fixed in modified Karnovsky’s fixative and dehydrated in ethanol. Scanning electron microscope (SEM) images were collected using secondary electron imaging (SEI) for morphology and 3D rendering analysis (Mountains Map 7) to assess plume height. Backscatter electron imaging (BSE) was used to analyze percent biofilm coverage on the coupons. **Results:** In BHI, USA isolates began to cover the surface more rapidly, but had notably less % surface coverage by 192 hrs compared to growth in TSB (Table 1). The clinical isolate had similar coverage rate and profile in both broths (Table 1). In BHI, USA300 resulted in peak plume heights of ~20 µm by 192hrs (Figure 1). USA400 resulted in taller plume heights (~50µm), while the clinical isolate had the tallest plume heights (~100µm; Figure 1). Plume heights were roughly 3x higher for each when grown in TSB. **Conclusion:** Little is documented regarding height profiles that biofilm plumes might reach and general coverage of growth substrates. Three strains of the same species were assessed for these outcomes. Data suggested that broth may influence the degree to which a surface is covered, and plume height may also be largely affected by broth type with significant variations between strains of the same species. These data provide basic science information, and may also influence future work where degree of maturation, plume height information (e.g., mechanical analyses) and susceptibility profiles may be of interest. **Figure 1:** Representative images of coverage and plume heights for each of the 3 isolates in BHI at 192 hrs.
Abstract Topic: Regulation of Biofilm Development

Abstract Body: The net amount of biofilm produced by a bacterium is a balance between biofilm production and destruction. In the genus Francisella, we have recently characterized two molecular mechanisms of negative regulation of biofilm: diffusible signal factor (DSF) and the secretion of beta-glycosidases (Chitinase). We demonstrated that DSF exerts its negative effect on Francisella biofilm through regulation of Francisella gene expression, including siderophores, relA and Chitinases by performing RNASeq and proteomics analysis. Beta-glycosyl hydrolyses (chitinases) are secreted enzymes that are negative regulators of biofilm production in several species of bacteria. We demonstrated the effect of chitinase inhibitors such as dequalinium and sanguinarine on Francisella biofilm formation and their diminished effect on ChiA and ChiB mutants. We demonstrated the effect of mutants in ChiA and ChiB on increasing biofilm formation in F. novicida through COMSTAT analysis of the biofilm. In a detailed proteomic post-translational modification (PTM) study, we have demonstrated acetylation PTM of some Francisella proteins, including ChiA, ChiB, and chitin binding protein, using mass-spectrometry analysis of bacterial proteins. Our analysis revealed that these proteins contain multiple naturally occurring acetylation sites, which may regulate the enzymatic activity of these proteins. Furthermore, chemical lysine acetylation of chitinases A and B results in silencing of the enzymatic activity of these proteins. Given our previous results demonstrating a role of chitinase as a negative regulator of Francisella biofilm production, this suggests that the post-translational modification of these enzymes may be a mechanism of regulation of chitinase function in Francisella. This suggests a new possible mechanism of chitinase activity regulation by acetylation, and thus another level of regulation of biofilm levels in Francisella. This work was supported by a 4VA grant to van Hoek and Nelson.
Horizontal gene (DNA) transfer (HGT) is a ubiquitous phenomenon in bacterial communities, and is often associated with the spread of bacterial antibiotic resistance and pathogenicity. However, horizontal RNA transfer (HRT) between bacterial cells remains an intriguing, but understudied, possibility. We aim to test whether bacteria can release and horizontally transfer RNA as an efficient strategy for within specie and interspecies communication. So far, we found that co-culture of GFP-labeled and mCherry-labeled non-competent Bacillus subtilis cells gave rise to dually labeled cells detected by flow cytometry and fluorescent microscopy, despite of the label being encoded in the same genomic locus. This raised the possibility that either RNA or proteins were horizontally transferred between B. subtilis cells. This cytoplasmic content exchange was most robust under biofilm conditions and did not require direct contact. We then systematically explored HRT in naturally relevant multispecies community; We performed deep sequencing of B. subtilis cells (acceptor) grown with two additional bacterial species sharing the same ecological niche (B. simplex and E. coli), serving as donors. The species were separated by an inlet to confirm the homogeneity of the sequenced acceptor. Our findings suggest horizontal RNA transfer from the donors into the B. subtilis acceptor, with a bias towards functional non-coding RNAs. The efficiency of the transfer was higher between the Bacilli, regardless of their relative proportion in the mixed community, indicating a potential bias towards HRT between related species. In addition, the abundance of the specific transferred RNAs was only partially correlated to their levels in the donor transcriptome, further supporting the existence of a selective HRT. Moreover we confirmed that the highly transferred RNAs were also present in the extracellular vesicles, indicating the molecular mechanism for RNA transfer. We now aim to decipher the potential contribution of RNA transfer to the fitness of complex communities under diverse environmental conditions.
Session Title: **MONDAY Poster Session 1**

**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 098

**Abstract Topic:** Social and Asocial Interactions in Biofilms

**Abstract Title:** Development of an *In vitro* Model Dental Plaque Biofilm Community to Study Bacterial Interactions

**Author:** Y. Khosravi¹, S. Palmer¹, M. Starke², M. Ward², P. Kumar¹, P. Stoodley²

1Ohio State University, Columbus, OH, 2Philips Oral Healthcare, Bothell, WA.

**Background:** Dental plaque biofilms are complex ecosystems formed from hundreds of interacting species. Initial colonizer such as *Streptococcus gordonii, Streptococcus oralis, Streptococcus mutans* and *Actinomyces viscosus* first attach to a host salivary proteins pellicle which forms on tooth surfaces. These organisms create microenvironments by producing lactic acid in the presence of sucrose and reducing redox potential, facilitating the proliferation of *Veillonella parvula* and the anaerobic periodontopathogens *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. Model biofilm communities are useful for studying interspecies interactions and potential therapeutics, but these are usually grown in anaerobic chambers. However, the mouth is not anaerobic, but anaerobic pockets form naturally within the biofilm. Here, we develop a model to grow a model biofilm community under air by allowing the biofilm to develop its own anaerobic microniches.

**Methods:** Human saliva/plaque was inoculated into a 12 well plate with hydroxyapatite (HA) disks and cultured at 37°C under air for 5 days with daily media exchanges. DNA from the biofilm was extracted using a commercial kit and 16s rRNA primers used to identify targeted species and genera. Conventional and qRT-PCR were used to identify species from the saliva/plaque biofilms at various days of growth. To confirm the PCR results that anaerobes could establish in the aerobically grown biofilms, selective media was used to culture *P. gingivalis* from the biofilm. **Results:** By day 4 all 7 species were detected in the static saliva/plaque biofilm model grown in air at 37°C, including the anaerobic species. qRT-PCR and semi-quantification by gel densitometry showed increasing an abundance of *Fusobacterium* species and *P. gingivalis*. *P. gingivalis* was established in the biofilm after day 2 and showed an increase as the biofilm matured. *Fusobacterium* species showed a slight increase as the biofilm matured, but had established as early as 1 day. Plating confirmed the growth of *P. gingivalis* on days 2, 3 and 4. Furthermore, Saliva/Plaque biofilms grown on HA disc for 4 days under air appeared patchy on the surface of the HA disc on day 1 but by day 4 were more uniform. **Conclusion:** Our model was able to form a mature biofilm populated by both early and late colonizing bacterial species of dental plaque grown under air by 4 days, demonstrating that the biofilm created its own anaerobic niches.
Interspecies Quorum Signaling by Nthi Disperses *M. CATARRHALIS* from a Dual-species Biofilm

**Author Block:**
E. M. Mokrzan¹, L. A. Novotny¹, K. L. Brockman¹, L. O. Bakaletz²;
¹The Research Institute at Nationwide Children's Hospital, Columbus, OH, ²The Research Institute at Nationwide Children's Hospital and The Ohio State University College of Medicine, Columbus, OH.

**Background:** Nontypeable *Haemophilus influenzae* (NTHI) is a predominant pathogen of multiple upper respiratory tract infections (URTI) including otitis media (OM). The chronic and recurrent nature of these diseases is associated with the presence of bacterial biofilms, which are highly recalcitrant to host immune effectors or antibiotics. These URTIs are frequently polymicrobial, and NTHI is commonly co-cultured from clinical specimens with *Moraxella catarrhalis* (Mcat). Our laboratory has developed a vaccine candidate for NTHI-induced OM and exacerbations of COPD that targets the majority subunit of NTHI Type IV pili, PilA. Antibodies against a recombinant, soluble form of PilA (rsPilA) can both disrupt and prevent the formation of NTHI biofilms *in vitro*, by a mechanism that involves both expression of PilA as well as quorum sensing via the *luxS* system and the release of AI-2. Here we explored the effects of antibodies against rsPilA on dual-species biofilms formed by NTHI and Mcat.

**Methods:** Dual-species biofilms formed by NTHI and Mcat at temperatures that mimic the nasopharynx (34°C) or the middle ear (37°C) were exposed to antiserum against either rsPilA or OMP P5 of NTHI, another adhesin.

**Results:** Antiserum against rsPilA, but not NTHI OMP P5, significantly disrupted NTHI+Mcat biofilms formed at either temperature. Supernatants from biofilms exposed to anti-rsPilA vs. naive serum contained significantly greater numbers of both NTHI and Mcat, which indicated that anti-rsPilA exposure led to dispersion of Mcat as well as NTHI from the biofilm. To determine the role of *luxS*-mediated quorum sensing in the dispersal of Mcat, we measured AI-2 levels in dual-species biofilms after exposure to anti-rsPilA. AI-2 was detected in supernatants from dual-species biofilms formed with Mcat+NTHI parent strain, but not Mcat+NTHI ΔluxS. Moreover, supernatants from NTHI parent+Mcat biofilms exposed to anti-rsPilA, which contained AI-2, induced the dispersion of Mcat from a monospecies biofilm. In contrast, supernatants from NTHI ΔluxS+Mcat biofilms similarly exposed to anti-rsPilA did not disperse Mcat from a monospecies biofilm. **Conclusions:** Mcat dispersed from NTHI+Mcat biofilms in response to the AI-2 quorum signal produced by NTHI after exposure to the immunological stressor, anti-rsPilA. These newly-dispersed bacteria were highly susceptible to antibiotics. Thus treatment strategies that combine vaccine-induced biofilm dispersal with traditional antibiotics to exploit the newly-dispersed, antibiotic-sensitive phenotype could reduce the antibiotic dosage required for treatment of these chronic diseases. These data strongly support the utility of rsPilA as a therapeutic vaccine antigen for polymicrobial biofilm-associated diseases including OM due to NTHI and *M. catarrhalis*. Funding: NIH-R01-DC003915 to LOB
Multicellular biofilms are one of the predominant modes of bacterial growth and thus the context in which cells often encounter and respond to each other in their immediate environment. It is therefore important to understand the evolutionary pressures acting on biofilms and how they impact intercellular interactions to better understand bacterial forms of multicellularity. We previously found that the soil bacterium *Bacillus subtilis* bases interactions on phylogenetic relatedness: very closely-related strains freely interact and coexist, while less-related strains are highly antagonistic and cannot form biofilms together. This kin discrimination behavior is mediated by the suite of antimicrobials present in unique combinations in each strain’s genome. Here we investigate the generality of this finding by testing the intraspecific interactions of biofilms of the opportunistic pathogen *Pseudomonas aeruginosa* isolated from patients, as well as the constitutively-filamentous species *Bacillus mycoides*. Preliminary results suggest that these two species do not follow the same phylogeny-based interaction rules as *B. subtilis*, demonstrating a lack of kin recognition that may be a product of their specific environments or multicellular growth modes. In the future we will compare these results to environmental isolates of *P. aeruginosa*, as there are very different ecological and evolutionary selective forces in pathogenic versus non-pathogenic niches, plus isolates of *Streptomyces* species since they are known to be major antimicrobial producers. These will provide additional contexts to test the broader implications of our hypothesis regarding intercellular interactions in the evolution of multicellularity.
Social and Asocial Interactions in Biofilms

A novel *P. mirabilis* Nuclease Affects Spatial Distribution within a Microbial Community

D. Sirias, K. A. Gibbs;

Harvard University, Cambridge, MA.

Advances in sequencing technology have made it possible to determine which microbes are present in a community; however, this does not inform on spatial arrangement, which can be shaped by beneficial and/or competitive interactions between cells. *Proteus mirabilis* provides us with a simple model to study spatial distributions because it forms a characteristic swarm structure under laboratory conditions. We have previously identified proteins that are communicated between cells that can affect the formation of this swarm. Here we have identified and characterized a novel nuclease of the PD-(D/E)XX superfamily. This nuclease protein contains homology to *rhs* genes, which encode effectors that are transferred between cells in a contact-dependent manner. We show that loss of this nuclease changes the spatial distribution of *P. mirabilis* strains within a mixed swarm. Through cell viability and *in vitro* nuclease assays, we have determined that this nuclease is toxic to cells by targeting genomic DNA. Homologous proteins are found in many bacteria, including in the human oral cavity, soil, and associated with plants. While these contact-dependent inhibition systems are associated with bacterial competition, we hypothesize that they also play a role in shaping the biogeography within these different microbial communities.
Bacterial protein acetylation is an abundant posttranslational modification that influences many key physiological processes, and while significant progress has been made towards the understanding of the impact of protein acetylation on bacterial physiology, much is yet to be discovered. For example, the oral commensal *Streptococcus gordonii*, thought to play an important role in oral biofilm development, is predicted to encode twenty-two protein acetyltransferases. This number is greater than the predicted number of two-component systems and corresponds to approximately 1% of the total number of *S. gordonii* genes, yet, no information exists on the role of protein acetylation on *S. gordonii* physiology. Here, we report that upon interaction with the major salivary mucin, MUC5B, *S. gordonii* down-regulates at least two genes that encode putative acetyltransferases (SGO_2030 and SGO_2031). This downregulation requires the two-component system (TCS) SGO_1180/81 implicating it in the ability of *S. gordonii* to sense MUC5B interaction. Dot blot analysis of total acetylated proteins shows that total protein acetylation level is higher when *S. gordonii* forms a biofilm on surfaces coated with saliva devoid of MUC5B (LDP) and that this increase in acetylation requires SGO_2031. Both acetyltransferase mutants showed a minor defect in biofilm formation on LDP-coated surfaces, but no growth defect was observed. Because *S. gordonii* is considered a pioneer colonizer of the tooth surface and thought to be a key player in the development and maturation of the microbial community that makes up the dental plaque, we investigated the ability of the acetyltransferase mutants to incorporate into an *ex vivo* plaque community *in vitro*. Compared to the WT, the SGO_2031 mutant, but not 2030, displayed a defect incorporating into the complex plaque community. Denaturing gradient gel electrophoresis analysis revealed subtle differences in community composition profile, suggesting that protein acetylation might play role in the ability of *S. gordonii* to be part of the oral microbial community and could help shape community composition. We are currently working to identify the acetylation target(s) of SGO-2031-dependent acetylation and determine the molecular mechanism behind their contribution to the overall dental plaque community.
**Abstract**

**Background:** This study was designed to explore the role of different phenotypes of *P. aeruginosa* in the development, stability and persistence of biofilm. **Methods:** A total of seventeen (17) waterborne biofilm producing strains of *P. aeruginosa* were studied. These isolates were identified on the basis of typical phenotypic characters, i.e. growth on cetrimide agar and by amplification of 16S rDNA. Tube method was used for development of biofilms on glass slides and growth and exopolysaccharides production was measured after 18h, 24h, 36h, 48h, 72h and 96h of incubation. The Crystal violet assay was used for quantification of biofilms. Population and phenotypic variance were studied by the drop plate method. The hydrophobicity of strains was evaluated by the bacterial adhesion to apolar solvent test. **Results:** Study showed that the subject isolates of *P. aeruginosa* adopted a biofilm lifestyle after 36h of incubation at 35°C. After 24h the adhesion started, but it was reversible and easily dispersed by simple washing. However, after 36h the irreversible adhesion, difficult to disperse, was noticed. The biofilm consortia harbor three different phenotypes: i. wild types, showed typical *P. aeruginosa* characters on Cetrimide agar; ii. Slow growers, showed poor pigmentation and take >36h for colony development, and iii. Small colony variants (SCVs) are metabolically inactive very slow growing and producing pinpointed non pigmented colonies. Interestingly, increase of incubation time of biofilm consortia results in strong adhesion and dominance of SCVs. Comparative analysis showed that these phenotypes i.e. SCVs were highly hydrophobic and persistent in biofilm consortia due to the production of excessive amounts of exopolysaccharides. **Conclusion:** This study showed that phenotypic heterogeneity is a characteristic feature of *P. aeruginosa* biofilms and all of these phenotypes have a major role in stability and persistence of biofilm consortia. **Keywords:** Biofilms, Hydrophobicity, *P. aeruginosa*, Phenotypes, SCVs
Synthesis and Assembly and Function of the Biofilm Matrix

Developing a Novel Biofilm Assay for Representative Surfaces: Assessment and Quantification of Biofilm Formation

by Acinetobacter baumannii


United States Military Academy, West Point, NY.

Biofilm formation is key to the prolonged survival of select bacteria, especially in a hospital setting. In an age of multidrug resistance, *Acinetobacter baumannii*, in particular, shows worrisome patterns of increased resistance to most classes of antibiotics. Rather than looking to treat antibiotic resistant bacteria, this research aims to develop a strategy to prevent the biofilm formation of such bacteria. First, this requires the development of an assay to measure and assess the amount of biofilm on various surfaces.

Using a series of growth and quantification assays, we modeled the presence of biofilm formation by inoculating metal washers (stainless steel, brass, and zinc) with a strain of *A. baumannii*, AB5075. We removed planktonic cells and used sonication at sequential time intervals, followed by serial dilutions to compare cell viability. Our results indicate a correlation between surface type and biofilm growth, as shown using scanning electron microscopy, crystal violet absorbance, and assessment of colony forming units (CFUs). It is pertinent to understand how surfaces contribute to biofilm formation in order to develop prevention and intervention strategies. Normalizing against our stainless steel results, our data show that zinc impairs biofilm growth, while brass increases biofilm formation. Additional experiments show that surface roughness contributes to biofilm propagation, and potential biofilm “knock-out” isolates of *A. baumannii* might have altered biofilm growth on stainless steel washers. This research is particularly relevant as the evaluated materials model surfaces routinely encountered in a patient’s environment. Future research will include qRT-PCR to assess biofilm associated gene expression in further validating and applying this surface assay.
Pseudomonas aeruginosa is a gram-negative bacteria capable of persisting in a variety of environmental niches due to its ability to form surface-adhered, protective communities called biofilms. The extracellular matrix encasing the multicellular aggregates in P. aeruginosa biofilms are comprised of exopolysaccharides (EPS), proteins, and DNA. P. aeruginosa produces three chemically distinct EPS: Pel, Psl, and alginate, which have been well-characterized in terms of their individual biofilm roles. Despite this, the functional contribution of matrix proteins to the community remains largely uncharacterized. Our lab previously described two matrix-associated proteins, CdrA and ecotin, which were both found to interact with the neutrally-charged P. aeruginosa EPS Psl. However, matrix proteins that associate with positively-charged Pel or negatively-charged alginate are relatively understudied.

Alginate-producing mucoid strains of P. aeruginosa are of particular interest due to their high resistance to antibiotics in addition to being associated with a worse prognosis in chronic cystic fibrosis infections. We show here for the first time that P. aeruginosa biofilms comprised of the negatively-charged EPS alginate retain a distinctive proteome. We specifically labeled matrix proteins within mucoid P. aeruginosa biofilms grown under continuous flow conditions, and then used LC-MS/MS to identify the alginate-associated proteome. Additionally, we isolated secreted proteins which interact with alginate by using bacterial alginate as bait to fish out interacting proteins within a cell-free system. Preliminary studies suggest that the proteins which are uniquely retained by P. aeruginosagalginate-rich biofilms serve a variety of roles ranging from nutrient acquisition, to structural and protective functions. This study expands our understanding of how exopolysaccharides can affect the protein composition of bacterial biofilms.
Session Title: **MONDAY Poster Session 1**

**Session Date/Time:** Monday, October 8, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 106

**Abstract Topic:** Synthesis and Assembly and Function of the Biofilm Matrix

**Abstract Title:** Biosynthesis of the Holdfast Polysaccharide in *Caulobacter crescentus*

**Author Block:** D. M. Hershey\(^1\), P. Azadi\(^2\), C. Heiss\(^3\), J. M. Troutman\(^4\), B. Scarbrough\(^5\), A. Fiebig\(^6\), S. Crosson\(^7\);

\(^1\)University of Chicago, Chicago, IL, \(^2\)Complex Carbohydrate Research Center, Athens, GA, \(^3\)UNC Charlotte, Charlotte, NC.

**Background:** Many organisms secrete specialized polysaccharides that facilitate the colonization of exogenous surfaces. The freshwater bacterium *Caulobacter crescentus* produces a carbohydrate-based structure called the holdfast that promotes tight physical attachment to a variety of solid materials. Holdfast production requires a group of *hfs* genes that encode predicted components of a polysaccharide assembly pathway. Missing details about its chemical structure, biosynthetic pathway and regulatory principles have limited progress in understanding the details of holdfast assembly.

**Methods:** We have leveraged the adhesive properties conferred by the holdfast to perform a saturating transposon mutagenesis based screen for genes that promote attachment to cheesecloth. To characterize the chemical structure of the polysaccharide we developed a method to extract the holdfast from *C. crescentus* cultures and analyze its carbohydrate content. Combining this composition information with knowledge of the biosynthetic enzymes allowed us to reconstitute the early stages of holdfast biosynthesis with purified components.

**Results:** Our analysis defined a complete set of genes required for adhesion that includes known *hfs* genes as well as newly discovered factors for polysaccharide biosynthesis and assembly. Of particular interest is the identification of a novel glycosyltransferase that is required for holdfast production, which we have named HfsL. We found that the holdfast polysaccharide contains a tetrasaccharide repeat with β-1,4 linked xylose, GlcNAc, glucose and mannose residues. We used this structure to predict the potential substrate pool for glycosyltransferase enzymes in the biosynthetic pathway and characterized a number of these reactions in vitro.

**Conclusions:** We have defined the chemical structure of the holdfast polysaccharide and identified the complete set of biosynthetic factors required for its biosynthesis. Using this information, we have reconstituted the glycosyltransferase steps in the pathway with purified components and defined each enzyme’s substrate specificity. This bottom-up approach of defining the genetic and chemical components in order assemble the pathway *in vitro* is providing invaluable insight into the mechanisms of surface polysaccharide production in bacteria.
Role of AlgL in Pseudomonas aeruginosa Alginate Biosynthesis

A. A. Gheorghita¹, S. Wong¹, F. Wolfram¹, A. M. Berezuk², M. Goodyear², C. M. Khursigara², P. L. Howell¹;
¹The Hospital for Sick Children, Toronto, ON, CANADA, ²University of Guelph, Guelph, ON, CANADA.

Pseudomonas aeruginosa is an opportunistic pathogen responsible for chronic lung infections in cystic fibrosis patients. These Pseudomonas infections are characterized by formation of surface-associated biofilms and overproduction of alginate exopolysaccharide. Alginate is synthesized, modified, and exported by a multi-protein complex that spans the inner and outer bacterial membranes. While most of the proteins within the alginate biosynthetic complex have an established role in exopolysaccharide production, the role of the alginate lyase, AlgL, remains poorly understood. In this study we determine the structure of AlgL and, through structural alignment with the homologous alginate lyase A1-III from Sphingomonas sp., identify active site residues important for alginate binding and catalysis. We demonstrate that both in a ΔalgL strain or when this strain is complemented with active site variants, induction of alginate expression is detrimental to the bacteria and results in a lethal phenotype. The active site point mutants Y256F, R249E, and R249A were found to negatively affect P. aeruginosa growth and viability in vivo by growth curve and colony forming unit analyses. Furthermore, transmission electron microscopy (TEM) images of whole cells demonstrated that the absence of AlgL and the catalytic point mutants Y256F, R249E, and R249A result in abnormal cell morphology, including membrane perturbations and build-up of substance within the periplasmic space. Approximately 5% of ΔalgL P. aeruginosa cells complemented with the K66A active site variant also demonstrated abnormal cell morphology. Structural determination of the K66A point mutant and alignment with Sphingomonas sp. A1-III show that K66A is part of a conformationally flexible lid loop region which probably interacts with alginate. The structural comparison suggests that this residue moves over 14 Å when the enzyme-substrate complex is formed. In vitro characterization of AlgL point mutant enzymatic activities is currently in progress. Combined, our results suggest that AlgL functions to degrade alginate that is not exported from the cell, thereby preventing its accumulation within the periplasmic space. Thus, AlgL appears to be required for P. aeruginosa viability during biofilm exopolysaccharide formation.
Investigating Novel Molecular Mechanisms of Biofilm Formation by Klebsiella Pneumoniae

C. B. Webb¹, J. A. Bengoechea¹, L. Hobley²;
¹Queen's University Belfast, BELFAST, UNITED KINGDOM, ²School of Biosciences, Nottingham, UNITED KINGDOM.

Background: The identification of Klebsiella pneumoniae as a pathogen of increasing concern by the World Health Organisation has revitalised interest in this increasingly antibiotic-resistant, Gram-negative pathogen. Many studies have shown correlation between in vitro biofilm formation by Klebsiella and virulence, but there is relatively little known about the molecular mechanisms responsible for biofilm formation. A variety of fimbriae types have been shown to be involved in biofilm formation and host cell attachment, but to date nothing is known about the polysaccharide components of the biofilm matrix of Klebsiella, nor about the regulatory processes governing its formation. Only by identifying these mechanisms can potential treatments targeting biofilm formation by Klebsiella be developed.

Methods: A mariner Tn5 transposon was used to generate over 6000 independently derived mutants, that after screening for altered biofilm formation (both decreased and increased biomass) resulted in the identification of almost 100 genes essential for biofilm formation in Klebsiella pneumoniae 52145.

Results: Genes identified included those for capsule synthesis and assembly of the O-antigen, both essential processes required for Klebsiella virulence in the host. In addition, we have shown a role in biofilm formation of two polysaccharides, cellulose and poly-glutamic acid, the production of which has never been identified before in Klebsiella. We are currently extending this work to further characterise the potential roles of these polysaccharides in Klebsiella virulence.

Conclusions: Overall our work has identified the presence of two previously unidentified polysaccharides in the Klebsiella biofilm, and has demonstrated a link between key virulence factors and biofilm formation, highlighting the importance of biofilm formation in Klebsiella virulence.
Session Title: **MONDAY Poster Session 1**
Session Date/Time: Monday, October 8, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 109
Abstract Topic: Synthesis and Assembly and Function of the Biofilm Matrix
Abstract Title: Mechanical Characterization of Curli Fiber Monomers and Dimers
Author: E. DeBenedictis, M. Dunbar, S. Keten;
Block: Northwestern University, Evanston, IL.

Curli fibers are functional amyloids that play a major structural and adhesive role in many biofilm extracellular matrices. These fibers are formed through self-assembly of curli specific gene A (CsgA) protein monomers, with curli specific gene B (CsgB) monomers anchoring the fiber to the cell surface. Curli have proven capable of assembly even with added mutations, and various curli mutants have been produced to date. However, the structure and mechanical properties of single fibers and their protein subunits are not well characterized, complicating our understanding of curli fiber network behavior in biofilms or engineered gels. To address this, we take a bottom-up approach to model curli mechanical behavior, beginning with all-atomistic representations of subunits CsgA and CsgB. We have conducted equilibrium and nonequilibrium molecular dynamics (MD) simulations to quantify resistance to mechanical unfolding of single subunits and dimerization energies of subunits made up of CsgA and CsgB. We find that when comparing beta-helical CsgA to alpha-helical motifs, beta-helical proteins require a similar order of magnitude of work to unfold but can pack more work to unfold per initial length. For quantifying curli subunit dimers, we perform free energy calculations using replica exchange umbrella sampling and extended adaptive biasing force simulations to obtain absolute binding energies for each dimer type, as well as estimations for the Young’s modulus of curli fibers. We find that the trend of binding energy magnitude follows: CsgB-CsgA > CsgA-CsgA > CsgB-CsgB. Our findings provide atomistic structures for dimer complexes towards building fiber models and quantify binding energies and PMFs that establish theoretical limits to the elasticity and strength of curli fibers.
**Abstract Title:** Eradication of *Pseudomonas aeruginosa* Biofilms by Chitosan

**Author:** J. Masak, M. Paldrychova, K. Lokocova, O. Matatkova, A. Cejkova;

**Block:** ICT Prague, Prague 6, CZECH REPUBLIC.

**Background:** *Pseudomonas aeruginosa* is an opportunistic pathogen, which easily forms biofilms on various types of surfaces. The biofilm phenotype is characterized by an increased resistance to environmental influences including resistance to antibiotics and other disinfectants. Considerable effort is therefore devoted to finding suitable, in practice useful, tools to inhibit cell adhesion, or eradicate mature biofilm. In this respect, the antibiofilm potential of various natural substances is often tested. One of the promising substances can be chitosan. Its biological activity is given by the presence of amino groups in the structure. Cationic nature enables chitosan to interact with negatively charged compounds. The antimicrobial activity of chitosan was confirmed many times, but information about its interaction with biofilm are relatively scarce. **Methods:** The cultivations were carried out in 100-well microtiter plates using Bioscreen C analyzer. The cell adhesion and biofilm stability under different concentrations and application methods of chitosan were quantified by crystal violet assay. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells. Concentration of biomass in medium above biofilm was determined as OD$_{600nm}$. Activity of N-acyl homoserine lactones (AHLs) involved in the regulation of biofilm formation was determined using *Agrobacterium tumefaciens* strain harboring a traG::lacZ/traR reporter gene responsive to AHLs. SYTO 13 and propidium iodide staining and spinning disc confocal microscopy (SDCM) were used for visualization of biofilm. **Results:** *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 15442 formed biofilm at the bottom of the microtiter plates after 30 min cultivation. Very low chitosan concentration ranging from 2 to 6 mg / l increased the amount of biomass in the biofilm and metabolic activity of the cells. However, the biofilm formation of both strains was significantly suppressed after 24 h cultivation when the chitosan concentration exceeded 20 mg / l. Application of chitosan at a concentration of 30 mg / l or higher enabled effective eradication of mature biofilm. The resulting effect of chitosan on biofilm depended also on the time of application and the duration of interaction with biofilm. The presence of chitosan in the medium slightly suppressed the level of AHLs regulatory molecules. **Conclusions:** We have proved that chitosan has a considerable antibiofilm potential. It significantly decreased cell adhesion and biofilm formation of *P. aeruginosa*, as well as eradication of the mature biofilm at practically usable concentrations.
**Abstract**

**Topic:** Antibiofilm Strategies

**Title:** Anti-Infective Potential of a Polyherbal Wound-Care Formulation (Herboheal) against planktonic and Biofilm Forms of Selected Pathogenic Bacteria

**Author:** P. Patel, C. Joshi, V. Kothari;

**Block:** Nirma University, Ahmedabad, INDIA.

**Background:** Bacterial biofilms present a major problem in wound care that augment the complexity of the disease conditions by slowing tissue repair at the wound site. Bacterial biofilms, where the pathogen exist in a protective coat of extracellular polymeric substances, are regulated by quorum sensing mechanism and have role in multidrug resistance by influencing the virulence and pathogenicity of a pathogen. Though, advanced wound-care is a big market worldwide, and many of the leading pharmaceutical companies are actively involved in this area, it is optimal to use the traditionally indicated formulations as a strategy to inhibit the biofilm effectively. In this study, a traditional formulation namely Herboheal indicated for wound-care was investigated for its anti-biofilm potential against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in vitro and in vivo, followed by studying its effect on *S. aureus* gene expression at the whole transcriptome level.

**Methods:** Parameters on which effect of Herboheal Formulation (HF) was investigated in vitro include: bacterial growth and biofilm formation, pigment production, cell surface hydrophobicity, susceptibility to serum and antibiotics, heamolytic activity, etc. Biofilm quantification was done by crystal violet assay, whereas its viability was evaluated using MTT assay. In vivo efficacy of HF was assayed using the nematode *Caenorhabditis elegans* as the model host. Molecular targets of the HF in *S. aureus* were elucidated through gene expression analysis at the whole transcriptome level. Additionally, effect of HF on mixed species biofilm of *P. aeruginosa* and *S. aureus* was also investigated. **Results:** HF(0.1-0.5%v/v) inhibited bacterial biofilm formation up to 40-55%. It had no effect on pre-formed *P. aeruginosa* biofilm, but could eradicate pre-formed *S. aureus* biofilm by ~63%. Mixed species biofilm formation by these two bacteria was inhibited by 34% in face of HF-challenge. CSH of *P. aeruginosa* and *S. aureus* was marginally decreased and increased under the influence of HF. HF-treated bacteria exerted significantly lesser virulence towards *C. elegans*. Transcriptome analysis revealed that several genes associated with adhesion (*icaR, icaB, icaA*), biofilm formation (*sigB*), determinants of CSH (teichoic/ lipoteichoic acid syntheses: *tagB, tagX*; SAFDA_2230, SAFDA_0051), capsular polysaccharide biosynthesis protein *capA_2*, etc. were differentially expressed in HF-treated *S. aureus*. **Conclusions:** This study validates the traditional use of *Herboheal* formulation in wound-care by proving its efficacy against gram-negative as well as gram-positive bacteria, most commonly involved in wound infections.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 005  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** Evaluation of Biofilm Disruption Technology to Disinfect C. Auris Monomicrobial and Candida - S. Aureus Polymicrobial Biofilms  
**Author Block:** M. F. Myntti¹, J. A. Vaquez²; ¹Next Science, LLC, Jacksonville, FL; ²Medical College of Georgia, Augusta, GA.

**Background:** *Candida* species cause infections in both immunocompromised and immunocompetent individuals, ranging from relatively benign superficial infections to life-threatening systemic infections. *Candida auris* is an emerging, multidrug resistant yeast that can cause invasive infections and has been frequently associated with healthcare outbreaks throughout the world. *Candida* spp. are commonly encountered wound and bloodstream pathogens and have been known to form monomicrobial and polymicrobial biofilms, especially in combination with *S. aureus*. This work evaluates the effectiveness of novel biofilm-disrupting technology in topical gel, wound wash, and surface disinfectant forms vs chlorhexidine and super-oxygenated water on *C. auris*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, and on polymicrobial biofilms composed of either *C. auris* or *C. albicans/S. aureus* or *C. glabrata* or *C. parapsilosis/S. aureus*.  

**Methods:** Prevention of *Candida* spp. biofilm growth is determined by zone of inhibition (ZOI) testing, whereby the relevant organism is seeded onto agar plates and the treatment applied to prevent growth. Efficacy is determined by the size of the zone created. Biofilm disinfection efficacy versus *Candida* spp. is determined by treating biofilm which has been pre-grown on SD agar plates. Treated area of the plates are excised and biofilm enumeration is determined by vortexing, serial dilution, and regrowth to obtain CFU values. Polymicrobial biofilms are created by growing *Candida* spp. and *S. aureus* on the bottom of 96-well microtiter plates. Monomicrobial biofilms of *Candida* spp. and *S. aureus* are used as controls. After biofilm growth, plates are washed with PBS to remove non-adherent cells and then the treatments are applied for 24 hours. Biofilms are resuspended and then sonicated to facilitate biofilm disruption and ten-fold dilutions are prepared for enumeration. The Student’s *t*-test (two-tailed, unequal variance) will be used to analyze the significance of difference between two experimental groups, with a P-value of 0.05 or less being considered significant.  

**Results:** Initial testing of the gel version of the biofilm disruption technology at a 50% dilution yielded multi-log reductions to the *Candida* biofilms in 24 hours of treatment (*C. auris*, 4.1 log; *C. albicans*, 3.9 log; *C. glabrata*, 4.1 log; *C. parapsilosis*, 4.4 log). 50% dilutions of the gel product have also demonstrated multi-log efficacy in treating mixed-species biofilms in 24 hours (*C. auris* + *P. aeruginosa*, 4.0 log; *C. auris*/S. *aureus*, 4.3 log). Additional data will be presented at the conference.  

**Conclusions:** The use of a novel biofilm disrupting technology has demonstrated efficacy in treating *Candida* biofilms and multi-species biofilms of *C. auris* and bacteria.
Antibiofilm Effects of a Novel Silver Gelling Fiber Dressing on *Pseudomonas aeruginosa* in Porcine Wound Infection Model

J. Gil¹, S. C. Davis¹, J. Li¹, C. Head¹, G. D. Glinos¹, M. Solis¹, A. Higa¹, E. Gerner², S. Hall³, K. Hamberg¹, I. Pastar¹; ¹University of Miami Miller School of Medicine, Miami, FL, ²University of Gothenburg, Sahlgrenska Academy, Göteborg, SWEDEN, ³Mölnlycke Health Care, Göteborg, SWEDEN.

The wound environment is a fertile ground for biofilm-forming pathogens. Once biofilms form within wounds, they become very challenging to eradicate and contribute to recurrent infections and inhibition of healing. The purpose of this study was to examine the effect of a novel gelling fiber dressing with silver using a well-established *in vivo* porcine wound biofilm model. Deep partial thickness wounds were inoculated with *Pseudomonas aeruginosa* ATCC 27312 and covered with a polyurethane film dressing to promote biofilm formation. Wounds were then divided into treatment groups: gelling fiber dressing with silver, gelling fiber dressing without silver; hydrofiber dressing with silver, benzethonium chloride and ethylenediaminetetraacetic acid and compared to untreated control. Microbiological, biofilm and histological wound assessments were performed from 104 wounds on days 3, 5 and 7 post infection. Treatment with gelling fiber dressing with silver resulted in significant reduction of *P. aeruginosa* biofilm when compared to all other treatment groups on every assessment time point (p < 0.05). In addition gelling fiber dressing with silver treatment resulted in detachment of biofilm from the wound, while wounds treated with gelling fiber dressing with and without silver showed more granulation tissue formation on day 3. Our data show that a new gelling fiber dressing with silver was effective in reducing biofilm associated *P. aeruginosa* *in vivo*. This study may have important clinical implications especially for wounds highly colonized with Gram-negative biofilm-forming bacteria.
**Background:** *Pseudomonas aeruginosa* has a large arsenal of virulence factors that are expressed in a coordinated and cell density-dependent manner by quorum sensing (QS). In addition, most antibiotics are not effective to chronic *P. aeruginosa* infections due to the high antibiotic resistance mediated by biofilm formation. Therefore, to cope with *P. aeruginosa* infection, there is a need for a substance capable of effectively controlling these two activities of *P. aeruginosa*, QS and biofilm formation. In this study, we tested a number of novel synthetic compounds for anti-QS and anti-biofilm activities.

**Methods:** We chemically synthesized a series of compounds (MHYs). To screen the compounds for the anti-QS and anti-biofilm activity, we carried out the reporter-based bioassays using various QS- or biofilm-specific promoter-lacZ fusions such as *lasI-lacZ*, PA1897-lacZ (for the QS activity), and *cdrA-lacZ* (for the measurement of the intracellular c-di-GMP level). We also measured the productions of several virulence factors and biofilm formation in *P. aeruginosa*. **Results:** We screened hundreds of novel synthetic compounds (MHYs) and discovered several promising compounds. MHY1383 and MHY1427 have only anti-QS activities, whereas MHY1387 has both anti-QS and anti-biofilm activity against *P. aeruginosa*. MHY1383 and MHY1387 have these activities at very low concentrations. All of these compounds lowered the production of virulence factors including proteases and pyocyanin, and MHY1387 significantly lowered the intracellular c-di-GMP levels. **Conclusion:** These compounds we found are expected to be developed as good drugs to prevent *P. aeruginosa* infection in that they exhibit anti-QS and anti-biofilm effects at very low concentrations.
session title: tuesday poster session 2

session date/time: tuesday, october 9, 2018, 4:15 pm - 5:45 pm

poster board number: 008

abstract topic: antibiofilm strategies

abstract title: anti-biofilm efficacy of N-acetyl cystiene (NAC) is pH-dependent

Author: V. Venkatakrishnan¹, M. Mehrotra¹, J. Ghigo², C. Beloin², A. Chauhan¹;
¹NIIT University, Neemrana, INDIA, ²Institute Pasteur, Paris, FRANCE, ³Tripura University, Suryamaninagar, INDIA.

background. Antibiotic lock therapy (ALT) serves as an adjunct therapy to catheter-related bloodstream infection. However, infections due to pathogens like S. aureus or P. aeruginosa are difficult to treat and are a serious threat to the patients. Currently used antibiotic lock solutions are associated with high rate of failure against biofilms, thus, warranting for new antibiotic locks. N-acetylcysteine (NAC) is a mucolytic agent commonly used for treating the lower respiratory tract infections in patients with COPD, CF and bronchiectasis. Moreover, several studies showed an intrinsic antimicrobial and anti-biofilm activity of NAC against some pathogens. We studied the in vivo potential of NAC alone or in combination with cefazolin and ciprofloxacin.

methods. We used an in vitro 96-well plate assay to study biofilm tolerance and tested various combinations of antibiotics and non-antibiotic adjuvants. Ciprofloxacin or Cefazolin was combined with NAC for 24 h and 48 h to reproduce the antibiotic lock therapy (ALT) approach. Biofilm biomass was determined by standard crystal violet (CV) staining method and killing of biofilm bacteria was measured by viable cell count quantification. We used catheter-associated rat model to determine the in vivo anti-biofilm efficacy of NAC alone or in combination of antibiotics.

results. NAC showed activity alone as well as in combination with antibiotics against biofilms formed by Gram-positive and Gram-negative bacterial pathogens. NAC adjusted to pH 7.0 lost its in vitro anti-biofilm activity and did not improve the efficacy of antibiotics against biofilms formed. Although NAC adjusted to pH 9.0 showed significant anti-biofilm activity compared to pH 7.0, at native pH (=1.5) NAC had maximum anti-biofilm activity alone or in combination. We also demonstrated that NAC (pH=1.5) eradicated in vivo biofilms only in combination with antibiotics.

conclusions. The NAC enhances the potential of ciprofloxacin and cefazolin against biofilms formed on catheters in pH dependent.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 009  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** A Topical Gel for Biofilm-Associated Respiratory Tract Infections- Translation from Bench to Bedside  
**Author Block:** K. Richter¹, S. Vreugde¹, T. Coenye², N. Thomas³;  
¹University of Adelaide, Adelaide, AUSTRALIA, ²Ghent University, Gent, BELGIUM, ³University of South Australia, Adelaide, AUSTRALIA.  

**Background:** Staphylococcus aureus is associated with recurring respiratory tract infections, such as chronic rhinosinusitis and cystic fibrosis. S. aureus has the capacity to form biofilms and small colony variants (SCVs), which are pathogenic subpopulations with a preferred intracellular lifestyle. S. aureus biofilms and SCVs are linked to antibiotic tolerance and resistance, and are challenging to eradicate. Despite aggressive antimicrobial therapies and surgery, infections often recur causing ongoing morbidity and significant healthcare costs. **Aim:** Preclinical validation of an antibiofilm and anti-SCV treatment targeting bacterial iron metabolism. **Methods:** The iron-chelator deferiprone (Def) and the haem-analogue gallium-protoporphyrin (GaPP), in solution and incorporated in a surgical wound gel, were tested for antibacterial activity using multidrug-resistant S. aureus SCVs in an intracellular infection model. The antibiofilm activity was assessed in vitro in the colony biofilm model and an artificial wound model, as well as in an in vivo infection model in nematodes (Caenorhabditis elegans). **Results:** While Def alone failed to show substantial antibacterial activity, GaPP and the combination of Def-GaPP demonstrated concentration- and strain-dependent antibacterial properties. Specifically, the Def-GaPP combination significantly reduced the bacterial load in an artificial wound model (1.4 log10 reduction) and increased the survival of S. aureus SCV infected nematodes (86% survival of infected, treated worms vs. 25% survival of infected, untreated worms over 3 days). When Def-GaPP were combined with ciprofloxacin (Cip) or gentamicin (Gent), the triple combinations exceeded the antibiofilm activity of the individual compounds in the colony biofilm model against Cip- and Gent-resistant strains (5.4 log10 reduction for Def-GaPP-Cip and 3.4 log10 reduction for Def-GaPP-Gent). Moreover, Def-GaPP-Gent eradicated intracellular SCVs in human bronchial epithelial cells. **Summary:** Def-GaPP showed significant activity against S. aureus biofilms and SCVs and potentiated the activity of Cip and Gent against resistant strains. Delivered in a wound healing gel, Def-GaPP progressed to an ongoing first-in-human pilot study (ACTRN12618000577213) for the treatment of chronic rhinosinusitis.
The control of biofilm formation on implants and orthopedic prostheses is still one of the major challenges concerning infection related to devices in the health field. The objective of this research was to investigate biomaterials with applicability in orthopedics, aiming for advances and facing challenges in the infectology area. The in vitro biofilm formation according to biomaterials (titanium and titanium coated with F18 bioglass), microorganisms (Staphylococcus epidermidis and Candida albicans) and incubation times (2, 4 and 8 hours) was evaluated by fluorescence microscopy. S. epidermidis (ATCC 12228) and C. albicans (ATCC 90028) biofilms were formed on proof bodies of titanium and titanium coated with F18 bioglass after 2, 4 and 8 hours of incubation at 37°C under orbital shaking. The image areas of proof bodies, in percentage, coated with biofilm (living cells) were evaluated by fluorescence microscopy. The data collected were submitted to statistical analysis using normality tests Shapiro Wilk, U from Mann-Whitney and t from Student through IBM SPSS Statistics (version 25) software and significance level α=5%. There was less biofilm formation by S. epidermidis and C. albicans (p<0.001) on titanium coated with F18 bioglass than on titanium, after 8 hours of incubation. However, there was more biofilm formation by S. epidermidis and C. albicans after 8 hours than in 2 hours of incubation, both on titanium and on titanium coated with F18 bioglass (p<0.05). In sum, titanium coated with F18 bioglass presented better antibiofilm activity in comparison with titanium, and the incubation times of 2 to 8 hours increased biofilm formation on both materials. Besides, future studies about F18 bioglass based on physicochemical, biochemical and microbiological aspects are important for the elucidation of action mechanisms related to biofilm control.
Antibiotic resistance is spreading at an alarming pace, and the risk that we fall into a new, pre-antibiotic era is real. Some pathogens are resistant to all known antibiotics. Attractive approaches that interfere with bacterial chemical communication (known as quorum sensing (QS)), have the potential to control pathogens without killing commensal bacteria. Numerous bacterial pathogens produce and utilize acyl homoserine lactones (AHLs) as chemical signal molecules to coordinate, in a cell density dependent manner, pathogen-critical bacterial behaviors including virulence and biofilm formation. Consequently, hydrolysis of AHLs with enzymatic quenchers, termed lactonases, inhibit pathogenicity. The main cause of morbidity and mortality in patients with cystic fibrosis (CF) is chronic lung infection. The most prevalent pathogen is *Pseudomonas aeruginosa* (up to 80% of adult CF patients). Many clinically relevant pathogens utilize quorum sensing for virulence, biofilm formation, and colonization. Lung colonization by *P. aeruginosa* is difficult to fight because of biofilm formation, even more so in cases of multidrug resistant infections. Therefore, new strategies to inhibit biofilm formation, fight or control pathogens are much needed. We investigated the ability of enzymatic quenchers to inhibit both virulence and biofilm formation of CF clinical isolates of *P. aeruginosa*. We used two enzymes, which have been engineered to be highly stable and highly active against AHLs. Because *P. aeruginosa* is known to utilize two QS circuits, one using 3-oxo C12 AHL, the other C4 AHL, we used two enzymes with different substrate specificity. For instance, we used an enzyme that disrupts only the 3-oxo C12 AHL based circuit (Ssopox-W263I) and a second enzyme that disrupts both circuits (GcL) used by *P. aeruginosa* for QS. We tested these enzymes on 39 clinical isolates of *P. aeruginosa*, and demonstrated that Ssopox treatment reduced biofilm formation in 23% of the strains by up to 88%, elastase in 54% of the clinical isolates by up to 65%, protease activity in 28% of the strains by up to 69% and pyocyanin production in 33% of strains. These results are consistent with recently published data using Ssopox-W263I on *P. aeruginosa* isolated from diabetic ulcers. Interestingly, as the enzymes target specific AHLs, we saw a separate pattern of quenching with treatment using an alternative lactonase, GcL. This study demonstrates the ability of these engineered enzymes to disrupt the complex communication system utilized by *P. aeruginosa* so as to reduce virulence and biofilm formation. With future studies on mixed species communities and combination therapy with antibiotics, we expect to provide the key data to assess the potential of signal disruption to treat and prevent infections relevant to CF patients, transition to animal model studies, and delineate the importance of signaling in chronic lung infections.
Discovery of Novel Anti-MRSA Agents by High Throughput Screening of Pre-Metabolized FDA-Approved Drug Library

FDA approved drug screening has become a common strategy for drug repurposing. Drug metabolites frequently have distinct biological activities, as exemplified by the discovery of sulfanilamide as the active metabolite of Protosil. In this study, we described and evaluated the use of microsome metabolized FDA approved drug library screening for the discovery of novel antibacterial agents. Screens were performed against a gram-positive pathogen - methicillin-resistant staphylococcus aureus (MRSA). Initial efforts were hampered by the presences of substantial levels of bacterial contamination in microsomal preparations from several vendors, but an effective library metabolization protocol was developed which eliminated this problem. These screens reveal a number of known FDA approved drugs with enhanced antibacterial activity after metabolism. One of these - capecitabine - was fractionated after a scaled up metabolism reaction, which identified 5-fluoro-5’deoxyuridine (Doxifluridine) as its active metabolite.
**Background:** Biofilms are aggregates of microbial cells, which attach to biotic and abiotic surfaces as a mode of survival. Biofilm formation causes increased tolerance to antimicrobials and are responsible for a large amount of persistent human infections. With the growing problem of antibiotic resistance, there is a need to identify alternative antimicrobials to tackle biofilm-associated infections. Essential oils (EOs) are natural plant products, which have known antimicrobial properties. The focus of this research was to investigate the antibiofilm activity of cinnamon essential oil against *Pseudomonas aeruginosa* biofilms.

**Methods:** *Pseudomonas aeruginosa* PAO1 in planktonic cultures were first challenged with cinnamon (**Cinnamomum zeylanicum**) EO using agar disk diffusion and broth microdilution methods to determine minimum inhibitory (MIC) and bactericidal (MBC) concentrations. A time-kill assay was used to assess contact time required for different concentrations of the oil to inhibit the bacteria and imaging using scanning electron microscope (SEM) employed to elucidate possible mode of action. Static biofilms grown in microtitre plates were treated with cinnamon EO to determine minimum biofilm inhibition (MBIC) and eradication (MBEC) concentrations. In addition, using the CDC Biofilm reactor model, mature PAO1 biofilms were challenged with different concentrations of the EO to determine antibiofilm activity. **Results:** Inhibition and killing of planktonic *P. aeruginosa* was achieved by cinnamon EO at concentrations of 0.125% (v/v). Results from the time-kill assay indicate inhibition can be achieved in as little as 2 minutes of contact time when treated with cinnamon EO at 2% (v/v), and electron micrographs indicate cell damage and loss of turgor. The MBIC and MBEC found using microtitre plate growth methods were 0.125% (v/v) and 2% (v/v), respectively. This MBEC was also reflected in CDC reactor-grown biofilms when challenged with EO after only 10 min of contact time, resulting in a reduction of biofilm bacteria by log7 colony-forming units per mL. **Conclusions:** Cinnamon EO is an effective antimicrobial against *P. aeruginosa* PAO1, capable of rapid killing at low concentrations. Its effects against biofilms when in liquid phase is very promising, and this study provides a sound basis for further investigation of the potential of cinnamon essential oil as an alternative to conventional antimicrobial agents.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 014  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** Antimicrobial and Anti-biofilm Activity and Machine Learning Classification Analysis of Essential Oils from Different Mediterranean Plants against *Pseudomonas aeruginosa* and *Staphylococcus* spp  
**Author Block:** M. Artini, R. Papa, G. Vrenna, M. Bozovic, R. Ragno, L. Selan; Sapienza University, Rome, ITALY.

**Background:** Compounds of natural origin still provide a high number of interesting structures, even in this era of combinatorial chemistry. Essential oils (EOs) represent a group of antimicrobial agents which are complex mixtures of volatile secondary metabolites. EOs show antimicrobial and antifungal properties and are also largely used in various cultures for medical and health purposes. Within the same variety, the EO composition can vary according to geographical region and seasonality. EOs from a variety of plants are also endowed with antibacterial activities as well as anti-inflammatory and antioxidant properties. In this work we investigate the antibacterial and anti-biofilm effect of EOs from three different Mediterranean plants and *Pseudomonas aeruginosa*. **Methods:** *S. aureus* 6538P and 25923, *S. epidermidis* RP62A and O-47, *P. aeruginosa* PaO1 strains were used. EOs were obtained from plants harvested in different seasons and conditions so as to obtain a total of 89 different samples. The chemical composition of each EO was determined by GC-MS. Determination of MIC for each EO on the bacterial strains was carried out. The action on biofilm formation was assessed by crystal. **Results:** Reported results demonstrate that EOs with different antimicrobial and anti-biofilm features were selected. Some of them inhibit bacterial growth at high concentration and possess good anti-biofilm activity at very low sub-MIC concentration. Other EOs were able to destabilize biofilm structure without killing cells. Furthermore, quantitative activity-composition relationships (QCAR) were developed through machine learning classification approaches with objective of discovery the chemical components mainly responsible for the anti-biofilm activity. **Conclusions:** Biofilm growth of *P. aeruginosa* and staphylococci is influenced by the presence of EOs extracted from plants harvested in different seasons. Application of an in-house python based machine learning protocol led to definition of a classification model able to discriminate EOs in active and inactive at a cut-off value of 50% of biofilm formation. Investigation of the most important components by means of feature importance and partial dependence plots seems to indicate estragole and phellandral as the chemical components mostly related to biofilm inhibition, while d-limonene, pulegone, and chrysanthenone seem to be related to biofilm production. The classification model is an example showing machine learning as tool to investigate complex chemical mixtures. Results obtained could enable the identification of blends of EOs with strong anti-biofilm efficacy applicable in many fields: airborne decontamination, products for dermatological and respiratory tract infections, etc.
Characterization of Extracellular Polymeric Substances from Marine *Streptomyces* sp. Cu Off24 and their Anti-Biofilm Activity

K. K. P. Sivaperumal, R. Tilagaraj; SRM Institute of Science and Technology, Kattankulathur, INDIA.

Extracellular Polymeric Substances (EPS) producing marine actinobacterium has been isolated from a marine natural biofilm and identified as *Streptomyces* sp. CuOff24 through 16S rDNA sequencing. Approximately 450 mg L$^{-1}$ EPS were produced and major content of carbohydrate followed by protein, nucleic acid and unidentified compounds (68.6%, 12.3%, 10.4% and 8.7% respectively) were quantified. The spectroscopy study also confirmed the presence of carbohydrate functional groups on the EPS surface and GCMS showed that the EPS comprised of mannose (45.2%), glucose (28.8%) and arabinose (26%). The EPS exhibited antibiofilm activity against a multi-drug resistance pathogenic strain of *Staphylococcus aureus* and *Klebsiella pneumoniae*. The high Carbohydrate compounds in marine actinobacterial EPS and their antibiofilm sensitivity would be make it suitable for prospective therapeutic and industrial applications.
**Abstract**

**Background:** The dearth of therapeutic antibiotics to counter staphylococci biofilm underscores a critical demand for antibiofilm therapeutics. The present study reports the antibiofilm prospect of zinc complexing salicaldehyde- (C1) and napthaldehyde-based (C2) synthetic amphiphiles and illustrates the development of antibiotic-loaded micelles based on self-assembly of C1 that foster synergy of two warheads and inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm formation on surgical suture. **Methods:** C1, C2 and their zinc complexes were characterized by analytical techniques. Orthopaedic stainless-steel wire (SS wire) was coated with C1 and surgical silk suture was coated with rifampicin-loaded (C1M-R) and vancomycin-loaded (C1M-V) C1 micelles. Bactericidal activity against clinical *S. aureus* MRSA 100 was determined by MIC, cFDA-SE leakage assay and microscopy. Antibiofilm activity was ascertained by MTT assay, crystal violet assay and microscopy. Cytotoxicity on human embryonic kidney (HEK 293) cells was determined by MTT assay. All experiments were performed in triplicates. **Results:** The MIC of C1 and C2 against MRSA was 40 µM and 320 µM, which was also corroborated by the higher membrane-targeting activity of C1. The MBIC$_{50}$ of C1 and C2 against MRSA was 40 µM and 160 µM, while a reduction in cFDA and congo red staining validated a dose-dependent biofilm inhibition. Amphiphile-zinc complex was visualized by microscopy and characterized by ESI-MS, FTIR, ITC and EDX. At doses equivalent to MBIC$_{50}$ of C1 and C2, MRSA biofilm growth was nearly 80% and 110%, respectively, upon addition of 50 µM C$_{12}$O$_{2}$Zn, indicating the relevance of zinc-complexation of amphiphiles in biofilm inhibition. SS wire coated with 0.3% - 5.0% C1 decreased MRSA biofilm viability from 97% to 9% and was non-toxic to HEK 293 cells. MRSA biofilm grown on collagen in presence of 80 µM C1 exhibited only 15% metabolic activity and 25% biomass, highlighting the prospect of C1 as an antibiofilm agent for potential wound site application. Driven by self-assembly, C1M-R and C1M-V having a particle size of 182 nm and 381 nm were generated. The relative anti-MRSA activity of C1M-R was 12-fold and 18-fold higher and that of C1M-V was 6-fold and 5-fold higher following 3 h and 6 h interaction with the cells, in comparison to free antibiotic. FESEM analysis indicated prevention of MRSA biofilm formation on C1M-R coated silk surgical suture, while eluates from C1M-R and C1M-V coated sutures were non-toxic to HEK 293 cells (nearly 85% viability), indicating the biocompatibility of the coated sutures. **Conclusions:** In the light of limited therapeutic options, the prudently designed synthetic amphiphiles that deter biofilm formation by zinc complexation and also facilitate generation of antibiotic-replete micelles by self-assembly emerge as potentially therapeutic material with enhanced capabilities against MRSA biofilm.
Session Title: **TUESDAY Poster Session 2**

**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 017

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Antibiotic Loaded Bead Spacing is Important in Controlling *Pseudomonas aeruginosa* and *Staphylococcus aureus* Biofilms in Periprosthetic Infections: an *In vitro* Study

**Author Block:**

D. H. Dusane¹, J. Brooks¹, P. Laycock², S. Aiken², E. McPherson¹, A. Sullivan¹, J. Granger¹, P. Stoodley¹; ¹The Ohio State University, Columbus, OH, ²Biocomposites Ltd., Staffordshire, Keele, UNITED KINGDOM, ³LA Orthopedic Institute, Los Angeles, CA.

**Background:** Antibiotic-loaded calcium sulfate beads (CS-B) are used in the management of periprosthetic joint infections (PJI). PJI is one of the most devastating and costly complications following total joint arthroplasty surgeries. In quiescent areas of the bone joint space, diffusion will be limited and will curtail the spread of antibiotics; therefore both the quantity and distribution of beads are important to ensure optimal antimicrobial concentration and coverage at these quiescent areas to reduce infection.

**Methods:** Lawn biofilms of bioluminescent strains of *Pseudomonas aeruginosa* (PA) Xen41 and *Staphylococcus aureus* (SA) SAP231 were grown on Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar respectively for 24 h. CS-B (10cc) were loaded with vancomycin (1000 mg) and tobramycin (240 mg). Based on the clinical relevance, beads with vancomycin + tobramycin and tobramycin alone were used against lawn biofilms of PA and vancomycin + tobramycin against SA. CS-B were placed in different configurations on a 9-cm petri dish: as a single bead in the center; as 16 beads clustered in the center; 4 groups of 4 clusters or 16 beads equally spaced arranged in a circle and beads distributed hexagonally considering the optimal distance of the bead that provides a zone of inhibition. Bioluminescence was used to measure metabolic activity over time using an *in vivo* imaging system (IVIS) and replica plating was used to assess the bacterial viability by plating onto a sterile TSA or BHI petri dishes.

**Results:** After 24h of placing the antibiotic beads on the lawn biofilms of PA and SA, the lawn was cleared and inhibition zones appeared around the beads. The cleared distance was similar for both single and clusters of beads. The cleared area continued to grow outwards to the edge of the petridishes with all cluster arrangements. After 3 days of CS-B placement, resistant colonies appeared within the cleared zone in the case of PA with tobramycin and tobramycin + vancomycin beads. No resistant colonies were observed on SA petridishes. There was no colony growth within 1 cm of the beads suggesting high concentration of antibiotic close to the beads. Replica plating of these petri dishes suggested that this area was sterile. The beads placed in a circle killed more of the biofilm present within the petri dishes than when concentrated in clusters. Beads placed hexagonally completely killed the lawn biofilms.

**Conclusions:** This *in vitro* study suggests that i) antibiotic resistance may arise in *in vitro* lawn biofilms of PA after treatment with tobramycin or combinations of vancomycin + tobramycin and ii) the spacing of antibiotic-loaded CS-B may be an important consideration during the treatment of biofilm associated infections at the surgical site.
Abstract Title: A Study of the Interaction between Fluorescently Labelled Silica Nanoparticles and Biofilms of *P. fluorescens* and *P. putida*

Author: H. Devlin, C. Barros, S. Fulaz, D. Hiebner, L. Quinn, S. Vitale, E. Casey;
University College Dublin, Dublin, IRELAND.

Engineered nanoparticles (NPs) for antimicrobial delivery to biofilms is an area of increased interest, particularly in order to increase the effectiveness of such antimicrobials. Numerous studies over the past decade have demonstrated the advantages and challenges of the approach (1). However, the role of the biofilm EPS matrix in NP transport is still poorly understood. A major reason for this is the physical and chemical complexity of the biofilm matrix and its temporal and spatial variations. A wide range of physicochemical parameters influence the uptake and retention of nanoparticles within the biofilm matrix. These include nanoparticle size and charge properties, biofilm topography and EPS composition (2). These aspects must be taken into account when studying biofilm - nanoparticle interactions. In order to gain a better understanding of these interactions, a series of experiments were undertaken using two genetically modified bacterial strains: mCherry-expressing *Pseudomonas fluorescens* and GFP-expressing *Pseudomonas putida*. The biofilms were cultured in both microtiter plates and on glass slides for 24 - 72 hours followed by exposure to fluorescently labelled silica NPs with different size and surface charge properties. Using high throughput fluorescent intensity measurements and confocal laser scanning microscopy, it was possible to investigate the uptake of silica NPs by the two bacterial strains and gain valuable understanding of biofilm - nanoparticle interactions. It was observed that positively charged silica NPs showed a significant increase in uptake compared to negatively charged particles. The increased uptake was most likely caused by the stronger binding of the positively charged amine groups on the surface of the NPs with negatively charged components within the EPS. The NPs also demonstrated different attachment distributions at the biofilm-liquid interface as observed through confocal laser scanning microscopy, where the positively charged NPs were seen to form larger aggregates at the biofilm-liquid interface whereas the negatively charged NPs demonstrated a more evenly distributed profile within the biofilm with reduced aggregation. The findings of this research will help with the future design of nanoparticles for antimicrobial delivery with specific modes of action towards components in the EPS. 1. Ramos M, Da Silva PB, Sposito L, De Toledo LG, Bonifacio BV, Rodero CF, Dos Santos KC, Chorilli M, Bauab TM. 2018. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. *International Journal of Nanomedicine* 13:1179-1213. 2. Nevius BA, Chen YP, Ferry JL, Decho AW. 2012. Surface-functionalization effects on uptake of fluorescent polystyrene nanoparticles by model biofilms. *Ecotoxicology* 21:2205-2213.
Streptococcus mutans is known as a key pathogen to cause oral caries preventing in plaque biofilms as well. Although, numerous synthetic and non-synthetic agents have been evaluated as potential treatments for oral pathologies, a few have shown the clinical applicability. Therefore, development of new effective strategies is still highly desirable. To address the need for novel agents operating against this widespread oral pathogen, we have focused our attention on 10-undecynoic acid as the representative of the acetylenic fatty acids. Using macro-broth susceptibility testing method we established MIC value. The MBC value was determined from broth dilution minimum inhibitory concentration test by sub-culturing it to BHI agar plates that do not contain the test agent. Anti-biofilm efficacy was tested in 96-well coated with saliva plates using BHI broth supplemented with 1% sucrose as a standard approach. Based on obtained results MIC value for 10-undecynoic acid was established to be 2.5 mg/ml and the MBC value to be 5.0 mg/ml. The minimal biofilm inhibitory concentration that prevents biofilm formation in 90% revealed to be the same as MBC value and also showed to kill mature biofilm, causing at the same time approximately 30% eradication of pre-existing biofilm. Thus, we concluded that 10-undecynoic acid might play an important role in the development of alternative or adjunctive anti-caries and anti-biofilm preventive and/or therapeutic approaches.
Biofilms and biofouling causes major problems in several water-intensive industrial sectors (incl. food and beverage). In addition, in hospital environments contamination and biofilm transferred infections are of major concern. Prevention of biofilm formation helps operation of industrial processes and diminishes maintenance costs and losses. Microbes are developing tolerance for currently used biocides. Hence, new alternative compounds and control methods are needed to replace biocidal products. We examined efficacy of Finnish plant extracts to prevent quorum sensing and biofilm formation of Chromobacterium violaceum, Pseudomonas aeruginosa and Escherichia coli strains. Adhesion of microbes and formation of biofouling was determined with crystal violet staining and microscopy. In addition, efficacy of plant extracts to weaken and destabilize outer membrane of Gram-negative bacteria was examined by a fluorescence assay (NPN uptake). Finnish berry extracts (incl. cloudberry extracts) were shown to inhibit quorum sensing and biofilm formation of Gram-negative bacteria. In addition, phenolic plant extracts destabilized outer membrane of P. aeruginosa and E. coli.
Antibiofilm Strategies

Effect of Antimicrobial Peptide Hy-a1, and Synthetic Analogue Lys-a1, on Antimicrobial and Antibiofilm Activity of Chlorhexidine against *Streptococcus mutans* ATCC 25175

M. S. Magalhães¹, V. A. De Freitas¹, E. N. Lorenzón², E. M. Cilli³, V. A. Carneiro⁴; ¹Universidade Federal do Ceará, Sobral, BRAZIL, ²Universidade Federal de Goiás, Goiânia, BRAZIL, ³Universidade Estadual Paulista, Araraquara, BRAZIL, ⁴Centro Universitário INTA, Sobral, BRAZIL.

**Background:** Dental caries and periodontal disease are pathologies associated with plaque formation, as a complex microbial community organized in biofilm. This consortium of microorganisms adhered to biotic or abiotic surface covered by matrix polysaccharides, produced by themselves, confers several adaptive advantages, such as greater resistance to the action of antimicrobial agents. Chlorhexidine digluconate (CHX) is considered the gold standard in chemical control of oral biofilm, but several side effects do not indicate for long periods. Peptides with antimicrobial activity (AMP) are molecules that are part of the innate immunity of living beings and represent a new perspective of combat oral biofilms. This work aimed to investigate the effect of the AMP Hylin-a1 (Hy-a1), and synthetic analogue Lys-[Trp6]hy-a1 (Lys-a1), on antimicrobial and antibiofilm activity of chlorhexidine against *Streptococcus mutans* ATCC 25175.

**Methods:** Initially, antimicrobial activity (MIC and MBC) of Hy-a1 and Lys-a1 was determined by microdilution in broth against *S. mutans*. The synergistic activity between the AMP and CHX against the microorganism in question was evaluated by the checkerboard assay. Then, the antibiofilm activity of the Hy-a1/CHX and Lys-a1/CHX combinations was evaluated by treatment, 5 minutes of contact, against preformed *S. mutans* biofilm (12 h), in 96-well plates. The control was performed with CHX 0.12%. After the treatment, the metabolic activity and biomass from biofilm were analyzed using XTT reduction and crystal violet techniques, respectively.

**Results:** The tested strain presented sensitivity for both peptides, as well as to control test (CHX). The concentrations of MIC were 0.23 μg.mL⁻¹ for individual CHX and 15.60 μg.mL⁻¹ for the two peptides, Hy-a1 and Lys-a1. The native peptide showed bactericidal effect (MBC) at the same of MIC, while the synthetic analogue presented MBC only at 31.25 μg.mL⁻¹, twice MIC. The bactericidal effect of individual CHX was verified only at 7.80 μg.mL⁻¹. Both AMP showed a synergistic effect with CHX. Hy-a1/CHX reduced the individual antimicrobial activity of CHX up to 30-fold, while Lys-a1/CHX reduced about 17-fold. In addition, the Hy-a1/CHX exhibited high antibiofilm activity, reducing metabolic activity in approximately 60%, and keeping unaltered the biomass of biofilm. Similar results were obtained with control group (CHX 0.12%). According Lys-a1/CHX treated groups, did not showed differ for metabolic activity and biomass from biofilm when compared to untreated group.

**Conclusion:** In the end, the results suggest that the association between Hy-a1/CHX represents a major alternative to control of *S. mutans* ATCC 25175 biofilm formation than Lys-a1/CHX. This would provide a great reduction of the concentration of CHX used, which could avoid undesirable side effects from it.

**Keywords:** Biofilm. Chlorhexidine. Antimicrobial peptides.
**Background:** The resistance of clinical infections associated with biofilms is one of the fastest-growing issues in the medical field today. It is estimated that up to 80% of infections have some biofilm-related aspect to them. A biofilm can be defined as the multicellular phase that is not recognized in complex bacterial lifecycles. The biofilm is characterized by the bacteria's production of a complex and remodelable extracellular matrix composed of polysaccharides, proteins, and DNA/RNA. It was previously believed that the high antibiotic resistance of biofilms was due to low penetration of antibiotics into the matrix; this, however, was demonstrated to be incorrect. The antibiotic recalcitrance of biofilms has since been shown to be an active metabolic process (Nguyen et al. 2011). The relA gene product plays a key role in this active antibiotic resistance. RelA and other RSH enzymes (RelA/SpoT homologs) are ATP: GTP (GDP) pyrophosphate transferases. When activated by amino acid starvation, RelA/RSH produces the stringent response, which activates “magic spot” alarmones. These “magic spot” alarmones are hyperphosphorylated guanosine compounds (guanosine (penta)tetraphosphate, collectively dubbed [(p)pGpp]). The formation of these alarmones causes a major change in cellular metabolism, turning cells into a persister state. These persister cells upregulate some genes (such as chaperones, toxin/antitoxin systems, and oxidative stress protection) and downregulate other genes involved in cell wall synthesis, translation, and DNA replication. Essentially, all antibiotics function by inducing oxidative stress. A persister cell’s upregulation of oxidative stress defense enzymes allows persister cell to be resistant to over 1000x the concentration of antibiotics in comparison to their planktonic counterparts. **Methods:** Using the recently published cryo-EM structure of RelA, *in silico* docking experiments were performed with a library >10^6 compounds. These compounds have yielded many hits, which were then run through our pipeline assay system for validation of RelA inhibitors and their anti-biofilm properties as a standalone treatment and in synergy with antibiotics. This pipeline validation system includes *in vitro* and *in vivo* (pppGpp quantification, direct binding studies using surface plasmon resonance, and novel approaches to biofilm quantifications. **Conclusions:** These compounds have been successfully tested and validated for their ability to interrupt the stringent response and re-potentiate antibiotics for the treatment of infectious bacterial biofilms. A library of small molecule lead inhibitors has been discovered for optimization by means of medicinal chemistry. These compounds will now be used to perform structural activity relationships to better understand the RelA/RSH enzymes, and further, develop compounds for the treatment of biofilm infections.
Session Title: TUESDAY Poster Session 2
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 024
Abstract Topic: Antibiofilm Strategies
Abstract Title: Evaluation of Biofilms Recurrent Contaminations Under Food Industry-like Stresses
Author Block: M. Baron-Yusty1, M. Soto-Ros1, P. Fernandez1, J. Huertas-Baquero1, E. Garcia-Gutierrez2;
1Polytechnic University of Cartagena, Cartagena, SPAIN, 2Quadram Institute Bioscience, Norwich, UNITED KINGDOM.

Abstract Body:

**Background:** Consumers’ demand for nutrient-rich natural products has led to an increased consumption of fruit and vegetable-based beverages that are used as liquid meals. For sensory and microbial quality assurance, industrial production comprises a short thermal treatment, followed by a fast cooling stage that is maintained during the filling process right until consumption. However, rec-contaminations of the product have been reported, leading to subsequent economic losses and potential risk for consumers. Bacterial biofilms have been indicated as responsible for persistent contaminations in the beverage industry, as bacterial cells are able to survive thermal treatment within biofilms. Deterioration of the surfaces on the processing machinery is a source of recurrent contamination. It facilitates biofilm development by exposing surfaces and creating sheltered microenvironments where cleaning process become ineffective, allowing bacterial cells to attach and grow, even under very poor conditions. Thus, it is mandatory to develop protocols that mimic industrial processes to assess bacterial strains’ abilities to survive and re-contaminate. Here, industrial cleaning conditions were simulated and, as a proof of concept, we propose a pipeline for standardizing the evaluation of bacterial survival in biofilm contamination in liquid food industry.

**Methods:** Being a raising concern in the food industry, *Listeria monocytogenes* CECT 4032 was used as a model organism for its ability to form biofilms. Subject to standard processing conditions followed by thermal treatment, survival of *L. monocytogenes* CECT 4032 was evaluated under starvation conditions in diluted nutrient broth. Bacterial cells that grew under starvation were exposed to acidic and caustic cleaning to simulate an industrial cleaning process, after which the samples were grown again on diluted nutrient broth and growth curves were measured on a bioscreen C. After the incubation, selected wells were plated on PCA to calculate the bacterial population (CFU/mL).

**Results:** Results show that, when in biofilm, survival curves of *L. monocytogenes* CECT 4032 exhibit tailing phenomena. After thermal treatment, the probability of survival of at least one cell was high. Heat treated cells of *L. monocytogenes* had the ability to survive at 1:100 optimum nutrient dilution. The cells that survived thermal treatment and went through harsh acidic and caustic treatments were able to grow (4.67 CFU/mL). Final starvation conditions stage highlighted that after the whole treatment, cells of *L. monocytogenes* were still able to survive (3.88 CFU/mL).

**Conclusions:** Our results show that this evaluation pipeline is effective in predicting the ability of *L. monocytogenes* CECT 4032 to survive after undergoing the standard industrial cleaning process even with low nutrient concentration, and therefore, becoming a source of recurrent contamination.
Development of a Novel Antibiofilm Resin-based Silver Nanocoating for Titanium Alloy Med. Implants

A. Besinis, M. Ktoridou, S. Farrell-Adams, M. Upton; University of Plymouth, Plymouth, UNITED KINGDOM.

It is currently estimated that the use of titanium implants in the United States orthopaedic market is valued at $15 billion. The American Academy of Implant Dentistry (AAID) estimates that the value of the American and European market for dental implants alone will rise to $4.2 billion by 2022. Nevertheless, periprosthetic joint infection and peri-implantitis, both caused by adherence of bacteria to the implant surfaces, remain the major complications and aetiologies of implant failure. The hypothesis of this study was that application of a suitable antibacterial coating can hinder biofilm formation on the implant surfaces. A dimethacrylate-based resin carrying dispersed silver nanoparticles was synthesised and subsequently applied in the form of a nanocoating to the surface of medical grade Ti6Al4V implants. A range of different optimisation techniques were investigated to improve bonding between the nanocoating and the surface of the implants, which is necessary for resisting the mechanical stresses experienced during implantation. The nanocoatings were fully characterised using confocal laser scanning microscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy. Their antibacterial and antibiofilm performance was tested against Streptococcus sanguinis and was quantitatively assessed by taking microbiological colony forming unit (CFU) counts and measuring the lactate produced by the microbes over 24 h. Scanning electron micrographs were also taken to evaluate the antibiofilm efficacy of the nanocoatings. Application of the resin-based silver nanocoatings to the surface of the implants resulted in a 2-log reduction in the number of biofilm-resident bacteria and a 4-log decrease in the bacteria remaining viable in the surrounding culture media. The antibiofilm activity of the nanocoatings was further confirmed by SEM analysis showing considerably less bacteria attached to the surface of the coated specimens compared to the controls. The nanocoatings were found to be highly stable (>99.6%) maintaining their integrity in biological fluids. The current study concluded that the application of a resin-based silver nanocoating provides the platform for creating an antibiofilm surface for medical implants, medical devices and other biomedical applications. Nanocoatings containing metal nanoparticles are an attractive alternative antibacterial coating strategy as they are not affected by acquired resistance of bacteria to the currently available antibiotics.
**Session Title:** TUESDAY Poster Session 2

**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 026

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Lysozyme Reduces the Viability of *Enterococcus faecalis* in Biofilms

**Author Block:** J. A. Harris, C. N. Rouchon, A. J. Weinstein, K. L. Frank; Uniformed Services University, Bethesda, MD.

**Abstract Body:**

*Enterococcus faecalis* is a gram-positive, gastrointestinal commensal and a leading cause of healthcare-associated infections. *E. faecalis* infections are difficult to treat because the organism forms biofilms and is resistant to many antimicrobial agents, including the antimicrobial enzyme lysozyme. Lysozyme is a muramidase that catalyzes the hydrolysis of the linkages between the N-acetylmuramic acid and N-acetylglucosamine subunits that comprise the backbone of peptidoglycan. *E. faecalis* lysozyme resistance is stimulated through a signal transduction cascade that involves activation of an alternative sigma factor via cleavage of the associated anti-sigma factor by the transmembrane metalloprotease Eep. Under planktonic conditions, strains lacking *eep* are more sensitive than wild-type strains to growth inhibition by lysozyme. Since bacteria in biofilms gain resistance to high concentrations of antimicrobials through biofilm-specific mechanisms, we investigated whether *E. faecalis* ∆*eep* biofilms would remain differentially susceptible to lysozyme as compared to biofilms of the isogenic wild-type strain (*E. faecalis* OG1RF).

Unexpectedly, we found that a three-hour treatment with chicken egg white lysozyme was associated with an increase in stained biomass of equal magnitude for both strains and concurrent decreased biofilm cell viability of 99.8% and 99.9% for OG1RF and ∆*eep*, respectively. The bactericidal effect of lysozyme on *E. faecalis* biofilms of both strains was even more pronounced when recombinant purified human lysozyme was used. In contrast, three-hour treatment with the cell wall-targeting antibiotic ampicillin caused no changes in stainable biomass or cell viability of either strain. LIVE/DEAD florescence staining showed a higher percentage of dead cells in lysozyme-treated OG1RF and ∆*eep* biofilms relative to biofilms treated with buffer alone. These data demonstrate that *E. faecalis* biofilms are susceptible to killing by lysozyme in a manner that is independent of Eep protease. In addition, these results suggest that *E. faecalis* biofilm cells lyse following treatment with lysozyme, and the increased biofilm staining observed following lysozyme treatment may be due to the release of DNA from the lysed cells. Consistent with this, ~3-fold more extracellular DNA was measured in association with lysozyme-treated biofilms than with biofilms treated with buffer alone. Finally, we found that lysozyme was effective in reducing the number of viable cells in biofilms of several other laboratory and clinical *E. faecalis* strains, including a vancomycin-resistant strain. In conclusion, lysozyme has the potential to be developed into a new therapeutic that can reduce the number of *E. faecalis* cells at the site of an infection where a biofilm has formed.
Session Title: **TUESDAY Poster Session 2**
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 027
Abstract Topic: Antibiofilm Strategies
Abstract Title: Catalytic Mechanism of the Diadenylate Cyclase CdaA from *Staphylococcus aureus* and Identification of a Potential Allosteric Site
Author Block: R. Meneghello¹, N. C. Mesquita¹, H. J. Wiggers², M. V. Navarro¹;
¹University of Sao Paulo, Sao Carlos, BRAZIL, ²State University of Western Parana, Paraná, BRAZIL.

**Background:** Microorganisms, specially bacteria, integrate signaling pathways mediated by distinct nucleotide-based second messengers, including (p)ppGpp, cAMP, cGMP, c-di-GMP, c-di-AMP and c-AMP-GMP. In this context, cyclic dimeric adenosine monophosphate, c-di-AMP, emerged as a key signaling molecule modulating several cellular processes, e.g. DNA integrity, sporulation and carbon metabolism. C-di-AMP synthesis and degradation are mediated by specific enzymes bearing diadenylate cyclase (DAC) or phosphodiesterase (GdpP, PgpH) domains, respectively. The first characterized DAC-enzyme, the DNA scanning protein DisA, revealed the basic mechanism of c-di-AMP synthesis. Within DisA octameric assembly, four face-to-face DAC-domain dimers are properly oriented to conduct metal-assisted cyclization of ATP bound to each protomer. When DisA N-terminal DNA-binding domain recognizes branched DNA, DAC-domain undergoes conformational changes, inhibiting c-di-AMP synthesis and cell division progression. Besides DisA, DAC-containing membrane-attached, CdaA, and sporulation specific, CdaS, cyclases are widely spread in Gram-positive bacteria. CdaA contains a regulatory N-terminal YojJ domain and forms hexameric rings mediated by YojJ-YojJ and DAC-DAC interfaces. Although the same conserved DAC-DAC interface shared by CdaA and CdaS homologs is found in DisA, this is distinct from the one mediating catalytic competent face-to-face DAC-domain dimers.

**Methods:** X-ray crystallography, biochemical and biophysical experiments performed with wild-type and site-directed mutants were integrated to propose a molecular mechanism of c-di-AMP synthesis. Using compound libraries, we applied high-throughput X-ray crystallography and luminescence-based screenings to identify CdaA inhibitors. Those were further characterized through biochemical assays. Results: CdaA DAC-domain structures showed a catalytic incompetent dimer, where the active site of each protomer faces opposite directions. Enzymatic and oligomerization experiments confirmed that the interface observed in the crystal structures is essential for the enzymatic activity. Moreover, the binary complex ATP+Mn2+ was found to concomitantly bind at the active site. The fragment screening assays revealed a hit molecule that binds specifically at the dimeric interface. Conclusions: Our results sheds light on the mechanisms underlying c-di-AMP synthesis and the dimeric interface was found to be a novel target site for the development of new antibiotics, especially against *S. aureus*.
Bacteria form biofilms to promote their survival, adhesion and persistence in the environmental niches that they occupy. In the model organism Burkholderia thailandensis (a model for the virulent organism to Burkholderia pseudomallei), the bacteria can be induced to form biofilms and this production can be measured by various assays. We are seeking novel ways to interfere with biofilm production in this organism, including antimicrobial peptides, antimicrobial compounds and anti-microbial or anti-biofilm surface chemistries. Our long-term goal is to develop anti-biofilm approaches that will be effective against the biothreat agent Burkholderia pseudomallei. Here, we demonstrate that B. thailandensis can reliably form biofilm using one particular growth medium (MVBM) and that biofilm is not significantly produced using other growth media such as LB or Nutrient Broth, while bacterial replication demonstrates the opposite pattern. We demonstrate that this biofilm production can be quantitatively measured using crystal violet as well as other dyes for staining and quantitation. Anti-biofilm approaches such as antimicrobial peptides or antimicrobial compounds can inhibit biofilm production. We screened a large number of antimicrobial peptides and found that an antimicrobial peptide from our library of peptides has anti-biofilm effects against B. thailandensis in both the D- and L-enantiomeric conformation. In addition, we have screened a library of FDA-approved drugs and identified several compounds that have significant anti-biofilm activity against this organism. These compounds were found to inhibit biofilm production without significantly inhibiting bacterial growth. Methods for testing coupons/discs of materials with potential anti-biofilm or antimicrobial surfaces were developed to quantitatively assess their anti-biofilm potential, compared to untreated coupons/discs. We are also attaching antibiofilm peptides to surfaces to make the surface have enhanced antibiofilm properties. Overall, we are taking multiple approaches to combat biofilm formation in B. thailandensis, in order to potentially develop an anti-biofilm approach that could eventually work against B. pseudomallei. This project was supported by a grant from 4VA to Ducker and van Hoek.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 029  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** NX-AS-911 Modulates Quorum Sensing to Attenuate P. aeruginosa and S. Aureus Biofilms and Virulence  
**Author Block:** D. Neef, D. Williams, H. Taleb, M. Williams, J. Preece, A. Bernard, W. Nichols, G. Dixon; Neem Biotech, England, UNITED KINGDOM.

**Background:** Bacterial biofilms are implicated in pathogenesis, transmission, host immune evasion and the reduction in the efficacy of antibiotics during infection. As a critical factor in serious and chronic infection, the health burden is incalculable especially in chronic wounds and respiratory diseases. Routine antibiotic treatment required in biofilm associated diseases can accelerate the acquisition of microbial resistance in clinically relevant species such as S. aureus and P. aeruginosa. Quorum Sensing (QS) pathways regulate biofilm formation and maintenance of biofilm-associated infections through the secretion of virulence factors. Whilst biofilms can be dispersed by agents and penetrated to a limited extent by antibiotics, neither treatment inhibits QS. This is an unmet need in the treatment of serious and chronic infections. Here we present the activity of QS Inhibitor (QSI) NX-AS-911 and its effects on the formation and eradication of biofilms and the secretion of virulence factors in P. aeruginosa and S. aureus.

**Methods:** The effects of NX-AS-911 on bacterial biofilms were assessed using in vitro biofilm formation and eradication assays on P. aeruginosa and S. aureus. The inhibition of quorum sensing activity by NX-AS-911 in P. aeruginosa and S. aureus was assessed by measuring the expression of monitor strains PaO1 lasB-GFP and S. aureus spa-lacZ respectively. The modulation of virulence factor secretion by NX-AS-911 was assayed by investigating the secretion of extracellular toxins, proteases and hemolysins in S. aureus and rhamnolipid and alginate in P. aeruginosa.

**Results:** NX-AS-911 is shown to inhibit the level of QS exhibited by both P. aeruginosa and S. aureus and concomitantly reduces the formation of biofilm in both bacteria. Eradication assays illustrate that pre-treatment with NX-AS-911 enhances the susceptibility of P. aeruginosa and S. aureus to both antibiotic and antiseptic treatment. Virulence assays demonstrate a correlated reduction in QS regulated virulence factor secretion.

**Conclusion:** NX-AS-911 inhibits quorum sensing, and the formation and maintenance of stable biofilms by, Pseudomonas aeruginosa and Staphylococcus aureus. NX-AS-911 re-sensitizes bacteria in biofilms to antibacterial agents and reduces secretion of virulence factors.
**Backgrounds:** Bacteria are well known to form complex multicellular structures on solid surfaces, known as biofilms, to survive in harsh environments. A hallmark characteristic of mature biofilms is the high-level antibiotic tolerance (up to 1000 times) compared to planktonic cells. However, the antibiotic susceptibility of the sessile cells during early-stage biofilm formation is not fully understood, hindering the development of new technologies for effective biofilm control. This study was motivated to systematically investigate the role of cell growth, extracellular matrix production, and cell-cell interaction in antibiotic susceptibility of attached cells. **Method:** To investigate the change in bacterial antibiotic susceptibility during early-stage biofilm formation, *Escherichia coli* cells were harvested every 30 min during the first 7 h biofilm formation in Lysogeny Broth (LB) and subjected to 1 h treatment with 200 µg/mL ampicillin or 5 µg/mL ofloxacin at 37°C. Antibiotic susceptibility of sessile or detached biofilm cells was quantified using colony forming units (CFU) and LIVE/DEAD® BacLight bacterial viability staining, and compared with that of planktonic cells in static biofilm cultures. Patterned biofilms were used to quantitatively study the role of cell-cell interaction in bacterial antibiotic susceptibility during early-stage biofilm formation. **Results:** We found that biofilm cells were not always more tolerant to antibiotics than planktonic cells in the same culture. During the first 2.5 h after inoculation, the antibiotic susceptibility of initially attached biofilm cells increased over time and was higher than that of planktonic cells due to active cell-surface and cell-cell interactions. After reaching the peak at 2.5 h, biofilm antibiotic susceptibility decreased when the attached cells started to produce biofilm matrix. However, the embedded biofilm cells remained active and thus more sensitive to antibiotics compared to planktonic cells if dispersed from the surface. By controlling the cell cluster size using patterned *E. coli* biofilm formation, cells involved in the interaction between cell clusters were found to be more susceptible to antibiotics than cells embedded in clusters. **Conclusions:** Collectively, the results from this study indicate that biofilm formation involves active cellular activities in adaption to the attached life form and interaction between cell clusters to build the complex structure of biofilms, which render these cells more susceptible to antibiotics. These results demonstrate that biofilm cells are not always more tolerant to antibiotics than planktonic cells in the same culture, which may help design better materials and surfaces to reduce biofilm formation and eradicate colonized bacterial cells.
The majority of microorganisms in nature, including those responsible for hospital-acquired infections and fouling of industrial processing equipment, live in association with surfaces as biofilms. Due to the secretion of proteins, extracellular DNA and lipopolysaccharides, biofilm communities are encased in a robust matrix which reduces their susceptibility to antimicrobial agents. The long term goal of my research project is to develop efficient, prodrug antimicrobial reagents that are able to permeate the biofilm matrix, as well as the cell membrane in order to eradicate biofilm colonization. This began with the literature review of simple phenolic essential oils that exhibit antimicrobial activity, followed by functionalization to increase potency. The minimum inhibitory concentration and biofilm eradication concentration for each essential oil and each derivative were then evaluated. Upon functionalization with allyl appendages, potency towards planktonic cells increased but decreased towards biofilms. Although it was found that trichloromethyl sulfonyl ester appendages did increase potency towards both planktonic cells and biofilm formations. A prodrug is a compound that is biologically inactive until metabolized. This design will serve two functions for these compounds; to increase permeability towards the biofilm matrix as well as the cell membrane and to achieve cellular retention of the compound. Ester appendages are being placed on select compounds to implement this prodrug design. Ester groups are commonly used in cellular dyes such as Calcein AM, where once inside the cell the ester groups are cleaved, and the resulting dye is trapped in the cell. Similarly after esterase cleavage, the antimicrobial compound will be negatively charged and become concentrated within the cell. A number of structurally diverse ester functional groups are being applied to selected compounds which have shown varying amounts of increased or decreased potency when compared to parent compounds. It is our hypothesize that the efficacy of antimicrobial agents towards biofilms will be efficiently restored through this strategic design and synthesis of derivatives with modulated polarity that are engineered to have high levels of cellular retention upon undergoing a cleavage event in the cell. This new class of prodrugs presents a wide array of potential applications, from the control of biologically induced corrosion to the incorporation into household cleaning products.
Session Title: TUESDAY Poster Session 2
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 033

Abstract

Topic: Biofilm Antimicrobial Tolerance
Title: Giving Up Attachment: Biofilms in Food Production Environments
Author Block: G. Dev Kumar¹, A. Barlow¹, D. Macarisin²;
¹University of Maryland, College Park, MD, ²FDA, College Park, MD.

Abstract Body:
The food industry is severely impacted by biofilm communities. Biofilm development by foodborne pathogens on food contact surfaces and processing facilities jeopardizes a safe food supply. Mitigation of bacterial biofilms is challenging because bacterial biofilms are comprised of a diversity of cellular phenotypes, such as slow growing or transient filamentous states with a decreased susceptibility to sanitizers. The current study evaluated several dye-retention methods to characterize the dynamics of biofilm formation by foodborne pathogens. The same staining techniques were employed to quantify bacterial biofilm mitigation by commonly used industrial sanitizers, peroxyacetic acid (PAA) and quaternary ammonium compounds (QAC). Laser scanning confocal microscopy (LSCM) was used to characterize phenotypes and exopolymeric substance (EPS) presence. It was observed that crystal violet staining is not representative of the quantity of EPS in Listeria monocytogenes and Salmonella enterica and does not correlate with the population of cells embedded in the biofilm matrix. PAA was significantly (P≤0.05) more efficient in controlling L. monocytogenes biofilms regardless of the strain origin, clinical, food or environmental. In Salmonella enterica, transient mono- and multicellular phenotypes were observed within the biofilm matrix, indicating different outcomes upon release from the biofilm matrix. LSCM analysis revealed the formation of multilayer cellular conglomerates embedded into EPS and not attached to surfaces. This observation indicates the ability of foodborne pathogens to form unbound pellicle-type biofilms that are free-floating in the fluid. Furthermore, the ability of enteric pathogens to form untethered biofilms represents considerable contamination risk for food processing facilities.
Session Title: **TUESDAY Poster Session 2**
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 034
Abstract Topic: Biofilm Antimicrobial Tolerance
Abstract Title: The Cell Wall Binding Domains of PlyG and PlyL are Highly Specific for *Bacillus Anthracis*
Author Block: D. Kemboi; University of Maryland-College Park, Rockville, MD.
Abstract Body: Endolysins are enzymes used by bacteriophages to digest the peptidoglycan and release their newly packed phage particles to infect other cells. These enzymes have two domains; the Enzymatic active domain (EAD) that cleaves the peptidoglycan and the cell wall binding domain (CBD) that attaches to the receptors on the cell surface. In most cases, the CBDs bind to the receptors independently of their EAD. In this study, phage lysin G and L (PlyG and PlyL) CBDs have shown tight and specific binding to the bacterial surface of *Bacillus anthracis*, a Gram-positive bacteria which is the etiological cause for Anthrax. Because of their specificity and high activity towards bacteria, endolysins have a high potential as the next generation of antimicrobials.
Nutritional Composition, Biochemical Characteristics & Antimicrobial Analysis of Plant Seed and Nut Oils

O. D. Yangomodou; University of Salford, Manchester, UNITED KINGDOM.

ABSTRACT

Plan
t oil significantly inhibited biofilm growth of S. aureus ATCC 6538 and MRSA 252 in a concentration-dependent manner more effectively than cashew oil. Walnut oil inhibited the growth of S. aureus biofilm formation at a concentration of 3.125 mg /ml. Pumpkin oil inhibited the growth of S. aureus biofilm formation at a concentration of 6.25 mg /ml. Walnut oil & pumpkin oil at 3×MIC effectively eradicated the established biofilm of ATCC 6538 and MRSA 252. Antibiotic susceptibility test result showed that S. aureus ATCC 6538 and MRSA 252 are susceptible to pure oil samples of walnut & pumpkin as well most conventional antibiotics tested. A clear zone of 20.02 ± 0.05 and 25.02 ± 0.01 was obtained for pure walnut oil. Antibiotic susceptibility test result showed that S. aureus ATCC 6538 and MRSA 252 are susceptible to pure oil samples of walnut and pumpkin as well most conventional antibiotics tested These oils could be used as alternatives to conventional antibiotics for the treatment of S. aureus infections.
Many bacteria live as populations on surfaces forming three-dimensional structure, called biofilms. Biofilms are bacterial populations covered by self-produced matrix of extracellular polymeric substances (EPS), and show different characteristics compared to the planktonic cells. Inside biofilms, microenvironments are formed that leads to the heterogenic gene expression and physiology of the cells. In addition, it has been reported that various spontaneous mutants can frequently be isolated from biofilms. Spontaneous mutations can be problematic by giving rise to multidrug-resistant bacteria in clinical settings. In this study, we focused on elucidating the emergence of spontaneous mutants from biofilms by using imaging analysis. In *Pseudomonas aeruginosa*, it has been reported that RecA is required for the spontaneous mutation in biofilms. However, the spatiotemporal pattern of this event is largely unknown. Here we used recA promoter reporter plasmid to detect the onset of DNA damage that would lead to mutation. *P. aeruginosa* PAO1 carrying the recA promoter reporter plasmid was observed in a flow cell system using a confocal laser microscope. Our results show that recA-expressing cells are mainly present in the bottom of biofilm. Since recA is induced by DNA damage, our data suggest that cells are more exposed to genotoxic stress at the bottom of the biofilm compared to the upper part. Oxygen gradient and accumulation of metabolite may cause these genotoxic stress. Future work will aim to elucidate this mechanism. We also show that the recA-expressing cells can be sorted from the rest of the population by using a cell-sorter. This will allow us to further characterize the cells and elucidate the consequence of the heterogeneous gene expression.
Abstract Title: SicA-dependent c-di-GMP Signaling Contributes to *P. aeruginosa* Biofilm Drug Tolerance in a SagS-dependent Manner

Author: B. Poudial, K. Sauer; Binghamton University, Binghamton, NY.

A hallmark of biofilms is their heightened tolerance to antimicrobial agents. While the nature of this tolerance has been deemed multifactorial and linked to biofilm biomass accumulation, recent findings suggest a role of c-di-GMP. However, no c-di-GMP modulating enzyme(s) contributing to the drug tolerance phenotype of biofilms has been identified. Here, we made use of RNA-seq to identify genes encoding diguanylate cyclases that are expressed in a biofilm-specific manner. Transcript abundance was confirmed by qRT-PCR. Strains inactivated in diguanylate cyclases were analyzed for biofilm formation, antibiotic susceptibility, and cellular levels of c-di-GMP. Using RNA-seq combined with biofilm formation and drug susceptibility assays, we identified SicA as an active diguanylate cyclase, with *sicA* inactivation correlating with significantly reduced c-di-GMP levels present in *P. aeruginosa* biofilms, and biofilms being rendered biofilms susceptible to antimicrobial agents. SicA was found to contribute to biofilm tolerance in a manner dependent on SagS, BrlR, and a small stress-related protein. While SicA contributed to biofilm drug tolerance, *sicA* inactivation had no effect on attachment and biofilm formation. Our findings suggest biofilm drug tolerance to be linked to a specific c-di-GMP modulating enzyme, SicA, with the pool of SicA-generated c-di-GMP only contributing to biofilm drug tolerance but not to biofilm formation. Moreover, our findings suggest SicA to be SagS-dependent.
**BACKGROUND:** Nontypeable *Haemophilus influenzae* (NTHi) is a predominant pathogen of otitis media (OM) globally, and the most common cause of chronic and recurrent OM. NTHi chronicity is due to the formation of highly recalcitrant bacterial biofilms. Upon entry into the middle ear, NTHi encounters a pH 7 climate; however, as the infection progresses, the conditions become pH 9. To adapt to changes in microenvironmental conditions, NTHi uses a novel genetic system, termed the phasevarion (phase variable regulon), to adapt to external factors. The NTHi phasevarion acts as a reversible, ON/OFF switch that regulates the expression of multiple genes. Previously, we demonstrated that the ModA2 phasevarion of NTHi strain 723 regulated biofilm formation based on microenvironmental conditions relevant to OM. Due to the known antibiotic resistant nature of biofilms, we hypothesized that the ModA2 phasevarion also influences antibiotic resistance.

**METHODS:** Biofilms formed by NTHi strain 723 *modA2* ON or OFF variants at 37°C/pH 7 or 37°C/pH 9 were treated with 1000 times the planktonic MIC of ampicillin, cefdinir, or amoxicillin/clavulanic acid, all of which are broad spectrum antibiotics commonly used to treat OM. We determined the relative antibiotic sensitivity of planktonic bacteria, bacteria resident within the biofilm and newly released bacteria disrupted by antibodies against a bacterial DNABII-binding protein, integration host factor (α-IHF), or dispersed by antibodies that target the NTHi type IV pilus (α-rsPilA). We examined biofilm architecture and relative percentage of live bacteria via viability stain. **RESULTS:** Antibiotic treatment of 24 hr biofilms formed by NTHi strain 723 revealed phasevarion-dependent differences in relative antibiotic sensitivity between *modA2* ON and *modA2* OFF variants. To assay the relative sensitivity of newly released NTHi, we determined the time point at which either α-IHF or α-rsPilA mediated disruption occurred at pH 7 or pH 9 for the ON and OFF variants. Ongoing work examines relative antibiotic sensitivity of ON and OFF variants post-biofilm disruption by either α-IHF or α-rsPilA. **CONCLUSIONS:** Biofilm contributes to antibiotic resistance; however, the role of the phasevarion in this resistant phenotype has yet to be determined. Our data demonstrates the importance of the ModA2 phasevarion in relative antibiotic resistance of planktonic versus biofilm-resident bacteria in ON and OFF variants. We are currently assessing the relative antibiotic sensitivity of ON versus OFF variants that have been newly released from a biofilm by the action of α-IHF or α-rsPilA given that newly released NTHi reveal markedly increased sensitivity to antibiotics. The work presented herein is designed to give insight into the role of the phasevarion in adaptation to environmental changes induced by exposure to broad spectrum antibiotics.

Support: NIH/NIDCD R01DC015688
Mechanical Instability and Interfacial Energy Jointly Drive Biofilm Morphogenesis

J. Yan, C. Fei, S. Mao, A. Košmrlj, N. S. Wingreen, B. L. Bassler, H. A. Stone;
Princeton University, Princeton, NJ.

Surface-attached bacterial communities called biofilms display a diversity of morphologies. Biological components required for biofilm formation are known, however, not if or how these essential constituents promote biofilm morphological features. Here, we combine mechanical measurements, mechanical modeling, quantitative image analyses, surface energy characterizations, and mutagenesis, to show that mechanical instabilities, including wrinkling and delamination, underlie the morphogenesis program of growing biofilms using *Vibrio cholerae* as our model system. We discover that interfacial energy is the key driving force for mechanomorphogenesis because it dictates the generation/annihilation of new/existing interfaces. Finally, we find that feedback exists between mechanomorphogenesis and biofilm expansion, which promotes an ordered set of sequential steps that yield the morphological pattern and shape the overall biofilm contour.
In natural environments like aquifers, lakes and within artificial enclosures like pipes/reactors; the lifecycle of biofilms are influenced by large physical forces resulting from aperiodic or periodic flows. The ability of biofilms to withstand such harsh environments is often attributed to their rheological resilience. However, until recently our insights into the rheological behaviour of biofilms have been mostly limited to studies undertaken at small strain amplitudes. In ecologically and industrially relevant scenarios, where large strain rates predominate; the biofilm response is nonlinear and characterization of these behaviours remains an unexplored challenge. Our experiments investigate the nonlinear behaviour of three different species of bacterial biofilms: *Bacillus subtilis*, a common soil dwelling microbe, *Pseudomonas fluorescens*, a biocontrol agent as well as a plant root coloniser and *Comamonas dentrificans*, which is abundant in activated sludge tanks. By using a rheometer operating in strain controlled mode and exploring the Pipkin space of strain amplitude (Weissenberg number) and frequency (Deborah number) we unravelled each biofilms’ distinct rheological response. By performing stress decomposition we are able to construct a series of elastic and viscous Lissajous plots that represent the state of the biofilm in Pipkin space. Furthermore, using Chebyshev polynomial analysis and by calculating the large/minimum strain moduli we show that intra cycle strain hardening and shear thickening are species dependent characteristics. Analysis of the elastic energy hysteresis plots show that certain species are more resilient (dissipate less energy) than others. In addition, by employing Sequence of Physical Processes (SPP) we reveal insights into the dynamic yielding and reformation events in each species of biofilm. Together these tools allow us to decipher distinct material characteristics that are akin to a unique human fingerprint (hence the term rheological fingerprint) and opens up new avenues to explore genetic/physico-chemical effects that result in alteration of the rheological properties of biofilms. In the future we also plan to consider how the species-specific properties of pure cultures will translate into the multispecies systems occurring in natural environments.
Bacteria respond to many chemical stimuli by using dedicated sensory components. However, the signals that activate many of these sensors have not yet been identified. In their natural environments, bacteria experience a variety of mechanical forces arising from their interaction with surfaces and flow. These mechanical cues have been rarely explored as regulator of bacterial decision-making. This is potentially due to a technological bottleneck, as there have only been little development of new tools for bacterial mechanobiology. Therefore, there is a need for in vitro systems that recapitulate the mechanics experienced by single cells in their natural ecological niches. Here, we present a toolbox dedicated to mechanomicrobiology studies. The purpose of each tool in the box is to faithfully reproduce key mechanical aspects of the bacterial environment during host infection. This includes (i) hydrogels mimicking the stiffness of eukaryotic cells and surrounding host tissues, (ii) microfluidics reproducing the flow of body fluids, (iii) surface receptors promoting attachment and affecting adhesion forces, and (iv) lipid bilayers mimicking the membrane of eukaryotic cells. More precisely, we leverage PEG-based hydrogels to reproduce relevant ranges of substrate stiffness encountered by single cells. These hydrogels are well defined chemically and mechanically, enabling the exploration of a large mechanical space, as well as spatiotemporal control of polymerization and incorporation of chemical moieties. We can implement hydrogel layers within microfluidic channels enabling the simultaneous study of the effect of fluid flow and elasticity of the substrate on phenotypes and cellular behaviors. As a proof-of-concept of this toolbox, we will present examples of mechanically-dependent modulation of *Pseudomonas aeruginosa* cellular behaviors. First, we show that surface specific twitching motility trajectories depend on the stiffness of the substrate. We could observe that higher stiffness promotes longer trajectories, likely due to a more efficient mechanical coupling of type IV pili with the substrate. Second, we show evidence that biofilm architecture depends on substrate stiffness. Biofilms growing on soft substrates contains heterogeneous bacterial clusters whose position is determined by initial attachment sites, while on hard substrate biofilms appear uniform. These distinct biofilms architectures can be attributed to differences in initial pili-dependent surface exploration. In summary, our approach is successful in reproducing key mechanical features of the natural environment of microbes, while enabling real-time monitoring of bacterial behavior at the single cell level. We thus hope our toolbox will stimulate the microbiology community to further investigate the role of mechanical forces in bacterial physiology.
Abstract

Understanding the material and mechanical properties of biofilms is crucial to developing future treatments for persistent chronic infections. Particle-tracking passive microrheology using individual bacteria as tracers was employed to investigate the viscoelasticity of *Staphylococcus aureus* biofilms grown in microfluidic cells that were subject to increasing hydrodynamic shears, specific biofilm-degrading enzymes and reduced-nutrient media. In this study, we have found that biofilm creep compliance (related to the inverse of viscosity) increases with height from the attachment surface and decreases universally in the presence of hydrodynamic shear. The addition of Proteinase K to the culture media substantially softened the biofilm, whereas DNase I showed no significant effects over the first 6 hours of growth. Spatial analysis of bacteria within the biofilm using Ripley’s K-function revealed biofilms under flow tend to spatial randomness with maturation at all heights, compared to static biofilms that remain statistically ‘clustered’. Reduced-nutrient media suggests a structural response by the biofilm to produce amyloid fibres, which have been imaged using super-resolution Stochastic Optical Reconstruction Microscopy (STORM).
Semi-flexible polymer networks have been emerging as promising bio-inspired materials in the application of underwater adhesives, biosensors, structural materials, and scaffolds for tissue engineering. Although the mechanics of semi-flexible networks has been investigated, their underlying adhesion mechanisms are not well understood. Understanding the structure-property relationship of semi-flexible polymer networks is important to advance the design of biomaterials based on semi-flexible polymer networks. A coarse-grained model of cross-linked semi-flexible polymer networks is developed to investigate the network mechanical and adhesive properties and this model is characterized by tunable crosslink density, polymer Young’s modulus and bending rigidity (persistence length). Our simulations reveal that an increase in network stiffness caused by a larger polymer persistence length or a larger crosslink density leads to a decrease in the interfacial energy between the network and the surface. While the work of adhesion (energetic cost to detach a material from a surface) of stiff materials such as glassy polymers is almost equal to the interfacial energy, the work of adhesion of deformable cross-linked polymer networks has to overcome not only the interfacial energy but also considerable absorbed strain energy. Networks with larger polymer persistence lengths leads to desirable lighter mass density and larger pore size, but at the expense of interfacial energy and work of adhesion. There is an optimal polymer persistence length to reach maximum work of adhesion normalized by network mass density. While network mass density and interfacial energy are insensitive to the change in crosslink density, networks with fewer crosslinks exhibit a smaller elastic modulus, absorb more strain energy during desorption and possess larger work of adhesion. However, if the crosslink density is lower than a critical limit, networks cannot maintain structural integrity under uniaxial stretching, despite that they exhibit slight variation in storage modulus under dynamic mechanical analysis. Our finding provides physical insight into the adhesive mechanisms of biofilm and shed light on the design of biomaterials based on semi-flexible polymer networks.
Membranes Bacterial Communities for Haloalkaliphilic Sulfate-reducing Bioreactors Revealed by 16S rRNA MiSeq Sequencing

J. Xing
Institute of Process Engineering, Chinese Academy of Sciences, Beijing, CHINA.

Biological technology used to treat flue gas is useful to replace conventional treatment, but there is sulfide inhibition. However, no sulfide toxicity effect was observed in haloalkaliphilic bioreactors. The performance of the ethanol-fed bioreactor was better than that of lactate-, glucose-, and formate-fed bioreactor, respectively. To support this result strongly, Illumina MiSeq paired-end sequencing of 16S rRNA gene was applied to investigate the bacterial communities in the membranes. A total of 389,971 effective sequences were obtained and all of them were assigned to 10,220 operational taxonomic units (OTUs) at a 97% similarity. Bacterial communities in the glucose-fed bioreactor showed the greatest richness and evenness. The highest relative abundance of sulfate-reducing bacteria (SRB) was found in the ethanol-fed bioreactor, which can explain why the performance of the ethanol-fed bioreactor was the best. Different types of SRB, sulfur-oxidizing bacteria, and sulfur-reducing bacteria were detected, indicating that sulfur may be cycled among these microorganisms. Because high-throughput 16S rRNA gene paired-end sequencing has improved resolution of bacterial community analysis, many rare microorganisms were detected, such as Halanaerobium, Halothiobacillus, Desulfonatronum, Syntrophobacter, and Fusibacter. 16S rRNA gene sequencing of these bacteria would provide more functional and phylogenetic information about the bacterial communities. Keywords: Haloalkaliphilic, Sulfate reduction, MiSeq, Bacterial community
Bacteria have a tendency to assemble in a matrix-encapsulated multicellular community called a biofilm. Biofilms form when planktonic (free-swimming) cells attach to a surface (living or inert) and secrete a polymeric substance known as the exopolymeric matrix. Biofilm bacteria are more resistant to antibiotics and the immune response of a host compared to planktonic (free-swimming) bacteria. Biofilms thus contribute to making pathogenic bacteria more persistent and threatening. Many studies have shown that biofilm development is affected by nitric oxide (NO), a diatomic gas molecule with well-understood signaling roles in mammalian cells. Data from our laboratory suggest that a novel NO sensing hemoprotein (NosP) initiates the biochemical signaling pathway that eventually leads to biofilm dispersal in many bacteria. While NosP exhibits similar biochemistry to other, better-studied hemoproteins, the structure of the protein, including details of the heme-binding site, is still unknown. We seek to gain knowledge of the structure of the protein using Raman spectroscopy and X-ray crystallography. This study will yield insight into the structural changes in NosP that occur during NO signaling.
Light- and Temperature-controlled Behavioral Patterns in *Pseudomonas aeruginosa* biofilms

**Author:** L. J. Kahl1, D. K. Morales2, L. Dietrich1;

1Columbia University, New York, NY, 2Weill Cornell Medical College, New York, NY.

**Background:** Most organisms are subject to Earth’s 24h day-night cycle. Rhythmic behaviors that anticipate daily changes in light and temperature are prevalent among both phototrophic and non-phototrophic organisms, but have not been described for non-phototrophic bacteria. We hypothesize that, during infection, pathogens could sense and respond to oscillations in light exposure or temperature in ways that facilitate survival. We are using a biofilm model of the pathogen *Pseudomonas aeruginosa* to probe this theory. The biofilm lifestyle contributes to persistence of *P. aeruginosa* infections. *P. aeruginosa* cells in biofilms employ various metabolic and structural mechanisms to maintain redox homeostasis in the face of limited oxygen availability. We are examining the physiological changes that occur in response to cyclic changes in light exposure and temperature, with a focus on redox metabolism as it relates to survival during biofilm growth.

**Methods:** We used the dye triphenyl tetrazolium chloride (TTC) to examine the respiratory activity of growing colony biofilms when exposed to 24h cycles of light/dark switching and a +/- 1°C temperature variation. RNAseq analysis was performed on samples isolated from biofilm growth that occurred during the light or dark intervals of this cycling. A targeted genetic screen was conducted to identify genes required for TTC ring formation. 

**Results:** Colony biofilm growth on medium containing TTC led to the formation of concentric, alternating rings of TTC reduction and indicated that respiratory activity is higher during growth in the dark (corresponding to a slightly lower temperature) relative to growth in the light. The RNAseq analysis comparing dark (-1°C) and light (+1°C) biofilm growth revealed a higher than 2-fold change in expression for roughly 10% of the genome. Central pathways such as oxidative phosphorylation, purine metabolism and the pentose phosphate pathway showed strong transcriptomic changes, correlated with phases of “light” or “dark” growth. Intriguingly, different terminal oxidase complexes, which allow *P. aeruginosa* to carry out aerobic respiration under specific conditions including low oxygen tension, were differentially regulated in response to the light/dark and temperature cycling. The genetic screen revealed two putative sensors for different wavelengths of the visible spectrum that are important for reduced TTC ring formation under conditions of light/dark cycling.

**Conclusions:** Since many aspects of host physiology, including immune activity and body temperature, are regulated by the host’s biological clock, we theorized that *P. aeruginosa* has evolved behaviors that anticipate rhythmic host activities and facilitate survival during infection. The phenotypic responses to cyclic light/dark switching and temperature variation described here could constitute adaptations to the host-associated lifestyle.
Many microbial infections in the body, including dental plaque, involve biofilms. Experiments focused on mimicking the removal of tooth associated biofilms have demonstrate distinct ripple patterns. We use this data to develop and validate theoretical models that describe the basic physics of the process. Additionally, insight gained from analyzing the physical process of rippling can lead to methods to enhance oral biofilm removal. We use a multiphase model that treats the biofilm as a mixture of two materials. By direct numerical simulation we show the fully nonlinear model provides accurately reflects the relationship between the apparent wavelength and the external forcing velocities. We also show how the model can be used to optimize the design of tooth cleaning devices.
Microbial biofilms are present on aircraft parts and can contribute to biodegradation of coatings and insulation. Lipases are industrially-relevant enzymes of which a subset contribute to polyurethane degradation of these aircraft coating materials. We devised a strategy to mine for microbial lipases in a cultivation-independent approach by leveraging structural domain homology using Hidden Markov Modeling (HMM). Microbial lipases cluster by sequence similarity and by the presence of catalytic residue motifs and structural lid domains; all of which contribute to function, specificity, and stability. While many lipases have been identified from cultured isolates, not all environmental isolates are easily grown in culture due to nutrient, symbiotic, or other as yet unknown growth requirements. We used lipase query sequences from experimentally verified polyurethane (Impranil®)-degrading Pseudomonas species to bioinformatically interrogate as a proof-of-concept publically-available environmental metagenomes. From marine metagenomics libraries, we recovered lipase homologs which harbored the key catalytic residues. One of the recovered homologs was from a hydrothermal vent microorganism, suggesting that this particular lipase may be thermostable. The predicted structure for the putative hydrothermal lipase aligned well with the Pseudomonas lipase structure and lid domain, critical to lipase activity. Many of the other recovered lipases were derived from uncultivated bacterial lineages or were annotated as ‘hypothetical’, stressing the value of this agnostic mining approach. We constructed and tested a synthetic plasmid construct that successfully expresses lipases in a Pseudomonas heterologous host. Constructs are tested for polyurethane-degrading activity using biochemical assays developed in our laboratory. Ultimately, our goal is to apply this mining approach to mine aircraft-specific metagenomics and metatranscriptomics libraries. Developing an HMM domain-centric based pipeline offers an optimized approach for assessing aircraft biofilm biodegradation enzyme activity.
Heterogeneous Local Polyurethane Degradation by *Papiliotrema laurentii* Biofilms Under Nutrient Limited Conditions

**Abstract Body:**
Understanding biofilm interactions with synthetic polymers under nutrient limited conditions is relevant to many cases of microbiological fouling and degradation of painted surfaces. *P. laurentii* was isolated from a polyurethane painted surface and screening showed high degradation activity of polyester polyurethane. We identified a cutinase as a contributor of enzymatic degradation and determined that *P. laurentii* can metabolize polyesters as a sole carbon source. To further analyze the biological - materials interactions, *P. laurentii* biofibiofilms were prepared by dropcasting cells onto polyurethane coatings and kept under >95% RH incubation without any additional nutrient sources. Samples were removed for analysis at time points up to 35 days. Infrared spectroscopy confirmed that preferential ester loss occurred in a polyester polyether polyurethane (Irogran) and no detectable polymer changes occurred for the biofilm on a polyether polyurethane. Brightfield microscopy showed slight changes in biofilm coverage on Irogran over time with both expanding and receding regions. The morphology and composition were further mapped down to the sub-cellular level using combined atomic force microscopy / infrared nanospectroscopy (AFM-IR). The deposited cells formed monolayer clusters showing both typical and novel cellular morphologies. Overall biomaterial content of cells was also comparatively reduced under the polymer degradative environment, suggesting that cells were physiologically impacted. Multiple polymer biodegradation processes were identified, consisting of diffusive and highly localized processes, with localized processes the most damaging to the polymer. Aggregation of degradation resistant Irogran components was also observed, a concern regarding release of micro and nanoplastics into the environment.
Model of Ciprofloxacin Killing Enhanced by Hyperbaric Oxygen Treatment in *Pseudomonas aeruginosa* Biofilms

P. A. Gade¹, T. B. Olsen², P. Ø. Jensen¹, M. Kolpen¹, N. Hoiby¹, K. Henneberg⁴, T. Sams⁴; ¹Dept. of Applied Mathematics and Computer Science, Technical University of Denmark, Lyngby, DENMARK, ²Dept. of Applied Mathematics and Computer Science, Lyngby, DENMARK, ³Dept. of Clinical Microbiology, Rigshospitalet and Costerton Biofilm Center, Dept. of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, DENMARK, ⁴Biomedical Engineering, Technical University of Denmark, Lyngby, DENMARK.

In chronic *Pseudomonas aeruginosa* (PA) biofilm lung infections the bacteria are protected from the immune system of the host and from antibiotic treatment. It has been demonstrated that the susceptibility of the bacteria to antibiotic treatment can be enhanced by hyperbaric oxygen treatment. Here we present a reaction-diffusion model that describes the combined effect of ciprofloxacin diffusion, oxygen diffusion and depletion, bacterial growth and killing, and adaptation of the bacteria to ciprofloxacin. In the model, the oxygen diffusion and depletion use a set of parameters derived from experiments. The description of ciprofloxacin killing uses parameter values from the literature in combination with our estimates. The complete oxygen model comprises a reaction-diffusion equation describing the oxygen consumption by using a Michaelis-Menten reaction term. The oxygen model performed well in predicting oxygen concentrations in both time and depth into the biofilm. At 2.8 bar pure oxygen pressure, HBOT increases the penetration depth of oxygen into the biofilm by a factor 4 and we see that hyperbaric oxygen treatment significantly increases the killing by ciprofloxacin in a PAO1 biofilm in alignment with the experimental results.

**Background:** NUFEB is a flexible and open source 3D framework for simulating the dynamics of biofilms at the micro-scale. NUFEB is based on the Individual-based Modelling (IbM) approach, where microbes are represented as discrete units and their behaviour changes over time due to different processes. This approach allows us to study population behaviours (e.g., biofilm morphology, detachment) that emerge from the interactions between individuals and their environment. The IbM implemented in NUFEB is constructed by combining the ideas described in [1, 2] and extending them to include hydrodynamics and parallelization. **Methods:** Microbes are implemented as rigid spheres. The computational domain is defined as a micro-scale 3D rectangular box with different compartments. Various sub-models are employed for describing Biology, Chemistry and Physics at different temporal scales. The biological sub-model includes microbe growth, decay and reproduction (EPS excretion and cell division). The chemical sub-model introduces pH dynamics and gas-liquid transfer to improve the accuracy of microbial growth. The physical sub-model models three key aspects and their interaction: nutrients, microbes and fluid. Nutrient distributions are governed by a convection-diffusion-reaction equation; Mechanical relaxation describes microbe motion and is carried out by the Discrete Element Method (DEM); An accurate modelling of hydrodynamics is implemented by using the Computational Fluid Dynamics-DEM approach. **Implementation:** NUFEB is built on top of the classical molecular dynamics simulator LAMMPS, extended with IbM features. LAMMPS offers a wide range of inter-particle interactions that can be directly applied to biofilm simulation (e.g, contact, adhesive forces). Chemical and biological processes are implemented as a set of ‘fix’ commands which can be invoked at each time step to update field quantities and microbe attributes. Solving hydrodynamics is done by communicating with the external CFD solver OpenFOAM. NUFEB is fully parallelized and allowing for the simulation of large numbers of bacteria (1E8 bacteria and beyond). The parallelization is based on a domain decomposition scheme that divides the domain into multiple sub-domains which are distributed to different processors. NUFEB can also log various biofilm properties into different formats (vtk, hdf5, etc) for post-processing. The tool has been verified and validated with a number of case studies including biofilm BM1, BM2. **Conclusions:** The primary objective of NUFEB is to implement an IbM that included biological, chemical and physical processes as well as individual microbes for biofilm modelling. Ongoing works aims to incorporate other features including quorum sensing, rod microbes, and GPU acceleration. [1] Jayathilake PG, Gupta P, Li B, et al. (2017) PLoS ONE 12(8) [2] Gonzalez-Cabaleiro R, Ofiteru ID, (2015). ISME J 9: 2630-2641.
**Abstract Title:** Genome of *E. coli* C - Old Model Organism with a New Application in Biofilm Research

**Abstract Body:**

*Escherichia coli* is a model prokaryote and a key organism for laboratory and industrial applications. *E. coli* C, a restrictionless strain, has been used for bacteriophage studies since the early 50’s. Its genetic map was constructed in 1970. Able to utilize the pentitol sugars ribitol and D-arabitol. *E. coli* C is the last of five strains designated as safe for laboratory research for which genome had not been sequenced. We found that *E. coli* C forms biofilm more efficiently than other laboratory strains. Genome comparison of *E. coli* C to these other strains will reveal differences in gene composition. Transcriptional activity comparison between planktonic and biofilm will reveal which genes play a role in biofilm formation. Methods. Biofilms on microscope slide and 96 well plate were grown and analyzed as described previously (Krol et al. 2013, Krol et al. 2014). DNA for sequencing was isolated using Qiagen Blood and Tissue DNA isolation Kit. Pacific Biosciences DNA sequencing was performed, and sequences were assembled by the HGAP Assembly 2 and annotated by Prokka. Comparison of *E. coli* C, K12, B, W and Crooks genomes was carried out by Roary, Mauve and Artemis. Total RNA were extracted from 4 day-old biofilm and planktonic cells by Trizol method. Ribosomal RNAs were depleted with Illumina RiboZero kit, library construction was completed utilizing Illumina’s TruSeq stranded RNA LT kit, without the poly-A selection. A single 1x75 sequencing was run on Illumina NextSeq Midi. Read alignments and gene feature mapping were performed with Hisat2, and Stringtie respectively. Differential expression analysis was performed with deseq2 in R. Results. *E. coli* C strain forms biofilm more efficiently than other laboratory *E. coli* strains. In minimal medium the difference is 1.5- to 3-fold, while in rich LB medium, biofilm formation is as much as 7.4-fold higher. *E. coli* C shows the highest genome similarity with the K12 strain. The chromosome is 4,617,024 bp and encodes 4,209 ORFs. Out of the 5686 genes, 3603 genes are shared by all 5 strains. Only 33 genes are present in all except *E. coli* C strains, but 177 genes are unique for C strain. Some of these genes *i.e.* encoding TolA, WzzB1 or fibrial-like adhesion protein might be involved in biofilm formation. Four day-old biofilm and an overnight planktonic cells were used to analyze gene expression profiles resulted in 51.5 million reads (25.7 biofilm and 25.8 control reads). Detailed results of that experiment will be presented and discussed.

**References.** Krol et al., 2013. Plasmid 70; 110 Krol et al ., 2014, J. Bacteriol. 196;129
Theoretical Understanding of Free Ammonia Inhibition on Nitrite Oxidizing Bacteria Suppression in Granular Sludge for Wastewater Treatment

Virginia Tech, Manassas, VA.

The suppression of nitrite oxidizing bacteria (NOB) is a major obstacle to establishing partial nitritation for deammonification or nitrite shunt in wastewater treatment. Stratification of ammonia oxidizing bacteria (AOB) and NOB is currently believed to be necessary for suppression of NOB in aerobic granular sludge. However, it is not known why AOB and NOB choose to stratify. Previous literature argues that high concentrations of residual ammonium nitrogen accelerate ammonium oxidation by AOB, consuming oxygen and limiting its penetration into the core of a stratified granule. Although this accounts for suppression of nitrite oxidation within the granule, this theory explains neither why stratification occurs nor how NOB at the granule surface is suppressed. It may be that stratification of AOB and NOB and suppression of nitrite oxidation at the surface are in fact interlinked. Further, suppression of surface NOB is believed to be due to free ammonia inhibition. In other words, free ammonia inhibits NOB at the surface, such that the only location where NOB may grow is in the layer beneath AOB. To understand more about this assumption, this paper seeks to establish a multi-layer biofilm model that for the first time accounts for both AOB/NOB stratification and free ammonia inhibition to provide a theoretical understanding on the effects of free ammonia along with other parameters (e.g., dissolved oxygen, pH, and temperature) on NOB inhibition. Further, this study reveals whether the free ammonia available in municipal wastewater is sufficient to inhibit NOB. The predictions of the model are verified by experiments in a benchtop reactor and data from literatures. The outcome from this study facilitates the design of nitrite oxidation suppression and shows if free ammonia inhibition is a viable means of NOB suppression in granular sludge systems for wastewater treatment.
Objective: To better understand Acinetobacter baumannii pathogenesis and to advance drug discovery against this pathogen, we developed a porcine full thickness, excisional, mono-species infection wound model. Approach: The research was facilitated with AB5075, a previously characterized, extensively drug-resistant A. baumannii isolate. The model requires cyclophosphamide-induced neutropenia to establish a skin and soft tissue infection (SSTI) that persists beyond seven days. Multiple 12 mm diameter, full-thickness wounds were created in the skin overlying the cervical and thoracic dorsum. Wound beds were inoculated with 5.0 x 10^4 colony forming units (CFU) and covered with dressing. Results: A. baumannii were observed in the wound bed and on the dressing in what appeared to be biofilm. When bacterial burdens were measured, proliferation to at least 10^6 CFU/g (log_{10}6) wound tissue was observed. Infection was further characterized by scanning electron microscopy (SEM) and peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) staining. To validate as a treatment model, polymyxin B was applied topically to a subset of infected wounds every two days. Then, the treated and untreated wounds were compared using multiple quantitative and qualitative techniques to include gross pathology, CFU burden, histopathology, PNA-FISH, and SEM. Innovation: This is the first study to use A. baumannii in a porcine model as the sole infectious agent.
Tissue-like 3-dimensional (3D) microbial communities called biofilms colonize a wide variety of biotic and abiotic surfaces and, in aggregate, constitute a major component of bacterial biomass on earth. As such, biofilms have a tremendous impact on the biogeochemistry of our planet and the biochemistry of higher living organisms. However, the spatial distributions of different genotypes or phenotypes that shape the emergent properties and capabilities of biofilms remain largely unknown. A critical barrier is that conventional imaging modalities are not able to resolve individual cells within thick 3D biofilms in a non-invasive manner. Lattice light-sheet microscopy is a new imaging technology that effectively combines low photo-toxicity and high spatiotemporal resolution making it a promising tool for live-cell biofilm research. We use lattice light-sheet microscopy to image the initial phases of biofilm formation by the exoelectrogenic bacterium *Shewanella oneidensis* MR-1 and the motility of the predatory bacterium *Myxococcus xanthus* in all three spatial dimensions. To measure biofilm remodeling due to cell growth, division, motility, and dispersal, we present progress towards automated cell segmentation algorithms that enable quantitative tracking of individual cells in developing biofilms. Resolving cellular level details in biofilms may provide crucial information to guide the development of predictive computational models of biofilm growth and reveal new strategies to control biofilms in natural and artificial environments.
Breast implant associated anaplastic large cell lymphoma (BIA-ALCL) is a rare T-cell derived lymphoma in tissue around a breast implants. Its remains unknown, but all cases have occurred in patients with textured or polysulphone implants. We showed textured implants support 72x more bacteria than smooth surface implants both in vitro and in vivo and found a linear relationship between biofilm load and lymphocyte activation in both a pig model and in specimens from patients with chronic implant infection. The microbiome of BIA-ALCL contain significantly more Gram-negative bacteria than the microbiome surrounding non BIA-ALCL implant capsules, suggesting that BIA-ALCL development is stimulated by chronic bacterial, particularly Gram-ve antigen stimulation, resulting in sustained T-cell proliferation that potentiates malignant transformation. We aimed to measure lymphocyte proliferation of BIA-ALCL tumour cells in response to plant (phytohemagglutinin -PHA), Gram-ve bacterial mitogens (lipopolysaccharide LPS) and Gram positive bacterial antigen Staphylococcal enterotoxin A (SEA). Methods Tumour cells (N = 9), isolated from patients’ with BIA-ALCL; ALK T-cell breast lymphoma cell lines (TLBR)(n= 3); primary cutaneous (pc)-ALCL cells (n=2); MT-4 cells (immortal T-cell line); and IL-2 stimulated-peripheral blood mononuclear cells (PBMCs) from patients with capsular contracture (CC, n=3) and 3 healthy controls were seeded at a concentration of 10^5, 10^5.5 and 10^6 cells per well, and stimulated with PHA, LPS and SEA at 5 or 10 μg/ml, over 72 hours. Mitogen-induced proliferation of lymphocytes was measured using a tetrazolium (MTT) colourimetric assay. Results BIA-ALCL tumour cells and TLBR cells respond significantly more to LPS than PHA (p < 0.05). In contrast, pc-ALCL cells and MT-4 cells had higher stimulation index values with the T-cell mitogen PHA (p < 0.05). Similarly, IL-2 stimulated-PBMCs from CC and control patients responded strongest to stimulation with PHA, with low proliferative responses to LPS (p < 0.05). The SEA response was similar to the PHA response in all cells except control patients who had lower responses. Conclusion These findings suggest a role for Gram-negative bacteria cell wall, of which LPS is a major component, providing further support to our hypothesis that a predominantly Gram-negative microbiome is the inflammatory trigger that leads to T-cell activation and sustained clonal proliferation.
Session Title: **TUESDAY Poster Session 2**

Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 057

Abstract Topic: Biofilms and Infection

Abstract Title: **Oral Infection of S. Typhimurium Leads to In vivo Expression of Curli and Generation of Anti-dsDNA Autoantibodies**

Author Block: A. Miller¹, N. Medeiros¹, S. Tursi¹, R. Wilson¹, A. White², C. Tukel¹; ¹Temple University Lewis Katz School Of Medicine, Philadelphia, PA, ²University of Saskatchewan, Saskatoon, SK, CANADA.

Abstract Body: Amyloids are proteins with a cross-beta sheet structure that fold into a quaternary fibrillar structure. They are found within the organs and tissues throughout the human body and have been linked to the development of a variety of diseases. Like humans, bacteria also produce amyloids. It is estimated that 40% of bacterial species produce amyloids and these proteins are major structural components of biofilms. Members of the Enterobacteriaceae family including *Salmonella enterica* serovar Typhimurium and *Escherichia coli* produce a beta amyloid called curli. Curli, encoded by two operons known as *csgBAC* and *csgDEFG*. Curli production can be induced in vitro at temperatures lower than 30°C, suggesting that the expression of the fibers are limited to environmental conditions and not in vivo during infection at 37°C. Previous reports suggest that intraperitoneal injection of purified curli or *S. Typhimurium*, that were triggered to express curli by growing the bacteria at 28°C, lead to the generation of anti-dsDNA and anti-chromatin autoantibodies. However, it is not known whether curli is expressed during natural *S. Typhimurium* infection, and whether this expression would be enough to trigger autoantibody generation. We used Nramp+ CBA and 129/SvJ mice that are resistant to *S. Typhimurium* infection to investigate curli expression in vivo. We found that curli is expressed in the cecum and colon of mice and this expression led to the generation of anti-dsDNA autoantibodies. In addition, we performed similar experiments in Nramp- C57BL/6 mice that are susceptible to *S. Typhimurium* and found in vivo expression of curli throughout the gastrointestinal tract.
**Pseudomonas aeruginosa** Evolves wsp Mutations that are Positively Selected for in Response to Chronic Infections

**E. S. Gloag**, C. W. Marshall², D. Snyder², S. B. Chaney¹, J. S. Harris¹, G. R. Lewin¹, M. Whiteley¹, V. S. Cooper², D. J. Wozniak¹

¹The Ohio State University, Columbus, OH, ²University of Pittsburgh, Pittsburgh, PA, ³Georgia Institute of Technology, Atlanta, GA.

**Background:** Chronic infections are persistent, despite extensive and drastic treatment strategies and are established due to the presence of microbial biofilms. The biofilms of *Pseudomonas aeruginosa* are often implicated in chronic infections, specifically in immune-compromised individuals. Complicating chronic infections is the ability of bacteria to adapt to an infection by evolving variants that are more fit and persist. Adapted variants typically display hyperbiofilm phenotypes and increased evasion of the host immune system and tolerance to antimicrobials. Despite our current understanding of the divergent phenotypes of adapted variants, studying their emergence in an infection is challenging. Here we analyzed *P. aeruginosa* evolution in a porcine full-thickness chronic burn wound model, which is a clinically relevant model of chronic infection.

**Methods:** Porcine full-thickness burn wounds were infected 3-d post thermal injury with wild type *P. aeruginosa*. Punch biopsies were taken at 3-, 14-, and 28-d post inoculation. Homogenized tissue was plated for CFUs to assess bacterial burden and screened for altered colony morphology variants. Whole genome sequencing was performed on selected variants to determine the genetic mutation accounting for the variant phenotype. Phenotypic assays were performed to determine the fitness benefits afforded to the isolated variants.

**Results:** 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 had driver mutations exclusively in the wsp pathway. The RSCVs had elevated levels of c-di-GMP and displayed increased biofilm formation compared to the ancestor strain. Furthermore representative RSCVs out-competed the ancestor strain under both planktonic and biofilm conditions. Some RSCVs also possessed secondary mutations. Of interest was a 14,299bp deletion in two PA14-derived RSCVs, which removed the remaining *psl* operon. Another two RSCVs contained a 60bp insertion at the CRISPR-Cas locus, which resulted in these RSCVs being resistant to phage infection from *P. aeruginosa* strains in the initial inoculum pool.

**Conclusion:** Our data indicates that *P. aeruginosa* experiences strong selective pressures to evolve in chronic infections. Adapted variants were isolated at early stages of infection, which challenges the dogma that variants arise due to chronic adaptation. We only isolated RSCVs with driver mutations in the wsp pathway. This indicates that the Wsp system experiences significant pressure in the wound to evolve adapted variants, despite other pathways being implicated in RSCV formation. RSCVs are routinely isolated from other chronic infections, particularly the pulmonary infections of cystic fibrosis patients. We predict that RSCVs may be an adaptation common to chronic infections.
Differentiation of Wound Associated Bacterial Biofilms Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) for the Detection of Volatile Metabolites

E. A. Slade¹, R. M. Thorn¹, A. Young², D. M. Reynolds¹; ¹Centre for Research in Biosciences, University of the West of England, Bristol, UNITED KINGDOM; ²Scar Free Foundation Centre for Children's Burns Research, Bristol Royal Hospital for Children, Bristol, UNITED KINGDOM.

Background: Rapid, non-invasive diagnosis of wound infection would allow clinicians to differentiate infection from the normal physiological inflammatory response in patients with non-specific symptoms. This would facilitate appropriate prescribing of antibiotics and reduce over-use. Microorganisms produce a wide range of volatile compounds (VCs) as a result of normal metabolism. Our previous research has shown that different types and concentrations of VCs, detected using Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) produced by a range of potentially pathogenic bacterial species can be detected and utilized to differentiate between species when grown as planktonic culture. This study shows that it is possible to discriminate between 3 species of bacterial biofilms, grown in a novel collagen based wound biofilm model.

Methods: Three strains each of Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes were grown in monoculture within a modified drip flow biofilm system using a collagen based growth matrix and simulated wound fluid (n=3 per strain). SIFT-MS in full scan (FS) mode and selected ion mode (SIM; to target specific VCs identified through GC-MS) was used to analyse biofilm headspace gases. Multivariate statistical analysis was employed to determine if bacterial volatile product ion profiles (from the resulting FS mass spectra) and volatile compound concentrations (through use of SIM) could be utilised to discriminate between species. Results: The novel wound biofilm model produced reproducible and steady-state biofilm monocultures (determined by cell viability). The biofilms were analysed using SIFT-MS in both FS and SIM modes and the resultant headspace gas data processed through Ward’s method of Hierarchical Cluster analysis and Principal Component Analysis. These were visualized by constructing dendrograms and scatter plots of principal component scores, with associated eigenvector plots. The resultant data sets show clear species specific differential clustering of microbial biofilms using both product ion (FS) and volatile compound (SIM) data. Conclusions: This study has shown that steady-state biofilm monocultures of S. aureus, P. aeruginosa and S. pyogenes grown within a novel biofilm wound model, can be differentiated based on analysis of headspace gases alone. We have shown that through utilizing SIFT-MS both volatile product ions and specific volatile compounds can be used to discriminate between bacterial species based on the analysis of headspace gases of continuous culture biofilms. This work lays the scientific foundations for development of a non-invasive diagnostic tool, based on recognition of biofilm associated volatile compounds for rapidly identifying bacteria associated with wound infection in the clinic.
Background: Skin is the largest organ of the body and acts as a barrier against external insults. An important part of the barrier is the skin microbiota, which offers protection by outcompeting or modulating the virulence of pathogens. However, a skin injury can disturb microbiota homeostasis, opening the door for colonization by opportunistic pathogens. For immunocompromised patients with healing impairment, colonization can lead to life-threatening chronic wound infections involving antimicrobial resistant, structured communities called biofilms. Furthermore, chronic wound biofilms tend to be polymicrobial, which are difficult to eradicate. Given that increased tolerance to antibiotics is an inherent characteristic of biofilms, new strategies are needed to treat and prevent these types of infection. Understanding the interactions between skin colonising bacteria (commensal and pathogen) to identify targets (potential biomarkers) that promote a healthy microbiota and reduce the likelihood of life threatening infections will enable treatment to be applied early and provide a model for testing topical application designed to promote healthy skin. The aim of this study was to generate a skin colonization model combining pathogens and commensal bacteria to study microbial-microbial and host-microbial interactions and to provide a platform to test different compounds of interest. Methods: Immortalised human keratinocytes (HaCat) were used as a support for the infection model. Cultures of Staphylococcus epidermidis and Micrococcus luteus (commensals) and Staphylococcus aureus and Pseudomonas aeruginosa (pathogens) were normalised at OD$_{600}$=0.01 and diluted accordingly. Total colonization time was set to 40h with $P$. aeruginosa being introduced after 20 h. HaCat cells were stained to observe monolayer’s structure. Confocal Z-stack biofilm images were analysed by COMSTAT and bio-volume, surface area and thickness were quantified. Results: A stable and reproducible polymicrobial colonization model has been generated. The presence of commensals protects the HaCat cells against the pathogens and significantly reduces bio-volume, surface area and thickness of the biofilm formed by $S$. aureus and $P$. aeruginosa. Conclusions: Preliminary data obtained from our model suggests that the beneficial role of the commensals is during the early stages of infection. Further investigation of the mechanisms involved will enable us to explore new ways to boost the commensals to the detriment of pathogens.
Abstract

Background: Clostridium difficile is an anaerobe that causes diarrhea and colitis following antibiotic therapy that disrupts the healthy microbiota. Bile salts modulate the infectious cycle of C. difficile by influencing germination, growth and toxin production. Furthermore, recent studies have shown that secondary bile salts such as deoxycholic acid (DCA) produced by the intestinal microbiota can protect against C. difficile infection. Recurrent C. difficile infections, thought to be related to spore formation, are also a major problem, but the exact mechanisms involved remain elusive. Among other intestinal pathogens, bile salts can act as a signal to switch growth mode, for example shift to biofilm formation. The objective of our study was to evaluate the effect of bile salts on biofilm formation by C. difficile.

Methods: Bacteria were grown in presence of bile salts and biofilm formation was evaluated in 24-well plates and staining with crystal violet. The biofilm matrix was then characterized using fluorescent dyes or lectins, enzymatic digestion and gel electrophoresis. The transcriptome of the induced biofilm was then analyzed and used to identify targets for gene inactivation. Results: We show that DCA induces biofilm formation in the presence of glucose and acidified growth medium. The DCA-induced biofilm matrix is composed of eDNA, protein and an unidentified exopolysaccharide; however, only eDNA is required to maintain biofilm stability. Furthermore, we demonstrate that a member of the intestinal microbiota, Clostridium scindens, enhances C. difficile biofilm formation by converting cholate into DCA. Our transcriptomic analysis indicates that long term exposure to DCA induces major changes in the metabolic pathways used by C. difficile. This was confirmed by reduced biofilm formation by strains lacking master regulators of metabolism (spo0A, cpaA or codY). Furthermore, our transcriptomic analysis combined with gene inactivation identified a novel lipoprotein required for biofilm formation.

Conclusions: Our data indicate that DCA exposure induces biofilm formation in C. difficile and is associated with a major metabolic reorganization. Based on these results and co-biofilm experiments, we hypothesize that the resident commensal microbiota can cause C. difficile to switch from a symptomatic infectious agent into a silent intruder, increasing the risk of relapse.
Session Title: **TUESDAY Poster Session 2**

### Session Details
- **Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
- **Poster Board Number:** 062

### Abstract Details
- **Topic:** Biofilms and Infection
- **Title:** Multi-species Biofilm Bacteria from Human Carotid Arteries Undergo Iron-induced Dispersion and Release Enzymes with the Potential to Degrade Vascular Tissue

#### Background:
Atherosclerosis is the main underlying cause of heart attack and stroke, which together claim approximately 15 million lives globally each year. While atherosclerosis is characterized as a lipid deposition disorder, there have been increasing reports suggesting infection and inflammation also play a role. Previous work in our lab showed the presence multi-species biofilm deposits within atheromatous tissues, including *Propionibacterium acnes*, *Pseudomonas spp.* and *Staphylococcus spp.* We believe that plaque-associated bacteria may influence the integrity of the fibrous cap, the boundary between the atheroma and arterial lumen. Studies have shown a sudden increase in a limiting nutrient such as iron, can induce biofilm dispersion, a process associated with the release of degradative enzymes. Although the presence of chelators generally limits the concentration of free iron in the human body, an interaction with norepinephrine can cause chelators such as transferrin to release free iron into the blood. We hypothesized that the level of iron released during stress would be sufficient to induce biofilm dispersion and result in the export of lipases and proteases capable of degrading arterial tissues. In the current work, we investigated multi-species biofilm dispersion and enzyme release in *in vitro* biofilms, as well as enzyme activity in human carotid explants.

#### Methods:
Tri-species biofilms comprising *P. acnes*, *P. aeruginosa* and *S. epidermidis* were grown in 24 well plates, challenged with 0.1 mM FeSO4 and analyzed for dispersion (*n=3*). Treated biofilm supernatants were assessed for enzymatic activity against lipid and protein substrates (*n=4*). Statistical analyses were performed using a t-test. Explanted carotid arteries were sliced into 20 µm sections, treated with 16S/23S rDNA fluorescently labeled probes specific to *P. acnes* and Eubacteria, and analyzed using confocal microscopy at locations either containing an atheroma (*n=2*), or at a site distal to an atheroma (*n=2*). Adjacent tissue sections were analyzed for protein degradation using *in situ* zymography and confocal microscopy.

#### Results:
Biofilms treated with FeSO4 showed 19% dispersion, compared to 10% in control biofilms (*p<0.01*). Supernatants from iron-treated biofilms had greater lipase activity (*p<0.05*), and protease activity (*p<0.005*) compared to controls. Bacteria were detected in atheromatous carotid tissue (*n=2*), and in tissue distal to the plaque in 1 of 2 explants. Enzymatic activity was greater within atheromatous tissues versus tissues that were distal to an atheroma.

#### Conclusions:
Bacteria from multi-species biofilms identified in carotid arteries and grown *in vitro* have been shown to disperse and release increased amounts of lipases and proteases compared to untreated controls. Such an event *in vivo* has the potential to influence plaque stability and possibly contribute to plaque rupture.
Abstract

Complex polymicrobial communities are present in the respiratory tract of cystic fibrosis (CF) patients, but chronic *Pseudomonas aeruginosa* (Pa) infections account for the majority of mortality. Chronic rhinosinusitis (CRS) is highly prevalent in CF; recent studies suggest the paranasal (PN) sinuses are a site of primary colonization and dissemination of Pa into the lungs. Despite a robust host immune response and aggressive antimicrobial treatment, Pa adapts via mutations that promote chronic respiratory infections. Respiratory viral infection leads to pulmonary exacerbation and has been linked with the development of Pa-dominant chronic infections. Our lab described how viral co-infection promotes colonization of airway epithelial cells (AECs) by Pa, through increased iron released from AECs that enhances Pa biofilms. However, the impact of viral infections on Pa pathoadaptation, and on the respiratory microbiome overall, is unknown. Our goal was to identify causal relationships between viral infection, Pa pathoadaptive evolution, sinus microbiota changes, and clinical disease severity. In a 2-year longitudinal study of 33 CF adults, we collected sinus swabs for bacterial culture, virus and metals analysis, and 16S microbiome sequencing at quarterly clinic visits and during exacerbations. We genotyped 140 Pa isolates and screened for in vitro phenotypes associated with chronic Pa infection. We used Tetrad, a program that searches for causal explanations represented by directed graphs, to construct Bayesian networks with directed edges between variables (nodes) in a combined patient clinical, microbiome, and Pa in vitro phenotyping dataset. We searched using both constraint-based (PC, FCI) and search-and-score (FGES) algorithms, then evaluated the networks based on their BIC score, bootstrapping, and biological plausibility. These searches reproducibly identified a causal relationship between respiratory viral infection and levels of iron present in patient sinus samples, in agreement with our previous in vitro and murine infection models. Validating the network with previously published findings, we identified directed edges between swimming motility and polysaccharide overproduction, as well as a loss of swimming motility and increased patient hospitalization. Using the model to generate hypotheses regarding drivers of CF disease progression, we are currently examining directed edges between in vitro phenotypes associated with Pa pathoadaptation, such as overproduction of the quinolone HHQ (“lysis and sheen”), and microbiome diversity indices by competing Pa with other sinus bacteria in a model of biofilm formation on polarized AECs (CFBE41o-). The long-term goal is to use machine learning to identify biomarkers and novel therapeutic targets for preventing chronic bacterial infections and CF respiratory disease progression.
Abstract

**Background:** *P. aeruginosa* is an opportunistic pathogen causing biofilm associated infection in immunocompromised patients. There are many internal factors involved in bacterial biofilm formation. The objective of this study was to investigate the natural variation in the antibiotic sensitivity, virulence and biofilm formation among the urinary catheter associated *P. aeruginosa* isolates, we also evaluated the role of host DNA on biofilm expansion by clinical isolates of *P. aeruginosa*. **Methodology:** *P. aeruginosa* isolates collected from urinary tract infections were identified by 16S rRNA gene sequencing. Sensitivity to antibiotics and healthy human serum were measured using standard procedures. Crystal violet staining and confocal laser scanning microscopy and scanning electron microscopy were used for biofilm studies. Ability to induce cell lysis was studied using Human Embryonic Kidney cells (HEK 293T). Association between virulence factors, biofilm and antibiotic resistance among the strains was analysed statistically. The presence of pyocyanin and H2O2 in the culture free supernatant was quantified by colorimetric method. The eDNA was quantified in the culture free supernatant by DNA quantifying fluorescent dye assay. **Results:** We observed 98 cases of CAUTI in 1,266 patients (Male, n=1008, Female, n=258, age: 48.4 ± 21.4), among them 18.3% (n=18) was *P. aeruginosa* infection. 94.4% (17/18) isolates were resistant to antibiotics including carbapenem (73.7%). All the isolates formed biofilm on all the materials tested. Intensity of biofilm on silicone latex material was significantly higher than polystyrene. The strains were highly virulent and could induce significant cytotoxicity to HEK cells (>60% cell killing) and showed very low sensitivity to healthy human serum. Biofilm formation was not associated with antibiotic sensitivity in some isolates. Variation among the factors contributing to biofilm was also observed. The host-DNA showed significant increase in the biofilm intensity by *P. aeruginosa* isolates. **Conclusion:** Present study showed the natural variation among the catheter associated *P. aeruginosa* isolates compared to the standard strains or of their mutants routinely used in the studies. Such diversity and antibiotic resistance among the isolates warrant newer strategies to target this ubiquitous opportunistic pathogen. Present study showed that the host-DNA can increase the biofilm rigidity and expansion during infection. The isolates showed high cytotoxicity on epithelial cells and was resistant to human serum which leads to the need of early detection and treatment for *P. aeruginosa* associated infections.
**Background:** *Pseudomonas aeruginosa* is responsible for much of the morbidity and mortality associated with cystic fibrosis (CF); therefore, studying the pathogenesis and immune response to *P. aeruginosa* is essential. The difficulty of modeling the infections in animals, however, hampers the study of chronic *P. aeruginosa* infections. In this project, we develop a model of chronic *P. aeruginosa* infections in ENaC-overexpressing mice to test the hypothesis that these infections produce Th2-biased responses that fail to clear the bacteria. **Methods:** To study how *P. aeruginosa* causes chronic infections in CF lungs, we developed a model of CF-associated lung infections in B-ENaC BALB/c mice. B-ENaC mice overexpress ENaC channels in their lungs, causing them to display CF-like mucus plugging, neutrophil infiltration, and airway inflammation. To characterize the immune response to infection, we intranasally infect 6-8 week-old mice with 3x10⁶ colony forming units (CFUs) and collect lungs at 3, 7, 10 and 14 days after infection for histologic and cytokine analysis. We section and stain lungs by H&E, and measure the cytokines IL-1B, IL-4, IL-17 and IFNγ, in lung homogenates using flow cytometry. Statistical tests include Fisher’s exact test and log rank tests. Wild type littermates serve as controls. **Results:** We found that both B-ENaC mice and their wild type littermates clear even large inoculums of planktonic *P. aeruginosa* within one week of infection. However, if we infect mice with small biofilm aggregates, B-ENaC mice are unable to resolve the infections effectively compared to controls, and most carry at least 10⁴ CFUs per lung one week after infection. Bacterial clearance in B-ENaC mice is delayed up to 14 days after infection. At least 90% of control mice survive infection, and 75% of their B-ENaC littermates survive (p = 0.17 by log-rank test). Approximately 90% of surviving B-ENaC mice carry at least 10⁴ CFUs per gram of lung one week after infection, whereas only 10% of controls remain infected (p = 0.001, Fisher’s exact test). On examination of H&E stained lung sections, infection with *P. aeruginosa* is characterized by increased inflammation, mucus production, and inflammatory infiltrate in the airways of B-ENaC mice compared to their wild type littermates. Uninfected B-ENaC mice demonstrate airway narrowing or plugging by mucus, while their uninfected wild type littermates exhibit no pathology. In our preliminary analysis of the cytokines produced in lungs during infection, B-ENaC mice show greater increases in IL-1B, IL-4 and IL-17, and decreases in IFNγ, relative to their wild type littermates. **Conclusions:** Our results show that B-ENaC mice are more susceptible to infection with biofilm aggregates than planktonic bacteria, and these mice show a delayed clearance of *P. aeruginosa*. The preliminary cytokine tests suggest that infection might cause a shift from Th1 to Th2/Th17-biased responses.
Session Title: TUESDAY Poster Session 2
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 066
Abstract Topic: Biofilms and Infection
Abstract Title: Antibiotic Resistance, Biofilm Formation and Safety Assessment of Bacteria Isolated from Selected Meat Processing and Production Surfaces in Ibadan, Nigeria
Author Block: O. Ajao; University of Ibadan, Ibadan, NIGERIA.

Bacterial contamination during meat processing is a major cause of food borne disease in Nigeria. Bacterial contamination of beef processing surfaces in Nigeria is usually an unavoidable aftermath in the processing of meat for human consumption, especially where there is poor standard of hygiene and sanitation practices. Biofilm formation has been a major source of concern in the food processing and production facilities and industries. It leads to persistent contamination of food, food borne disease and offset of food spoilage. Also, microbial antibiotic resistance has been major health implications both to humans and animals. Biofilms aid bacterial tolerance to antibiotics thereby leading to chronic infections Microbiological analyses of swabs taken from meat processing table (before and after processing) was carried out and the antibiotic susceptibility of representative isolates determined using Kirby-Bauer disc diffusion assay and results interpreted using CLSI standard, 2016. Biofilm formation was determined using the crystal violet binding assay. Haemolysin, Gelatinase and DNase test were also conducted using standard procedures to determine the virulence properties. The data obtained were subjected to analysis of variance (ANOVA) and p < 0.05 was considered to be statistically significant. Total heterotrophic counts before meat processing ranged from $17.5 \times 10^5\text{cfu/g}$ to $5.95 \times 10^5\text{cfu/g}$ while the values after processing ranged $19.3 \times 10^5\text{cfu/g}$ to $8.85 \times 10^5\text{cfu/g}$. A total of sixty-four bacteria isolates was obtained and fifty-two were observed to produce biofilms. The 52 isolates were selected and further identified by biochemical and physiological characterization. The selected bacteria isolates were in the following proportion: E. coli (28.9%), S. aureus (21.2%), Pseudomonas spp. (7.7%), Bacillus spp. (15.4%), K. pneumoniae (13.5%) and Proteus spp. (13.5%). The Gram negative isolates displayed 90.9% resistance to Ampicillin, 28.1% to Cefotaxime, 25% to Ceftaxidime, 28.1% to Cefpodoxime, 38.5% to Sulphmethoxazole, 15.2% to Ciprofloxacin, 96.9% to Tetracycline and 54.5% to Chloramphenicol. The Gram positive isolates showed 57.9% resistance to Ampicillin, 31.6% to Chloramphenicol and Ceftaxidime, 26.3% to Cefotaxime, 36.8% to Cefpodoxime, 84.2% to Vancomycin and 15.8% to Sulphmethoxazole. All Gram positive were susceptible to Ciprofloxacin. All S. aureus showed resistance to Vancomycin and Oxacillin. 11 out of 15 E.coli showed DNase activity, 7 K. pneumoniae isolates produced gelatinase and haemolysin. 50% of the Bacillus spp. Showed hemolysis activity while 8 out of 11 S. aureus strains showed hemolysis activity. The meat processing surfaces in this study were contaminated with potentially pathogenic and antibiotic resistant bacteria that may constitute public health risk. This study reiterates the need for safe and hygienic practices during meat processing.
Session Title: **TUESDAY Poster Session 2**
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 067
Abstract Topic: Biofilms and Infection
Abstract Title: Evaluation of the Interplay Between the Membrane Metalloprotease Eep and the Cellular Host Innate Immune Response in *Enterococcus faecalis* Biofilms
Author Block: S. D. Schaffer, C. N. Rouchon, K. L. Frank;
Uniformed Services University of the Health Sciences, Bethesda, MD.

*Enterococcus faecalis* is an opportunistic, healthcare-associated pathogen that causes a range of infections. Biofilm formation is a major factor that contributes to the severity of *E. faecalis* infections. Our lab has shown that Eep, a transmembrane metalloprotease, is associated with biofilm formation in *E. faecalis*. Specifically, transcription of the *eep* promoter was increased in *in vitro*-grown biofilms and in two rabbit models of biofilm-associated infection (infective endocarditis and foreign body abscesses). We also showed that a Δ*eep* strain has an altered cell distribution phenotype during biofilm development. In addition to its role in biofilm formation, Eep processes substrates in planktonic cells that promote resistance to lysozyme and uptake by phagocytic cells, thereby indirectly modulating the host innate immune system. Based on this, we hypothesize that Eep may promote *E. faecalis* resistance to the cellular response of the host innate immune system in biofilm-mode cells. Our approach to test this hypothesis will employ *in vitro* and *in vivo* methods. Our *in vitro* assays will measure the effect of Eep on survival of planktonic- and biofilm-mode cells within macrophages and after exposure to hydrogen peroxide. Our current results indicate that planktonic-mode isogenic wild-type (OG1RF) and Δ*eep* strains survive at identical levels in macrophages over the course of a 96-hour infection, thereby emphasizing the importance of testing both growth forms. Our *in vivo* assays will use flow cytometry to evaluate how the host innate immune response to OG1RF and Δ*eep* changes over time in a rabbit foreign-body abscess biofilm infection model. We are currently optimizing a flow cytometry panel of antibodies to measure macrophage, monocyte, dendritic cell, and granulocyte immune cell populations in abscess samples collected from the biofilm infection model. Together, these methods will provide new insight on the role of Eep in enterococcal host-pathogen interactions during biofilm infection. This may aid in the discovery of improved methods to treat enterococcal infections, particularly those infections complicated by biofilms.
Composition and Properties of Holdfast Adhesin in the marine bacterium *Hirschia baltica*. The dimorphic α-proteobacterium *Hirschia baltica* is a marine budding bacterium in the Caulobacterales clade. *H. baltica* uses a polar adhesin, the holdfast, located at the cell pole opposite the reproductive stalk for surface attachment and cell-cell adhesion. Holdfast adhesins have been best characterized in *Caulobacter crescentus*, a freshwater member of the Caulobacterales, and little is known about holdfast composition and properties in marine Caulobacterales. Environmental factors, such as ionic strength, pH, temperature, and shear forces affect bacterial adhesion, and therefore marine bacteria must have developed holdfasts with different composition and structures than their freshwater counterparts. Here we use *H. baltica* as a model to characterize holdfast properties in marine Caulobacterales. We show that freshwater and marine Caulobacterales use similar genes in holdfast biogenesis, that these genes are highly conserved among these strains, but that some of their properties are different. We show that *H. baltica* produces larger holdfast than *C. crescentus* and that those holdfasts contain N-acetylglucosamine and galactose monosaccharide residues and proteins, but lack DNA. We also show that *H. baltica* holdfast tolerates higher ionic strength than *C. crescentus* holdfast. We conclude that marine Caulobacterales holdfasts have binding and physicochemical properties that maximize binding at high ionic environments.
Honey, I shrunk the Culture Plate! 3D Interferometric Non-Destructive Imaging Pinpoints Transition from Planktonic Culture to Biofilm

C. Larimer\textsuperscript{1}, M. Brann\textsuperscript{2}, J. Powell\textsuperscript{1}, M. J. Marshall\textsuperscript{1}, J. D. Suter\textsuperscript{1}, R. S. Addleman\textsuperscript{1};
\textsuperscript{1}Pacific Northwest National Laboratory, Richland, WA, \textsuperscript{2}U of Chicago, Chicago, IL.

The interference of light has been foundational to scientific research since Isaac Newton first studied ring patterns in optical glasses in 1717. On the other hand, the agar culture plate has played a crucial role in bacteriology since the origins of the discipline. In this work, we developed a process to use white-light interferometric (WLI) imaging for enhanced non-destructive characterization of bacterial colonies and biofilms growing on miniaturized agar culture plates. WLI is a 3D surface imaging technique that has not been used extensively in microbiology. It has extremely high vertical resolution (3-5 nm) which is far below the resolution of diffraction-limited imaging techniques even when capturing large fields of view (up to 5 mm). We used WLI to track volumetric changes in hundreds of unlabeled \textit{Pseudomonas fluorescens} and \textit{Bacillus thuringiensis} colonies spread across 5 mm culture disks with volumetric resolution of \~50 zeptoliters (5x10^{-20} l). In the early hours (approximately 1-6 hrs) after plating the culture, it was possible to observe individual cells multiplying into colonies. Tracking colonies as they grew from single cells into biofilms revealed, with high temporal resolution, the emergence of EPS through characteristic changes in surface roughness.

We also developed a technique to measure, in parallel, the independent growth rates of several hundred plated colonies and a method to count colony forming units in a culture within 1-2 hrs of plating. The results indicate that WLI is capable of accurate enumeration of CFU counts from agar plates while also enabling accurate quantification of other morphological and phenotypic attributes, such as transition into a biofilm state. The highly sensitive 3D and volume information was also used to identify sub-lethal effects of antibiotics on colonies and biofilms. Finally, we showed that morphological characteristics of groups of bacteria and the way they spread can be used to differentiate some species when co-cultured. Based on these results, bioimaging with WLI was demonstrated as a powerful tool for non-destructive study of the structures that evolve during early stage biofilm development.
Session Title: **TUESDAY Poster Session 2**

**Session Date/Time:**
Tuesday, October 9, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:**
071

**Abstract Topic:**
From Planktonic To Biofilm and Back

**Abstract Title:**
Characterization of FleN Interaction with the Flagellum Assembly Machinery in *Pseudomonas aeruginosa*

**Author Block:**
W. C. Generoso, B. Y. Matsuyama, M. V. Navarro; University of São Paulo, São Carlos, BRAZIL.

**Background:** *Pseudomonas aeruginosa* is an important human opportunistic pathogen, with wide resistance against several known antibiotics. The high *P. aeruginosa* adaptability and its dynamism in switching between planktonic and biofilm lifestyles further lead to intermittent chronic infections. The bacterial enhancer binding protein (bEBP) FleQ is the main transcriptional regulator controlling both the expression of flagellar proteins and the *pel* operon. FleQ controls its target operons in completely different manners but involving the signaling molecule c-di-GMP and FleN. At the flagellar operons, FleQ-mediated gene activation undergoes the classical mechanism of bEBPs, and its activity regulation occurs via interaction with FleN; a MinD-like ATPase encoded by the FleQ-regulated operon *flhFfleN*. FleN acts as an antiactivator of FleQ, generating a feedback regulation in flagella development. Although the molecular mechanisms underlying FleQ activity modulation by FleN are unknown, such regulation is crucial for flagella number control. However, it is not sufficient to fully understand *P. aeruginosa* phenotype of a single, polar flagellum. In *Campylobacter jejuni*, a FleN homolog takes part of the basal flagellum assembly machinery, triggering its polar localization. Although *P. aeruginosa* FleN lacks an N-terminal segment important for interaction and activation of FlhF, it could interact with other components of flagellar machinery. Therefore, this work aimed to expand the knowledge of FleN in the regulation of FleQ activity and involvement in flagellum basal assembly machinery. Thus, we investigated and characterized FleN-FleQ interaction and searched for potential FleN interaction partners within flagellum assembly machinery.

**Methods:** The flagellum assembly factors FlhF, FliM, and FliN; FleQ and FleN from *P. aeruginosa* PA14 were employed for the *in vitro* interaction experiments. Accordingly, heterologously expressed proteins were produced in *E. coli* host system. The interactions were evaluated via chemical crosslinking with EGS and further explored with microscale thermophoresis experiments. ATP and GTP were included in the reactions according to the cofactor necessities of each protein.

**Results:** Beyond the known interaction with FleQ, FleN and FliM interaction were observed in the *in vitro* experiments. FlhF or FliN did not to interact FleN, unlike the reported for other single flagellum species. Moreover, FleN interaction with FleQ and FliM occurs independent of the presence of ATP, although the cofactor addition altered the interaction affinities.

**Conclusions:** Our results indicate that FleN is not only triggering feedback regulation of the flagella assembly but is also engaged itself in the assembly machinery in *P. aeruginosa*. However, further experiments are needed to understand FleN involvement into the assembling of the single flagellum in *P. aeruginosa*.
Grapping Hooks Involved in Biofilm Development

Screening for Genes Involved in Biofilm Formation in the Gut Anaerobe Bacteroides Thetaiotaomicron

N. Béchon, J. Mihajlovic, C. Ivanova, J. Ghigo;
Institut Pasteur, Paris, FRANCE.

Despite the medical, environmental and industrial relevance of biofilms formed by anaerobic bacteria, biofilms have been mostly studied in aerobic conditions using aerobic model bacteria. Bacteroides thetaiotaomicron, a Gram-negative, strict anaerobe, is one of the dominant commensal members of human intestinal microbiota. Whereas B. thetaiotaomicron adhesion and biofilm formation capacity could be important for colonization and maintenance in the intestine, the molecular basis of biofilm formation in this commensal anaerobe are poorly understood. Moreover, the widely used and genetically amenable reference strain B. thetaiotaomicron VPI 5482 only displays poor biofilm forming capacity. Using random transposon mutagenesis and multiple rounds of positive selection for B. thetaiotaomicron mutants developing biofilm in continuous-flow biofilm microfermenters, we identified mutations in the operon BT1338-56 leading to increased biofilm formation. This operon corresponds to capsule 4 locus (cps4), one of the 8 capsule operons described for this bacterium, suggesting that, when expressed, capsule 4 could mask a putative B. thetaiotaomicron adhesion factor. A second round of random transposon mutagenesis in Δcps4 mutant screening for loss of biofilm capacity led us to identify a capsule regulator and a putative lipopolysaccharide biosynthesis locus as candidates potentially involved in adhesion in a Δcps4 background. This study could contribute to a better understanding of biofilm formation dynamics in B. thetaiotaomicron.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 073

**Abstract**

**Topic:** Grapping Hooks Involved in Biofilm Development  
**Abstract Title:** Study for Biofilm Formation on a Various Materials in Water System  
**Author Block:** H. Yu, K. Cho, H. Lee, S. Kang, S. Park; Coway Co. Ltd., Seoul, KOREA, REPUBLIC OF.

**Abstract Body:**

**Backgrounds:** In the aquatic environment, the occurrence of biofilm is a common phenomenon. However, if biofilm is not properly removed, the problem such as degradation of water quality or generation of bad tastes and odors may arise. Especially the biofilm is a critical issue in drinking water system such as water purifier because it is directly linked to hygiene problems. In recent years, a lot of research on biofilm has been progressed; however, study for biofilm generated by Heterotrophic Plate Count (HPC) bacteria has been limited. The purpose of this research is to analyze biofilm effects caused by HPC bacteria on the surface of various materials using a Drip Flow Biofilm Reactor®.

**Methods:** All test materials were selected of components among water purifier or tubing. Samples were prepared in two shapes of plate and tubing. Thirteen materials were used in this test: NORYL, PP, PET, PTFE, Tritan, LDPE, Stainless steel(SUS), Silicone, TA, TES, PL, PN and TYGON®. The sizes were 100 x 15 mm (Surface area = 1,500 mm²) for plates and Φ4.15 x 100 mm (Surface area = 1,300 mm²) for tubing shape of samples. The experiment was conducted for 5 to 30 days and flow rate was 3 ml/min.

**Results:** After 30 days, large amount of the biofilm on surface of TYGON® and silicon was formed to the extent that it was visible to the naked eye. In contrast, fewer biofilm was formed on the surface of Teflon materials; PTFE, TES, and TA. Also the lowest number of biofilm was formed in PP and NORYL. Unusually, Biofilm formation on the surface of SUS was higher than that on general plastic materials such as LDPE and PP. It means SUS is not hygienic; it’s improper to use stainless steel instead of plastic considering the cleanliness in water systems. Supposing that the amount of biofilm formation on LDPE is 1, the highly polluted materials were TYGON®(55.7), Silicon(1.9) and SUS(1.7). By contrast, unremarkably polluted materials were TES(0.11), TA(0.08), PET(0.05), PP(0.05) and NORYL(0.02). Analysis of surface roughness and contact angle were conducted to study for influence factors for biofilm formation on the surface of some materials of NORYL, PP, PET, PTFE, Tritan, LDPE, SUS and silicon. The contact angle of Silicon and PTFE were similar (109 and 106, respectively), but the biofilm formation on the surface of silicon was greater than the PTFE. Also the surface roughness of samples were mostly 0.1 – 0.4μm, except PTFE(2.3 μm). The results show that the surface roughness and contact angle are not affected by biofilm formation on the surface of materials. **Conclusion:** Through this study, the biofilm formation rates on the surface of various materials were compared. However, it has not yet been determined which material properties affect the biofilm formation of the surface. Thus, further studies are needed to identify factors affecting biofilm formation on the surface and to reduce biofilm in water system.
Host Microbe Biofilms

Biological Conversion of Methane to Methanol from Biofilm Producing Methanotrophic *Methylomonas Koyamae* LM6

L. Madhavaraj, K. Kim, G. Han; Centre for Industrialization of Agriculture and Livestock Microorganisms, Jeonbuk, KOREA, REPUBLIC OF.

Methane is converted to methanol by methane monoxygenase of methanotrophs. In this study, a methanotrophic bacterial strain was isolated from a rice paddy soil that has an ability of producing extensive biofilm, characterized by whole genome sequence analysis & named as *Methylomonas koyamae* LM6. Using this isolate as a whole-cell biocatalyst, optimal bioconversion conditions were investigated: temperature, pH, reaction time, CH₄/air mixing ratio, & reaction mixture volume to headspace ratio were found to be 30°C, 7.0, 24 h, 60/40, & 5:1, respectively. Due to low methane solubility in the reaction mixture, a new strategy was introduced to produce a high methanol concentration by using an organic solvent, 1-octanol. Under optimal condition, methane/air mixture was supplied to 500 mL Erlenmeyer flask, which contained 100 mL nitrate mineral salt (NMS) medium and 2 mL 1-octanol, & the flask was agitated at 180 rpm for 8 h at 30°C. With 1-octanol, more than 1.5 times higher methane was solubilized in the NMS medium. After removal of 1-octanol, 10 mL of methane solubilized NMS medium in tightly sealed 70 mL glass serum vial was treated with not only 0.6 mg dry cells/mL & 80 mM Na-formate (reducing power source), but also 60 mM potassium phosphate, 60 mM MgCl₂, 90 mM NH₄Cl and 3 mM EDTA as methanol dehydrogenase inhibitors, & then incubated at 30°C for 24 h without additional methane. Through this reaction, 53 mM methanol was produced, & this concentration was the highest concentration reported so far. These results demonstrated that 1-octanol acts well as a methane solubilizer without cell damage to convert methane to methanol.
Mechanical Environment Regulates E. coli Pathogenesis

S. Moorthy, E. A. Klein; Rutgers University-Camden, Camden, NJ.

UPEC infection in vivo is characterized by initial invasion of bladder umbrella epithelial cells followed by endosomal escape and proliferation in the cytoplasm to form intracellular bacterial communities (IBCs). In contrast, UPEC infection in tissue culture systems results in bacteria being trapped within LAMP1-positive endosomes where proliferation is limited. Based on previous reports demonstrating that depolymerization of the actin cytoskeleton enabled UPEC to escape endosomes in vitro (Eto et al, Cell Microbiol, 2006), we further dissected the cytoskeleton signaling pathway to determine that the inhibition of the Rho-GTPase family member RhoB and its effector PRK are sufficient to increase cytoplasmic bacterial growth. While these data required various pharmacological and genetic manipulations to disrupt RhoB signaling, we were interested in physiologically relevant mechanisms that may regulate RhoB in vivo. We have previously shown that tissue stiffness is a regulator of actin dynamics; therefore, we hypothesized that substrate stiffness may play a role in UPEC endosomal escape. Using functionalized polyacrylamide substrates, we found that at low (physiological stiffness), UPEC can escape the LAMP1-positive endosomes and proliferate rapidly in the cytoplasm of 5637 bladder epithelial cells. Furthermore, consistent with our findings regarding Rho-GTPase signaling, RhoB protein level is significantly reduced at physiological stiffness. Our data suggest that tissue stiffness is a critical regulator of bacterial pathogenesis. Due to the ease of doing genetic and pharmacological manipulation in cell culture, this model system may provide a useful tool for studying the intracellular life cycle of uropathogens.
**Abstract**

**Introduction:** Gingival epithelial cells serve as a physical barrier and also fulfill an active role in innate host defense by secreting different soluble mediators like cytokines. The epithelium is in constant contact with bacteria. It represents the first line of defense against developing biofilms. Host immune responses to biofilms play a key role in periodontal disease pathogenesis. Periodontal diseases occur from a dysregulation between the bacterial biofilm and the immune response. The challenge to oral bacterial biofilms can trigger the release of cytokines and lead up to chronic inflammatory responses. It is known that different bacteria differentially stimulate the epithelial cells and also alter the biofilm composition. In our study we use a co-culture model of mono- and multispecies biofilms in combination with epithelial cells. We aim to investigate the interactions between commensal and pathogenic oral bacteria to the immune response. Additionally, we will examine how the different combinations of bacteria differently influence a multispecies biofilm in composition and depth. **Methods:** Biofilms developed with *Streptococcus sanguinis* (Ss), *Corynebacterium durum* (Cd) and *Porphyromonas gingivalis* (Pg) were cultured in vitro under anaerobic conditions on Thermanox™ coverslips. Oral epithelial cells (OKF4) were co-cultured in vitro with bacterial biofilms for 6h under aerobic and anaerobic conditions. Changes in epithelial cell viability was measured by Calcein AM stain. Biofilms depth was determined via crystal violet stain assay. Culture supernatants were collected for analyses of cytokine content. Gene expression of epithelial cells and the composition of the biofilm was examined via q-PCR. **Results:** The first results of our project revealed, that, although both are commensal bacteria, *S. sanguinis* causes a different immune response of epithelial cells compared to Cd. We also observed differences in biofilm depth after co-culturing of Ss and Cd compared to the respective single species growth. To further improve our setup we will introduce the pathogen Pg. Furthermore, first progress was achieved in establishment of a q-PCR based setup to quantify the species composition of the tested multispecies biofilms. **Conclusion:** Biofilms trigger the immune response of the host which can lead to periodontal diseases. The extent of the host response depends on the composition of the oral polymicrobial biofilm. Due to the high variety of oral bacterial species in such biofilms, it is extremely difficult to study the different interactions and the complex effects to the host immune response. Thus, we selected only a few commensals and one pathogen to develop our biofilm model. Due to its simplicity, we hope to address the big question of how commensal and pathogen bacteria influence the composition of a biofilm and further how they influence the host immune response more easily.
Colonisation and chronic lung infection by the opportunistic pathogen *Pseudomonas aeruginosa* (PA) is the leading cause of morbidity and mortality in cystic fibrosis patients (CF). A critical key determinant of PA pathogenicity is the switch from planktonic to biofilm mode of growth which facilitates chronic infections and makes PA eradication extremely difficult even with antibiotic therapy and robust immune attack. Neutrophils are essential for protection against PA but neutrophil-dominated inflammatory responses can also compromise organ function through tissue damage. The overarching aim of this project is to investigate the interplay between PA and neutrophils and how it is affected by biofilm formation. We characterized the interaction of purified human neutrophils with PA fixed biofilms with regard to morphology, cell-association, phagocytosis, and NETs formation. Preliminary live and confocal microscopy data indicated that when exposed to PA 18hr fixed biofilms, neutrophils increased in size and could clear PA biofilm. Furthermore, biofilm formation impaired neutrophil movement. Future work will involve analysis of how biofilm composition influences neutrophil behavior. Investigation of the interplay between PA and innate immune cells and how it is affected by biofilm formation will give us an insight into novel approaches to combat PA infection.
Session Title: **TUESDAY Poster Session 2**
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 079
Abstract Topic: Host Microbe Biofilms
Abstract Title: Dietary Fatty Acids Modulate *C. albicans* Biofilms on Biotic and Abiotic Surfaces
Author Block: M. C. Garcia-Sherman, S. T. Siddiqui;
Block: Cuny Brooklyn College, Brooklyn, NY.

*Candida albicans* is a major species of the fungal component of the human microbiome; commonly colonizing oral, gastrointestinal and genital niches of the human body. *C. albicans* also forms biofilms on medical materials found in devices such as catheters and dentures. These niches occupied by *C. albicans* can result in a pathogenic interaction with an immunocompromised host. The human diet can modulate immune response, inflammation levels and the human microbiome. The average American diet is high in omega-6 fatty acids but low in omega-3 fatty acids. Here we demonstrate that an omega-3 fatty acid, docosahexaenoic acid (DHA), inhibits switching from yeast to the pathogenic-associated hyphal morphology. Treatment of *C. albicans* with DHA mitigates biofilm formation on abiotic surfaces. Furthermore DHA also prevents binding of *C. albicans* to human oral epithelial cell monolayers. Our results suggest that DHA interferes with *C. albicans* morphogenesis, adhesion to biotic and abiotic surfaces. These findings serve as a basis for future studies to determine whether DHA decreases pathogenic interactions between *C. albicans* and its host.
Session Title: TUESDAY Poster Session 2
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 081
Abstract Topic: Regulation of Biofilm Development
Abstract Title: One Step Forward with Dry Surface Biofilm: TMT-Based Quantitative Mass Spectrometry of Staphylococcus aureus Reveals Novel Regulators of Biofilm Formation
Author Block: A. Rahman1, D. Chowdhury1, A. Amirkhani2, M. Molloy1, D. Pascovici2, M. Baker1, M. Mempin1, X. Song2, H. Hu1, K. Vickery1; 1Macquarie University, Sydney, AUSTRALIA, 2Australian Proteome Analysis Facility, Sydney, AUSTRALIA.

Background: Recent findings of biofilms on dry hospital surfaces emphasises the failures in current cleaning practices and disinfection. Therefore, we aimed to construct a comprehensive reference map followed by identifying marker proteins between traditional hydrated biofilms (wet) and Dry Surface Biofilm (DSB), and then perform pathway analysis, subcellular localisation and protein-protein interaction network mapping. Methods: S. aureus reference strain (ATCC 25923) was grown in tryptic soy broth to produce a 24-hour planktonic culture, and over 12-days in the Centres for Disease Control biofilm reactor to produce the wet and DSB. Protein extraction, fractionation, reduction, alkylation and digestion steps were performed prior to Multiplex labelling using Tandem Mass Tag (TMT) 10-plex reagent, respectively. TMT-based Mass spectrometry (MS) was performed, and protein identification and relative quantitation of protein levels were performed using Proteome Discoverer (version 1.3). Statistical analysis was done using the TMTPrePro R package. Results: We identified 1636 total biofilm extractomes. Among them, 113 and 134 significant differentially regulated proteins were identified compared to planktonic bacteria in wet and DSB, respectively. Of these, proteins significantly up-regulated in wet include glucosamine-fructose-6-phosphate aminotransferase encoded by glmS, and argininosuccinate synthase encoded by argG are cytoplasmic enzymes involved in alanine, aspartate and glutamate pathway. These proteins are involved in energy metabolism. In contrast, cytoplasmic membrane proteins associated with sulfur, pyruvate, and nucleotide sugar metabolism are significantly down-regulated in wet. Proteins significantly up-regulated in DSB include PTS glucose transporter subunit IIBC encoded by ptaA, UDP-N-acetylmuramate-L-alanine ligase encoded by murC and UDP-N-acetylenolpyruvoylglucosamine reductase encoded by murB. These three proteins are all linked with peptidoglycan biosynthesis pathway. PtaA is involved in amino sugar metabolism and responsible for energy metabolism. In particular, murC and murB are responsible for cell wall formation and may play a role in biofilm formation. Proteins associated with nitrogen metabolism, biosynthesis of secondary metabolites and amino acids are significantly down-regulated in DSB. Conclusions: This is the first report using high throughput TMT-based MS determining proteins in S. aureus DSB. Our result showed significant abundance variation compared to planktonic bacteria in wet and DSB. In this study we identified novel regulators of S. aureus biofilm formation in DSB. Current study will be helpful in designing advanced, targeted disinfectants and detergents to remove biofilms from dry environments. Keywords: S. aureus, Bacterial Biofilms, Dry Surface Biofilm, Bioreactor, Tandem Mass Tag, Mass Spectrometry
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 082

**Abstract**  
**Topic:** Regulation of Biofilm Development  
**Title:** Microbial Biofilms: A Promising Bioinoculant Technology for Agriculture  
**Author Block:**  
1ICAR-Central Institute for Cotton Research, Nagpur, INDIA; 2ICAR-Indian Agricultural Research Institute, New Delhi, INDIA.

**Background:** Limited published information regarding the basic aspects of biofilm formation in agriculturally important microorganisms is available, despite the awareness and significance of bacterial-fungal biofilms in clinical and industrial settings. An investigation was undertaken to analyse the interactions between a diazotrophic bacterium (*Azotobacter chroococcum* - Az) and a filamentous fungus (*Trichoderma viride* - Tv), with emphasis on the structural and temporal changes during biofilm formation, nature of EPS, and gene expression. **Methods:** Aggregation assay was done through centrifugation, and the growth and biofilm formation was monitored through Bioscreen C lab system and Crystal Violet assay respectively. The composition of EPS isolated through ethanol precipitation, was determined both photocolometrically and using ATR-FTIR spectroscopy. Mesocosm experiments (with wheat, cotton and chickpea) were conducted at National Phytotron Facility. Soil nutrients, plant defense enzymes and soil biological parameters were quantified following standard methods. Whole transcriptome sequencing was done through Illumina NextSeq500 and the transcriptome was validated through qRT-PCR. **Results:** Manipulation of growth media led to significant increase in growth, aggregation and biofilm formation. Biofilm EPS showed significantly higher concentrations of proteins, acetyl groups and uronic acids, while planktonic EPS recorded higher amount of carbohydrates. Supplementation of different concentrations of L-amino acids revealed that 40 mmol l⁻¹ was the most effective in enhancing growth and biofilm formation. L-Glu and L-Gln favoured planktonic growth, while L-Trp and L-Thr, enhanced aggregation and biofilm formation. Whole transcriptome sequencing, revealed differential gene expression profiles in the individual (Az, Tv) and biofilm (Tv-Az). Gene expression analyses (Az or Tv vs. Tv-Az biofilm) revealed the up- and down-regulation of a large number of genes, particularly those related to *T. viride*. Pot studies using Tv-Az biofilm revealed significant enhancement in colonisation ability, plant growth, soil parameters and activity of plant defense enzymes as compared to individual inoculations (Az, Tv) in all three crops. **Conclusions:** Our study illustrates the potential of Tv-Az biofilm as a promising biofertilizing option in agriculture, for enhancing rhizosphere colonisation of applied bioinoculants leading to improved plant growth and availability of soil nutrients.
Quorum Sensing (QS) is a bacterial cell to cell communication mechanism, which is responsible for regulation of gene expression that mediates the production of virulence factors in a cell density-dependent manner. Inactivation of quorum sensing signal molecules of pathogenic bacteria has been proposed as a novel biotherapeutic method to fight against bacterial diseases. In this study, potential quorum quenching property of virulence factor production of shrimp pathogen *V. harveyi* by Bacillus spp. was investigated. 118 isolates of Bacillus spp. from aquaculture ponds and mangrove soil samples were screened for their ability to degrade synthetic AHLs; C4-HSL, C6-HSL, C8-HSL, C10-HSL and C12-HSL. Based on their potential to degrade all the five synthetic AHLs tested, seventeen Bacillus isolates were selected for further study. The AHL degradation potential was highest in *B. subtilis* MFB10, *B. lentus* MFB2 and *B. firmus* MFB7 and among these *B. subtilis* MFB10 exhibited maximum activity (78%). The supernatant of the three isolates of Bacillus spp. tested suppressed the production of various virulence factors of *V. harveyi* VH201 such as protease, lipase, phospholipase, caseinase and gelatinase and also the isolates led to a reduction of 75%, 73%, 70% and 65% in the biofilm formation of *V. harveyi* VH201. Also, the reduction in the expression of virulence genes such as metalloprotease, serine protease and haemolysin was confirmed by PCR analysis. Thus the present study suggests the potential of AHLs degrading Bacillus spp. as an alternative to antibiotics in shrimp hatcheries for controlling the luminescent Vibriosis.
Streptococcus pneumoniae (pneumococcus) is an opportunistic pathogen that causes otitis media, sinusitis, pneumonia, meningitis and sepsis. The progression to this pathogenic lifestyle is preceded by asymptomatic colonization of the nasopharynx. This colonization is associated with biofilm formation; the competence pathway influences the structure and stability of biofilms. However, the molecules that link the competence pathway to biofilm formation are unknown. Here, we describe a new competence-induced gene, called briC, and demonstrate that its product promotes biofilm development and stimulates colonization in a murine model. We show that expression of briC is induced by the master regulator of competence, ComE. Whereas briC does not substantially influence early biofilm development on abiotic surfaces, it significantly impacts later stages of biofilm development. Specifically, briC expression leads to increases in biofilm biomass and thickness at 72h. Consistent with the role of biofilms in colonization, briC promotes nasopharyngeal colonization in the murine model. The function of BriC appears to be conserved across pneumococci, as comparative genomics reveal that briC is widespread across isolates. Surprisingly, many isolates, including strains from clinically important PMEN1 and PMEN14 lineages, which are widely associated with colonization, encode a long briC promoter. This long form captures an instance of genomic plasticity and functions as a competence-independent expression enhancer that may serve as a precocious point of entry into this otherwise competence-regulated pathway. Moreover, overexpression of briC by the long promoter fully rescues the comE-deletion induced biofilm defect in vitro, and partially in vivo. These findings indicate that BriC may bypass the influence of competence in biofilm development and that such a pathway may be active in a subset of pneumococcal lineages. In conclusion, BriC is a part of the complex molecular network that connects signaling of the competence pathway to biofilm development and colonization.
Abstract Topic: Regulation of Biofilm Development

Abstract Title: Substrate Binding Protein DppA1 of ABC Transporter DppBCDF Increases Biofilm Formation in Pseudomonas aeruginosa by Inhibiting Pf5 Prophage Lysis


Abstract Body: Filamentous phage impact biofilm development, stress tolerance, virulence, biofilm dispersal, and colony variants. Previously, we identified 137 Pseudomonas aeruginosa PA14 mutants with more than 3-fold enhanced and 88 mutants with more than 10-fold reduced biofilm formation by screening 5850 transposon mutants (PLoS Pathogens 5: e1000483, 2009). Here, we characterized the function of one of these 225 mutations, dppA1 (PA14_58350), in regard to biofilm formation. DppA1 is a substrate-binding protein involved in peptide utilization via the DppBCDF ABC transporter system. We show that compared to the wild-type strain, inactivating dppA1 led to 68-fold less biofilm formation in a static model and abolished biofilm formation in flow cells. Moreover, the dppA1 mutant had a delay in swarming and produced 20-fold less small-colony variants, and both biofilm formation and swarming were complemented by producing DppA1. A whole-transcriptome analysis showed that only 10 bacteriophage Pf5 genes were significantly induced in the biofilm cells of the dppA1 mutant compared to the wild-type strain, and inactivation of dppA1 resulted in a 600-fold increase in Pf5 excision and a million-fold increase in phage production. As expected, inactivating Pf5 genes PA0720 and PA0723 increased biofilm formation substantially. Inactivation of DppA1 also reduced growth (due to cell lysis). Hence, DppA1 increases biofilm formation by repressing Pf5 prophage (Frontiers Microbiol. 9:30, 2018).
Agrobacterium tumefaciens is a plant pathogen that attaches to diverse surfaces via a single cellular pole using a polysaccharide adhesin, the unipolar polysaccharide (UPP). UPP production is strictly surface-contact dependent, but this can be bypassed by artificially elevating cytoplasmic levels of cyclic diguanylate monophosphate (cdGMP). One of the primary regulators of UPP production is the dual function diguanylate cyclase (DGC)-phosphodiesterase (PDE) protein we have designated DcpA, which has two transmembrane segments and a large periplasmic domain, in addition to its cytoplasmic catalytic domains. Null mutations in *dcpA* result in elevated cdGMP, loss of surface-contact dependence, increased attachment, and aggregation due to overproduction of UPP and cellulose. Under laboratory conditions DcpA is maintained in a PDE-biased state, governing low UPP production under non-inducing conditions. In contrast, DcpA exhibits strong DGC activity in null mutants of *pruR*, encoded immediately upstream of *dcpA*, and *pruA*, unlinked to the *pruR-dcpA* operon. PruA is a pteridine reductase that reduces the small co-factor 1,2-dihydromonapterin to 1,2,3,4-tetrahydromonapterin. PruR is homologous to molybdopterin-binding proteins in the SUOX family, but lacks the conserved cysteine that conjugates molybdenum. Null mutations in *pruA* or *pruR* result in strong elevation of DcpA DGC activity, and ectopic expression of *pruR* complements the *pruR* mutation, but does not rescue the *pruA* mutant. Chromatographic and mass spectrometric analyses reveal that a hexahistidyl-tagged version of PruR expressed in *A. tumefaciens*, co-purifies with a monapterin. PruR is a periplasmic protein and is targeted to the periplasm, via an N-terminal signal sequence. Whole-cell crosslinking experiments reveal that PruR and DcpA form a complex. Thus, a tentative model is that periplasmic PruR binds to a monapterin co-factor (synthesized via PruA activity), and through direct interaction with the DcpA periplasmic domain, pterin-associated PruR regulates the balance between PDE and DGC activity. PruR homologues are found in diverse proteobacteria and all share the N-terminal signal sequence, the absence of the Cys residues and the presence of several additional uniformly conserved residues. Strikingly, DcpA-type proteins (dual transmembrane domain DGC-PDEs) are found immediately downstream of most of these *pruR* homologues, suggesting widespread functionality of the pterin-dependent cdGMP regulatory circuit.
**Session Title:** TUESDAY Poster Session 2  
**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 087  
**Abstract Topic:** Regulation of Biofilm Development  
**Abstract Title:** The Antimicrobial Peptide Polymyxin B Induces a Drop in Biofilm Formation and Motility in *Vibrio cholerae*  
**Author Block:**  
S. Giacomucci, X. Perron, M. Duperthuy;  
Université de Montréal, Montréal, QC, CANADA.

**Background:** The acute diarrheal disease cholera, caused by *V. cholerae*, remains a significant public health problem, causing large numbers of infections and deaths annually in the world. During the infection, *V. cholerae* colonizes the surface of the small intestine. There are increasing number of evidence that *V. cholerae* is forming biofilm-like structures in vivo during the infection of the small intestine. One of the step in the transition from planktonic to biofilm lifestyle in *V. cholerae* is the down-regulation of the genes involved in motility coupled with an upregulation of the genes necessary for biofilm formation. The intestinal epithelium is the site of synthesis of many antimicrobial peptides, whose expression can be constitutive or inducible by microorganisms. In addition, bacteria from the microbiota are also secreting antimicrobial peptides. Therefore, we hypothesized that the antimicrobial peptides can be signalling molecules triggering the transition from the planktonic to the biofilm lifestyle in *V. cholerae*.  

**Methods:** A determination of the minimal inhibitory concentration in polymyxinB, a standard antimicrobial peptide, has been performed. After verification of the viability of the bacteria using flow cytometry analysis and growth curves coupled with colony forming unit counting, we determined the work concentration of polymyxin B as ¼ of the minimal inhibitory concentration. Then, standard crystal violet quantification of the biofilm, soft agar motility test, electronic microscopy and western blotting analysis were performed.  

**Results:** After 24h we surprisingly observed a drastic significant drop from 20 to 70% - depending on the *V. cholerae* strain considered - in biofilm formation in presence of polymyxin B. Similarly, a significant decrease of 20 to 35% in motility was also observed after 24h. A western-blot analysis demonstrated that the quantity of flagellin attached to the cells decreased in presence of polymyxin B associated with an increase in the free flagellin in the supernatant, suggesting a detachment of the flagella from the cells. Observation in electronic microscopy confirmed that result. We could also observe that a bulb appears at the tip of the flagella that remain attached to the cells. To our knowledge, a similar flagellar structure with bulb has only been observed in *Campylobacter* and never in *V. cholerae*.  

**Conclusions:** The presence of polymyxin B at sub-lethal concentration induces a decrease in *V. cholerae* motility probably due to a modification of the flagellar structure leading to the detachment of the flagella. In addition, polymyxin B also induces a drastic drop in biofilm formation. Whether the two phenotypes are linked or not is currently under investigation. In parallel, we are also investigating if the biofilm regulation pathway are involved in the decreased biofilm formation observed in presence of polymyxin B.
Yeast of the genus *Candida* are the most common cause of hospital-acquired fungal infections worldwide. *Candida parapsilosis* is the second or third most common *Candida* pathogen, depending on geographic location, and predominantly affects the very old and the very young. Biofilm formation is a key virulence factor in *Candida* species, which are frequently isolated from device-associated infections among hospitalised patients. A key step in biofilm formation is adhesion to a surface which requires the expression of cell surface proteins like adhesins. Although putative adhesins have been identified in the *C. parapsilosis* genome, their mechanism and regulation is poorly understood. In this study, we screen a library of transcriptional regulator deletion mutants to identify proteins that are involved in the regulation of adhesion to a plastic surface. We found four genes (*RFX1*, *RFX2*, *SFL2* and *NRG1*) that are required to regulate adhesion on multiple abiotic surfaces *in vitro*. Deletion any of these genes significantly increased the adhesion capacity of *C. parapsilosis* CLIB214 *in vitro*. Extensive transcriptomic analyses focusing on *NRG1*-mutant strains suggests a role for Nrg1 in the regulation of several adhesin-like proteins and proteins involved in GlcNAc metabolism. Finally, we use of a CRISPR-based approach to demonstrate the varying phenotypic effects of Nrg1-depletion in different clinical isolates of *C. parapsilosis*. 
Regulation of Biofilm Development

Abstract Title: What Fluorescent Reporters Tell about Biofilm Development: Classics versus Moderns

Author Block:

A. Monmeyran¹, P. Thomen¹, H. Jonquière¹, C. Douarche², A. Gautier³, N. Henry¹;
¹Sorbonne University, CNRS UMR 8237, Laboratoire Jean Perrin, Paris, FRANCE, Paris, FRANCE, ²Université Paris-Sud,
Université Paris-Saclay, CNRS, Laboratoire Fluides, Automatique et Systèmes Thermiques, Paris, FRANCE, ³P.A.S.T.E.U.R.,
Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, Paris, FRANCE.

Abstract Body:

Imaging era has reached biofilm field for years now, bringing about valuable qualitative information about complexity and heterogeneity of these living materials. These days, the advances in computational informatics, ever more widely available, support an exciting shift towards more quantitative approaches which require an improved degree of control of the markers. Here, we focus on living biofilm microscope imaging using genetically-encoded fluorescent reporters to monitor real-time biofilm development. We compare, in an Escherichia coli model biofilm growing in a millifluidic microfabricated device, the reports provided by the classic green fluorescent protein (GFP) and by the ‘modern’ fluorescence-activating and absorption-shifting tag (FAST), respectively. We show how oxygen gradients that establish as biofilm grows, alter classical reporter response. By contrast, FAST which fluorescence relies on a distinct mechanism avoids this problem. Finally, we discuss how the understanding of the reporter specific-dependence on the environmental conditions enables deriving new information about biofilm formation mechanism. References Plamont MA, et al. (2016) Small fluorescence-activating and absorption-shifting tag for tunable protein imaging in vivo. Proc Natl Acad Sci U S A 113(3):497-502. Monmeyran A, et al. (2018) The inducible chemical-genetic fluorescent marker FAST outperforms classical fluorescent proteins in the quantitative reporting of bacterial biofilm dynamics. Sci Rep 8(1):10336.
Burkholderia pseudomallei is a sapronotic disease agent that transitions from the environment to cause severe infections in humans and animals. During this transition, B. pseudomallei encounters and responds to rapidly changing conditions. Environmental sensing systems that control cellular levels of c-di-GMP promote pathogen survival in diverse environments. C-di-GMP is a nearly universal second messenger that controls the production of biofilms, virulence factors, and motility in many bacteria including B. pseudomallei. The goal of our research is to elucidate the regulatory control of the extracellular polymeric substances (EPS) that are produced by B. pseudomallei during infection and biofilm formation. B. pseudomallei produces diverse EPS components that include exopolysaccharides and capsular polysaccharides and there is currently a large gap in our knowledge concerning the identity and roles of these EPS components. To gain a more comprehensive understanding of the regulation of EPS genes that contribute to biofilm formation and evaluate their role in pathogenesis, we have taken multiple approaches that includes screening transposon mutants for loss of biofilm production, bioinformatics discovery of EPS components, global transcriptional analysis of genes during biofilm formation, and targeted deletion of polysaccharide biosynthetic clusters. Our recent efforts have demonstrated the role of c-di-GMP in the regulation of biofilm formation in B. pseudomallei and identified the becA-R exopolysaccharide biosynthetic cluster, which produces a crucial component of the B. pseudomallei biofilm matrix. We are currently investigating five additional polysaccharide biosynthetic clusters that share homology to gene clusters from other Burkholderia spp. and are predicted to serve critical roles in transmission, dissemination, and protection of the bacteria in the various environments and host niches that B. pseudomallei can occupy.
Regulation of Biofilm Development

Exploring the ecology and biodiversity of *Phormidium* (cyanobacteria) river biofilms

I. Echenique-Subiabre\(^1\), A. Zancarini\(^2\), M. W. Heath\(^3\), S. A. Wood\(^4\), C. Quiblier\(^5\), J. Humbert\(^6\);
\(^1\)Universidad Mayor, Santiago, CHILE, \(^2\)University of Amsterdam, Amsterdam, NETHERLANDS, \(^3\)Greater Wellington Regional Council, Wellington, NEW ZEALAND, \(^4\)Cawthron Institute, Nelson, NEW ZEALAND, \(^5\)Muséum National d’Histoire Naturelle and Université Paris Diderot, Paris, FRANCE, \(^6\)INRA, Université Pierre et Marie Curie, iEES Paris, Paris, FRANCE.

**Background:** River biofilms dominated by *Phormidium* (filamentous cyanobacteria) are receiving increased attention worldwide because of a recent expansion in their distribution and their ability to produce anatoxins, potent neurotoxins leading to animal mortalities. While planktonic cyanobacterial blooms have been the subject of numerous studies, mainly due to their detrimental effects on the ecosystems, only a limited number of studies have focused on their benthic counterparts. Consequently, there is limited data on the ecology and biodiversity of cyanobacterial biofilms.

**Methods:** In order to identify the environmental conditions that favour their development and potential toxicity, the composition and structure of bacterial communities (BCs) associated with *Phormidium* was investigated. Biofilms were collected during the summer season of 2013 and 2014 in the Tarn River (France) and in eight rivers across New Zealand. Biofilms biomass and cover was quantified as well as biodiversity and anatoxin concentrations using high-throughput sequencing and mass spectrometry respectively.

**Results:** This study revealed that *Phormidium* biofilm development was exclusively detected in riffle areas. In the Tarn River *Phormidium* cover was positively correlated with water temperature, depth, and anatoxin concentrations. The highest anatoxin concentrations were recorded at the upstream sites of the river and at the end of the summer period. The structure of the BCs from both countries was well conserved at the order level and 28% of the OTUs containing 90% of the reads were shared by these BCs. A strong and significant distance-decay relationship was found in BCs from New Zealand rivers but the Bray-Curtis dissimilarities between French and New Zealand BCs were in the same order of magnitude of those found between New Zealand BCs.

**Conclusions:** The spatio-temporal pattern of anatoxin production in the Tarn River highlights the importance of biofilm maturity and the local environmental conditions on anatoxin variability. The conserved structure of BCs suggests that micro-environmental conditions occurring within *Phormidium* biofilms strongly shape the associated communities. Local environmental conditions seem to have more impact on BCs than dispersal capacities of bacteria.
Xylella fastidiosa Utilizes a β-1,4-endoglucanase to Modulate Exopolysaccharide Production and Biofilm Development

C. A. Castro, B. M. Ingel, M. Roper; University of California, Riverside, RIVERSIDE, CA.

*Xylella fastidiosa* is a gram-negative bacterium that causes numerous severe diseases in economically important crops, residing in the host xylem and in the mouthparts of its insect vectors where it produces exopolysaccharides (EPS) and forms robust biofilms. We investigated the role of a β-1,4-endoglucanase (EngXCA2) secreted by *Xf* subsp. *fastidiosa*, the causal agent of Pierce’s disease of grapevine, in phenotypes associated with biofilm development: surface attachment, cell-cell aggregation, EPS production, and 3D biofilm architecture. ∆engXCA2, an EngXCA2-deficient mutant strain, was impaired in cell-cell aggregation and produced significantly more EPS than the wild-type strain *in vitro*. In addition, ∆engXCA2 was significantly compromised in attachment to glass and was unable to develop a full biofilm relative to the wild-type strain. Furthermore, confocal laser scanning microscopy images of 4-day-old biofilms show that ∆engXCA2 biofilms are thinner than wild-type biofilms, which was confirmed by measuring average and maximum biofilm thickness. Therefore, we hypothesize that EngXCA2 facilitates the turnover of EPS during the *Xf* biofilm developmental cycle by dismantling the predicted β-1,4-glucan backbone of EPS.
**Background:** *Vibrio fischeri* expresses a complex set of two-component regulators to control biofilm formation, a critical event in the symbiotic colonization by *V. fischeri* of the Hawaiian squid, *Euprymna scolopes*. Overexpression of the sensor kinase *rscS* induces biofilm formation dependent on the symbiosis polysaccharide (*syp*) locus, and results in the formation of wrinkled colonies, pellicles in static liquid cultures, and enhanced symbiotic biofilms. Activated *RscS* is thought to donate a phosphoryl group to the Hpt domain of hybrid sensor kinase *SypF*, which promotes phosphorylation of the response regulator and transcriptional activator *SypG*. Recently identified sensor kinase *BinK* is a negative regulator of biofilm formation, also acting at the level of *syp* transcription. Numerous regulators are involved in biofilm formation, but until recently, no environmental signals were known to control biofilm formation by *V. fischeri*. **Results:** We recently reported the identification of calcium as a signal that promotes biofilm formation by biofilm-competent strains, including *rscS* overexpressing cells and *binK* mutant cells. Under shaking liquid growth, calcium induced both *syp*-dependent biofilms, resulting in cohesive cellular clumps, and bacterial cellulose synthesis (*bcs*)-dependent biofilms, resulting in surface attached rings. Calcium also induced formation of *syp*-dependent cohesive wrinkled colonies, and significantly increased transcription of both *syp* and *bcs* in liquid culture. In the absence of *BinK*, calcium-induced biofilms were shown to have three independent inputs - *RscS*, *SypF*, and newly identified sensor kinase *HahK*, all dependent on the Hpt domain of *SypF*. Deletion of these regulators resulted in disruptions to biofilm formation. *HahK* is in an operon downstream of nitric oxide-responsive sensor kinase *HnoX*. Investigation of *HnoX* revealed that it decreases *syp*-dependent biofilm formation through inhibition of *HahK*. Finally, we determined that inhibition by *HnoX* was dependent on nitric oxide, a result that reveals nitric oxide as a second environmental signal controlling biofilm formation in *V. fischeri*. **Conclusions:** This work identifies calcium and nitric oxide as the first known environmental signals to control *V. fischeri* biofilm formation. Calcium induces two distinct types of biofilms, dependent on *SYP* and cellulose polysaccharide, while nitric oxide inhibits *syp*-dependent biofilm formation. Effects of calcium and nitric oxide both occur, at least in part, at the level of transcription. Furthermore, this work reveals two new regulators, sensor kinases *HahK* and *HnoX*, as additional components of this complex pathway. The roles of both calcium and nitric oxide in biofilm formation by *V. fischeri* appear to be distinct from other *Vibrio* species, highlighting the diverse mechanisms by which these ubiquitous molecules can control bacterial behavior.
Reciprocal Control of Surface Attachment and Motility by the PdhS2 Two-Component Sensor Kinase of *Agrobacterium tumefaciens*

J. F. Pinto¹, J. L. Eagan², D. Crosby¹, S. Brar¹, T. Singletary¹, C. Fuqua², J. E. HEINDL¹;

¹University of the Sciences in Philadelphia, Philadelphia, PA, ²Indiana University, Bloomington, IN.

A core regulatory pathway that directs developmental transitions and cellular asymmetries in *Agrobacterium tumefaciens* involves two overlapping, integrated phosphorelays. One of these phosphorelays putatively includes four histidine sensor kinase homologues, DivJ, PleC, PdhS1, and PdhS2, and two response regulators, DivK and PleD. In several different alphaproteobacteria, this pathway influences a conserved downstream phosphorelay that ultimately controls the phosphorylation state of the CtrA master response regulator. The PdhS2 sensor kinase reciprocally regulates biofilm formation and swimming motility. In the current study the mechanisms by which the *A. tumefaciens* sensor kinase PdhS2 directs this regulation are delineated. PdhS2 lacking a key residue implicated in phosphatase activity is markedly deficient in proper control of attachment and motility phenotypes, whereas a kinase-deficient PdhS2 mutant is only modestly affected. Genetic interactions between the DivK/PleD response regulator pair and PdhS2 are revealed, unmasking one of several connections between PdhS2-dependent phenotypes and transcriptional control by CtrA. Epistasis experiments suggest that PdhS2 may function independently of the CckA sensor kinase, the cognate sensor kinase for CtrA which is inhibited by the DivK/PleD response regulator pair. Gene expression analyses support PdhS2 regulating both motility and levels of the intracellular signal cyclic diguanylate monophosphate (cdGMP) through CtrA transcriptional control, thereby affecting production of adhesive polysaccharides and attachment. We hypothesize that in *A. tumefaciens* the CtrA regulatory circuit has expanded to include additional inputs through addition of PdhS-type sensor kinases, likely fine-tuning the response of this organism to the soil microenvironment.
Proteomics of Cell-cell Communication in Lactic Acid Bacteria from Porcine GI Tract

O. A. Aiyegoro¹, A. A. Adegoke²;
¹Agricultural Research Council- Animal Production Institute, Pretoria, SOUTH AFRICA, ²University of Uyo, Akwa-Ibom, Uyo, NIGERIA.

Lactobacillus reuteri ZJ625 mechanisms of cell-cell communication were investigated in this study when co-cultured with either Lactobacillus reuteri VB4 or Lactobacillus salivarius ZJ614. Almost 80% of Lactobacillus reuteri ZJ625 cells were killed when co-cultured with either Lactobacillus reuteri VB4 or Lactobacillus salivarius ZJ614 as stationary phase of growth was reached. Almost the same proteins were induced in all co-cultures at the mid-exponential phase of growth, and the number of induced proteins significantly increased at stationary phase of growth in all co-cultures. In all, ten proteins were over expressed in the co-cultures; these proteins have been reported to play critical roles in stress response mechanisms. The LuxS-mediated signalling was involved in the regulation of most of these proteins in this study. QPCR showed that the expression of luxS gene decreased as the cultures approached stationery phase of growth in co-cultures. It could be inferred from the study that: the type of microbial co-cultures dictates the synthesis of proteins, also two compounds: 2(3H) dihydrofuranone-5-ethyl and 2(3H) dihydrofuranone-5pentyl were identified as putative signalling molecules when Lactobacillus reuteri ZJ625 cells were co-cultured with either Lactobacillus reuteri VB4 or Lactobacillus salivarius ZJ614 Keywords:Proteomics; Porcine; Cell-cell communication; Co-cultured; LuxS; Microbial interaction; Lactic acid bacteria
Biomaterials designed to heal bone fractures often serve as a nidus for chronic infection. The primary barriers to alleviating these infections are the development of biofilms that are tolerant to systemic antibiotics. Cell-cell communication through the quorum sensing (QS) accessory gene regulatory (agr) pathway has been shown to contribute to biofilm regulation. While some agr inhibitors have shown promise, the large diversity in peptide pheromones that drive QS presents a challenge. Thus, it is necessary to develop materials that delay the agr-mediated biofilm development. In this work, we investigated (1) the effect of surface geometry of an implanted device on the kinetics of tolerance development in \textit{S. aureus} and (2) the relationship between the quorum sensing accessory gene regulatory (agr) system and tolerance development across different surface geometries. Finally, we determine the ideal implant structure capable of delaying tolerance development on medical implants. Convex substrates were 3D printed using PLA extrusion. Concave scaffolds were synthesized by casting a settable poly(ester urethane) (PEUR) within the 3D convex structure and extracting the PLA. Inoculations of \textit{S. aureus} UAMS-1 WT and agr knockout strains were seeded on the 3D structures and allowed to develop biofilm communities for 6, 24, and 48 hours before 24-hour treatment with vancomycin. Cells surviving vancomycin treatment were quantified and classified as tolerant cells. \textit{S. aureus} cells seeded on 3D concave morphologies demonstrated minimal decrease in surface density following vancomycin treatment after 24 hours of biofilm establishment. Conversely, cells seeded on a convex 3D geometry exhibited a more susceptible phenotype after 24h of biofilm development despite having a similar surface density to 3D concave morphologies when left untreated. \textit{S. aureus} cells on both morphologies demonstrated similar tolerance profiles when allowed 48h of biofilm development, suggesting that a tolerant community will eventually develop on both surfaces. \textit{S. aureus} agr knockout cells grown on concave substrates demonstrated a significantly smaller community of tolerant cells compared to the WT control, while no difference was seen between the WT and agr knockout strains on convex surfaces. Thus, substrate morphology was shown to influence the kinetic development profile of biofilms on a surface, and convex morphologies have shown capabilities of delaying biofilm treatment recalcitrance by interrupting QS-mediated cell tolerance development.
Bacteria in chronic lung infections alternate between surface-attached and planktonic lifestyles. Adaptation in such a dynamic environment can select for ecological diversity that can increase survival and fitness of the population. To understand the molecular mechanisms underlying this adaptive diversity, we propagated *Burkholderia cenocepacia*, a pathogen associated with chronic lung infections, in biofilm conditions for >1000 generations. Multiple coexisting lineages that evolved within biofilm populations acquired single nonsynonymous mutations affecting different domains of RpfR, a key modulator of cyclic-di-GMP (cdG) levels in various proteobacteria. RpfR has both GGDEF and EAL domains which function as diguanylate cyclase and phosphodiesterase, respectively. Thus, RpfR can synthetize as well as degrade cdG. High cdG generally promotes exopolysaccharide production and inhibits motility and in *B. cenocepacia* it binds to the RpfR-GtrR complex, repressing the influence of RpfR on GtrR-regulated genes such as fucose-binding lectins. Further, the RpfR PAS domain interacts with the diffusible signal factor cis-2-dodecenoic acid, which stimulates phosphodiesterase activity and degrades cdG. We ask if the different mutations selected in *rpfR* regulate these cdG-mediated processes differently. Specifically, what functional advantage do these mutations provide that results in their selection and maintenance in the population? To achieve this, we used phenotypic and transcriptomic assays to quantify the growth, biofilm productivity, polysaccharide composition and gene expression differences in various mutants. We find that evolved mutants have higher fitness in biofilms, vary in their cdG concentrations as well as display structural differences in biofilm architecture. The RNAseq results display downregulation of genes conferring motility and fucose binding lectins, which coincide with an increased presence of fucose in the matrix of evolved mutants. When grown in cocultures, certain isogenic mutants can invade each other when rare, which implies that they could stably coexist. Moreover, the increased biofilm productivity of mixed cultures suggests complementary interactions and a potential display of division of labor between the genotypes. Taken together, we demonstrate that distinct genotypic changes in one gene- *rpfR*, result in diverse ecological roles contributing to the evolution of synergistic biofilm assembly. These results not only reveal RpfR is at the core of biofilm regulation in this bacterium but also indicate that modifying the regulation of RpfR mediated pathways can generate adaptive diversification.
**Abstract**

**Background:** Horizontal gene transfer via conjugation is a very important mechanism of gene spreading in bacterial populations, and is of particular concern for the emergence of antibiotic resistant bacteria. While transmission of conjugative plasmids were described as being particularly efficient in biofilms, the molecular mechanisms underlying this enhanced transfer is not fully elucidated. Additionally, nothing is currently known on the transfer of genome-encoded integrative and conjugative elements (ICEs) in these multicellular structures. *B. subtilis* is host to ICEBs1, a well-characterized ICE that is thoroughly characterized. This Gram-positive bacterium is also known to form robust biofilms under the appropriate conditions, which allows us to precisely evaluate the impact of biofilm and extracellular matrix on conjugation. **Methods:** For this study, the non-domesticated NCIB3610 *B. subtilis* bearing an antibiotic resistance marker on ICEBs1 (donors) or cured of ICEBs1 (recipient) were used. Conjugation assays were performed using solid and liquid media differing in their capacity to induce biofilm, and several deletion mutants of biofilm-related genes allowed us to determine the importance of the extracellular matrix. Excision rates were evaluated using a qPCR approach. **Results:** Our results clearly demonstrate that biofilm formation increases ICEBs1 transfer efficiency by at least a 100-fold. This efficient transfer is maintained even at low donor:recipient ratios. Evaluation of transfer rates through time shows a clear temporal correlation between formation of the biofilm and ICEBs1 transfer. However, biofilm formation does not trigger an increase of ICEBs1 activity, since excision levels in the donor cells remains constant through time. This result suggests that the biophysical context of biofilms favours ICEBs1 conjugative transfer. Indeed, cells constitutively secreting component of the extracellular matrix can transfer ICEBs1 very efficiently in conditions that normally do not induce biofilm formation. Additionally, production of the extracellular matrix, particularly from the recipient cells, is essential for efficient ICEBs1 transfer in biofilms. Presence of exopolysaccharides in the matrix was slightly more important than amyloid-like fibers in favouring conjugation. **Conclusions:** Our study shows that biofilms favour high efficiency conjugative transfer of ICEBs1 between cells due to the presence of extracellular matrix. These results provide a new perspective on the transfer of Gram-positive ICEs, many of which exhibit inefficient transfer in vitro but are nevertheless harboured by biofilm-prone bacteria. Our work provides important insights on horizontal gene transfer in biofilms, a widely preponderant bacterial lifestyle.
Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects cystic fibrosis (CF) lungs and chronic wounds by forming biofilms. Emergence of phenotypically diverse isolates within P. aeruginosa biofilms has been already reported. However, the dynamics between isolates evolved within biofilms and the possible effects on the progression of chronic infection and treatment outcomes are poorly understood. Here we tested how the P. aeruginosa strain PAO1 evolves in biofilms over 50 days using a bead biofilm model in combination with synthetic sputum medium. Our evolution experiment showed that within biofilms, the emergence of distinct P. aeruginosa morphotypes occurs. We studied interactions between selected evolved morphotypes to understand how these impact upon the phenotype of diverse populations. We observed varying levels of cooperation and conflict between certain morphotypes in biofilms. We also tested the antibiotic tolerance of evolved populations and individual morphotypes, and observed a population level increase in tolerance to certain antibiotics, despite no previous antibiotic exposure. We used MALDI-TOF analysis and confocal imaging to observe both physical and chemical interactions between morphotypes. A greater understanding of interactions between diverse morphotypes within populations could form the basis of new strategies towards treatment and control of biofilms, and also provide explanations as to how and why phenotypic diversity and antibiotic resistance evolves during long term chronic infection.
Competition Sensing Regulates Matrix Production Formation, Virulence and Antibiotic Tolerance in *Salmonella* Biofilms

Bacteria use complex regulatory networks to cope with stress, including antibiotics, but the function of these networks in natural habitats is poorly understood. The competition sensing hypothesis states that bacterial stress response systems can serve to sense ecological competition but studying regulatory responses in mixed culture is challenging. Here we solve this problem by using differential fluorescence induction to screen the *Salmonella Typhimurium* genome for loci that respond, at the single-cell level, to life in a competitive biofilm community. This reveals the presence of competing strains drives up the expression of genes involved in biofilm matrix production (CsgD pathway), epithelial invasion (SPI1 invasion system), and antibiotic tolerance (*TolC* efflux pump; *AadA* aminoglycoside 3-adenyltransferase) and the associated phenotypes. We study mutants in key regulatory pathways and link these competitive responses to major stress responses, as predicted by the competition sensing hypothesis. Moreover, inactivation of the Type VI secretion system (T6SS) of a competitor annuls the responses to competition, indicating that T6SS-derived cell damage activates these stress response systems. Our work provides critical support for the competition sensing hypothesis by showing that bacterial regulatory networks detect and respond to competition in a manner important for biofilm formation, virulence and antibiotic tolerance. This also reveals microbial consortia can become tolerant to antibiotics and other stresses because bacteria are defending themselves from competitors, not because they are cooperating with one another.
Mechanisms and Impact of Horizontal Gene Transfer in a Model Microbial Community

Title:
Mechanisms and Impact of Horizontal Gene Transfer in a Model Microbial Community

Author:
G. Heussler¹, K. Bonham², R. Dutton¹

¹University of California San Diego, San Diego, CA, ²Broad Institute, Harvard School of Public Health, Boston, MA.

Abstract:
These include proteins putatively involved in processes such as iron-siderophore transport & lactate utilization. We hypothesize that these genes can confer a selective advantage in the iron deplete and lactate rich cheese medium. Here, we focus on a widespread genetic element termed RUSTI (iRon Uptake and Siderophore Transport Island). Our data suggest this element is encoded on an active Integrative and Conjugative Element (ICE). By tracking this ICE during the initial colonization and aging of the cheese medium, investigating the regulation of the RUSTI genes, and assaying the effect RUSTI confers on the fitness of the recipient bacteria, these data provide insights into how HGT can influence microbial communities including during the colonization of a new environment.
The emergent behavior of microbes in multispecies communities can lead to robust biofilm formation, a phenomenon that causes major problems in many segments of our society, including in the clinic. As many as 80% of infections in the human body are associated with biofilms [1], which often include more than one microbial species. Despite the prevalence of multispecies communities in disease and medical device infections, we lack robust in vitro models and metrics to standardize multispecies biofilm measurements and facilitate reproducible, comparable experiments. Our objective was to evaluate the potential of metabolic profiling to establish reproducibility criteria for biofilm function, by measuring repeatability of intracellular and extracellular metabolomes for a clinically relevant in vitro co-culture model. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are pathogens often co-located in human disease states including wound infections and chronic lung infections of cystic fibrosis (CF) patients. Coinfection involving these species typically worsens patient outcomes and can lead to antibiotic resistance as compared to monoculture infections. The two species were grown as mono- or co-cultures for 24 h in artificial sputum medium to mimic nutrients in CF lung infections and improve clinical relevance versus typical in vitro growth media. We integrated and optimized protocols for microbial culturing and quantification, quenching, and metabolite extraction. Global metabolite profiles were analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS). Principal component analysis indicated that profiles of *P. aeruginosa*, *S. aureus*, and the co-culture are statistically differentiable (n = 9) and highly repeatable across all three levels (day, biological and technical replicate). In addition, we found 575 features unique to the co-culture, which are under identification analysis. Overall, our results suggest that LC-MS/MS metabolic analysis is a promising approach to monitor reproducibility of multispecies biofilms. Our approach enables comparison and combination of results across laboratories toward a collective improved understanding of interspecies interactions, their role in disease, and novel therapeutics to target these systems.
Session Title: **TUESDAY Poster Session 2**

**Session Date/Time:** Tuesday, October 9, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 104

**Abstract Topic:** Synthesis and Assembly and Function of the Biofilm Matrix

**Abstract Title:** The Formation of Functional Mineral Scaffolds Within Biofilms is Biologically Controlled

**Author Block:** I. Kolodkin-Gal, A. Keren-Paz, I. Karunker-Hazan; Weizmann Institute of Science, Rehovot, ISRAEL.

**Abstract Body:** Multicellular bacterial communities, known as biofilms, have been thought to be held together solely by a self-produced organic extracellular matrix. Our study of two phylogenetically distinct bacteria: *Bacillus subtilis* and *Mycobacterium smegmatis*, identified a novel mechanism maintaining biofilms - an active production of crystalline calcite scaffolds. We demonstrated the existence of calcite scaffolds in bacterial biofilms and their roles in stabilizing biofilms, and in limiting penetration of small molecule solutes as antibiotics. In addition, we demonstrated that calcite mineral scaffolds play a conserved role in the assembly of complex communities. Inhibiting enzymes promoting biomineralization, hampered biofilm formation and restored diffusion of small molecules into the biofilm (Keren-Paz et al., 2018; Oppenheimer-Shaanan et al., 2016). Furthermore, our recent results demonstrate that both in vitro and in clinical scenarios, biofilms formed by the Gram-negative pathogen *Pseudomonas aeruginosa* are held together by biogenic calcite minerals. To identify the cellular pathways involved in biomineralization, we performed an unbiased transcriptome analysis of the biofilm cells responding to a soluble calcium source. The transcriptome architecture suggested a role for cell envelope synthesis and remodeling during biomineralization, as well as for increased calcium storage within the cells. These results support the hypothesis that crystal formation initiates intracellularly. Consistently, high resolution Scanning Transmission Electron Microscopy (STEM) of the biofilm cells indicated that the minerals are produced within discrete foci in the periplasm of dedicated cells, released from the cell, and finally mature by interactions with the organic extracellular matrix. Our work provides insights into the relationship between the behavior of single cells, community structure and biofilm function.
Biofilm formation by the opportunistic pathogen *Pseudomonas aeruginosa* involves the secretion of polysaccharides or other polymers that structure the extracellular matrix and protect bacteria from antimicrobials and host immunity. The Pel polysaccharide is important for biofilm development by *P. aeruginosa*, yet very little is known about the initial stages of Pel biosynthesis and export. In this study, we elucidate a complex of the proteins PelDEFG that is key for the earliest steps of exopolysaccharide production. By using a combination of genetic dissection, cellular fractionation, co-immunoprecipitation, and bacterial two-hybrid analyses, we demonstrate that these proteins associate, and bring together in a single molecular machine all the functionalities necessary for Pel polymerization and its transport across the cytoplasmic membrane. We find that binding of the bacterial secondary messenger cyclic diguanylate (c-di-GMP) to the receptor PelD modulates interactions within this complex, suggesting a means to post-translationally regulate Pel biosynthesis in response to changing intracellular c-di-GMP levels. Further, we identify direct interactions between PelD and c-di-GMP metabolizing enzymes, including the phosphodiesterase BifA, which implies a mechanism for maintaining specific control over Pel biosynthesis independently of other c-di-GMP binding effectors in the cell. Overall, the mechanism for Pel biosynthesis inferred from these findings deviates from established exopolysaccharide secretion mechanisms in Gram-negative bacteria, leading us to propose that the Pel apparatus defines a new mechanistic sub-class of the synthase-dependent exopolysaccharide secretion pathway. This work also expands our fundamental understanding of how c-di-GMP signaling specificity can be attained in the unrestricted environment of a bacterial cell, as well as how c-di-GMP can allosterically regulate the functionalities of membrane-associated protein complexes.
The Extracellular DNA Lattice of Bacterial Biofilms are Structurally Related to Holliday Junction Recombination Intermediates

**Author:** S. D. Goodman, A. Devaraj, L. Mashburn-Warren, L. O. Bakaletz;

**Nationwide Children's Hospital, Columbus, OH.**

**Background:** Bacteria adopt a biofilm state that represents multicellular microbial communities adherent to each other as well as to an abiotic or biotic surface. Bacteria in a biofilm are surrounded by extracellular polymeric substances, primarily comprised of exopolysaccharides, extracellular DNA (eDNA) and proteins. eDNA is ubiquitous and a pivotal component to maintain the structural integrity of bacterial biofilms. We have shown previously that eDNA in biofilms formed by multiple bacterial species is organized into a lattice-like structure that is stabilized by DNABII proteins. DNABII proteins are a family of DNA binding proteins that exhibit high affinity to pre-bent DNA. Sequestration of these proteins from the bacterial biofilm matrix with a DNABII-specific antibody (α-DNABII) destabilizes the matrix and thereby results in disruption of the biofilm structure. Since the cross strand organization of eDNA bears a striking resemblance to Holliday-junction (HJ) DNA and the DNABII proteins naturally bind to HJs, we hypothesized that eDNA lattice in bacterial biofilms are structurally related to HJ recombination intermediates.

**Methods:** To incorporate RuvA, we added RuvA (450 nM) to pre-formed biofilms at 16h and 24h in the presence of α-DNABII. RuvA dependent helicase RuvB (1130 nM), HJ-specific endonucleases RuvC (100 nM) or RusA (350 nM) were added at 24h. At 40h, the biofilms were stained with LIVE/DEAD® or labeled with appropriate antibody for immunofluorescence and analyzed using confocal laser scanning microscopy. **Results:** The addition of the prototypic HJ-specific DNA binding protein RuvA, both readily incorporated within the biofilm matrix and prevented α-DNABII-mediated disruption of bacterial biofilms formed by uropathogenic *E. coli*, nontypeable *Haemophilus influenzae* (NTHI), and *Staphylococcus epidermidis*. Next, we assembled the HJ-specific endonuclease complex RuvABC at the RuvA-bound HJ DNA sites, which resulted in collapse of the biofilm structure. Additionally, treatment of bacterial biofilms with another HJ-specific endonuclease RusA, also resulted in total collapse of the biofilm structure of multiple bacterial species. Addition of RusA also prevented the formation of the complex web-like eDNA lattice structure in biofilms formed by NTHI. As a final confirmation for the presence of HJ DNA structure within the biofilm matrix, we labeled bacterial biofilms with a monoclonal antibody directed against cruciform DNA that recognizes HJ DNA and observed uniform distribution of HJ DNA throughout the biofilm matrix. Addition of RusA to biofilms at seeding also dramatically decreased the cruciform DNA within the biofilm matrix. **Conclusion:** Collectively, these data indicated that the eDNA lattice of bacterial biofilms are structurally related to HJ recombination intermediates and are critical to the structural integrity of bacterial biofilms.
Session Title: **TUESDAY Poster Session 2**  
Session Date/Time: Tuesday, October 9, 2018, 4:15 pm - 5:45 pm  
Poster Board Number: 107  
Abstract Topic: Synthesis and Assembly and Function of the Biofilm Matrix  
Abstract Title: Volatile Fatty Acids Production by Anaerobic, Autotrophic Bacteria in a Syngas-based Membrane Biofilm Reactor  
Author Block: D. C. Calvo, B. E. Rittmann, C. I. Torres; Arizona State University, Tempe, AZ.

**Introduction:** Acetogenic bacteria utilize the Wood-Ljungdahl pathway to convert hydrogen, carbon dioxide, carbon monoxide, and a wide range of sugars to volatile fatty acids (VFA) and alcohols, especially acetate and ethanol. VFA production using syngas (H₂, CO₂ and CO) has been widely reported; however, gas hydrophobicity has limited the production rate of this process. The Membrane Biofilm Reactor (MBfR) is a unique technology that delivers syngas directly to microorganisms that live as a biofilm on the outer surface of gas-transfer membranes. Direct transfer to the biofilm overcomes interfacial gas-liquid mass-transfer limitations. Today, the MBfR is used for waste treatment, especially for oxidized contaminants such as perchlorate, uranium and chromate. Here, we develop an MBfR capable of producing VFAs using the H₂ and CO present in syngas as electron donors and CO₂, CO, and bicarbonate as carbon sources. **Methods:** We operated three 120-cm³ glass MBfRs with a continuous medium flow containing 120 mM of bicarbonate. Digester sludge was the inoculum. Reactors 1 and 2 (R1 and R2) had 130 cm² of composite hollow fibers (polyurethane-polyethylene) and hydraulic retention times (HRT) of 40 h and 14 h, respectively. Reactor 3 (R3) had 2.15 cm² of asymmetric Matrimid®-based fibers and an HRT of 14 h; this fiber was synthesized specially for this project by our collaborators at Georgia Institute of Technology, and it can achieved >10000-fold faster gas delivery than the composite fiber. Syngas pressure inside the membranes was 5 psig. We tracked production/consumption of H₂, CH₄, CO, and CO₂ by gas chromatography, measured bicarbonate concentration by ion chromatography, and measured VFA concentrations by high performance liquid chromatography. Gas and liquid samples were taken daily. We also sequenced the 16S ribosomal RNA gene in biofilm samples and performed qPCR to assay total 16S ribosomal RNA gene for all bacteria, mcrA gene for methanogens, and the FTHFS gene for homoacetogens. Sequencing results were analysed using Quantitative Insights Into Microbial Ecology - Quiime®. **Results and Discussion:** All reactors achieved steady state and substantial VFA concentrations. Notably, R1 generated products up to 7 carbons chain length, while R3 had the highest acetate concentration, 67 mM. The longer HRT of R1 seemed to play a key role in chain elongation. Gas chromatography showed that H₂ was below 5% and CH₄ was negligible in the gas output in all reactors. Although general bacteria decreased from about 2x10⁴⁴ to 8x10⁴⁴ gene copies/cm² and methanogens decreased to undetectable, acetogens increased from negligible to 5x10⁴⁵ gene copies/cm². This enrichment of the biofilm with homoacetogens is desired. **Conclusions:** This study proves that the syngas-based MBfR is a viable platform for VFA production and enrichment of acetogens in a biofilm. Reaching steady state is promising for large-scale applications.
Abstract

The Gram-negative bacterium *Pseudomonas aeruginosa* is found ubiquitously and is an opportunistic human pathogen in people with weakened immune-system. Due to its ubiquity, abundance, pathogenicity, and ability to form biofilms it is often used as a model organism to study biofilm development. The biofilm lifestyle enables efficient resources sharing, protection from predation and changing environmental conditions. Biofilm bacteria often have an increased resistance to antibiotics. People with the genetic disease Cystic fibrosis (CF) are often afflicted with chronic infections of *P. aeruginosa*. The prognosis of CF patients becomes markedly worse with the appearance of an alginate over-producing phenotype, called the mucoid variant, in the late stages of infection. This mutant greatly increases the environmental viscosity and the alginate encapsulated cells also have increased resistance to environmental stresses; these factors contribute to the deterioration of the patient’s health. It is reported that the *P. aeruginosa* mucoid variant and non-mucoid wild type coexist in *in vivo* biofilms. However, there is little understanding of the spatio-temporal appearance of the mucoid mutant and localization of alginate within those biofilms. In this study we use the *P. aeruginosa* PAO1 mucoid variant to clarify the ecology of the mucoid variant in 3-dimensional biofilms starting at the single cell level. We utilize flow-cell channels to observe the development of the mucoid-variant biofilms and image the localization of alginate using immuno-staining and confocal microscopy. We find that alginate secretion increases with the elapsed time: in the early stages of biofilm formation, we observe a heterogeneous distribution of filamentous alginate strands decorating small microcolonies, while at later times the alginate completely covers the mature biofilm. Interestingly, we observe the appearance of different phenotypic populations from the edges of plated mucoid colony biofilms. These phenotypically different cells exhibit surface motility patterns similar to WT suggesting that a reversion variant appears under certain conditions present in a mucoid biofilm. To visualize this change, we constructed a revertant variant reporter strain in the PAO1 mucoid variant background that expresses DsRed when overproducing alginate or sfGFP when the reversion mutation has occurred. We imaged the appearance of this reversion mutant at the base of a mature mucoid colony using this reporter strain and confocal microscopy. We aim to observe biofilm formation using this reporter mutant to clarify the localization of alginate production and, simultaneously, the appearance of revertant variant in biofilms. We hope that these results will contribute to development of a deeper understanding of the ecological implications of the appearance of the mucoid phenotype.
Biofilms are groups of microorganisms that adhere to one another on a surface, through a self-produced matrix of extracellular polymeric substance. This mesh provides protection against environmental pressures, host immune responses and antimicrobial agents. As almost all bacteria establish biofilms as a strategy for survival and persistence, understanding how they form is key to the development of new compounds to combat antibacterial resistance. Biofilm growth by virulent strains of *Escherichia coli* is a major contributor to the establishment of diseases such as hemorrhagic colitis, neonatal meningitis, urinary tract infections, pneumonia and sepsis. Matrix components such as SslE have been identified as fundamental for biofilm maturation; however, the actual interactions that this molecule mediates are not well understood. SslE is a ~165-kDa protein found on the bacterial surface and within outer membrane vesicles but details of its structural features are unclear. It does, however, possess a C-terminal zinc-metalloprotease-like domain, which influences colonization of the small intestine through degradation of mucins. This facilitates bacterial penetration of the mucus layer, adhesion to host receptors and delivery of toxins. In this work we are developing an interdisciplinary approach to identify interactions that are essential for the establishment of biofilms in *Escherichia coli*. We are using the non-pathogenic *Escherichia coli* strain W as a model system to (a) determine the structure of the lipoprotein SslE, (b) understand its role in establishing colonization and (c) why it is required for biofilm maturation. We are using structural biology approaches such as crystallography and NMR to solve SslE structure and observe its interaction with substrates. We have developed mucin degradation assays to observe and compare the mucinase activity of the full-length protein and its isolated domains. We have built a biofilm flow-cell system to observe difference in phenotypes of the wild type vs SslE mutants using confocal microscopy. We have also developed a static biofilm plate assay where we are being able to observe biofilm maturation, when according to literature, this would not be possible for our system. Altogether we are using microbiology and biochemistry techniques to validate and complement each other on our quest to understand SslE function in establishing a mature biofilm and its importance in degrading mucin. In the future our findings will be further tested in the enterotoxigenic *E. coli* strain H10407.
Synthesis and Assembly and Function of the Biofilm Matrix

**Abstract**

**Background:** CsgA, also known as a major curli component, is a secreted protein ubiquitous in biofilms of gram-negative bacteria (Zhou et al., 2012a). Thanks to its ability to create durable fibers, CsgA is a dominant proteinaceous scaffold of biofilms. In fact, CsgA belongs to amyloids, proteins that form fibers during a spontaneous aggregation. CsgC, a sequence-specific inhibitor, is able to arrest aggregation of CsgA in periplasmic space (Evans et al., 2015). The presence of pre-formed amyloid fibers can accelerate aggregation of other amyloids. This process is known as cross-seeding. It is extremely sequence specific and can be restricted by a difference in a single amino acid (Zhou et al., 2012b). CsgA can be in vivo cross-seeded by its nucleator protein, CsgB, but also other CsgA fibrils. **Methods:** We recently published AmyloGram (Burdukiewicz et al., 2017). It is an efficient tool for prediction of amyloid proteins as CsgA and CsgB. Among others, our algorithm produces a simplified amino acid alphabet for amyloid proteins. It groups amino acids together into six groups based on their physicochemical features. We combined sequences of around 500 triplets CsgA-CsgB-CsgC from closely related gram-negative bacteria and used EVmutation software (Hopf et al., 2017) to find out which residues are especially susceptible to simultaneous mutations. **Results:** Both CsgA and CsgB are characterized by a regional structure of five repeated motifs. We found out that the general motif (S-X5-Q-X-G-X2-N-X-A-X3-Q) (the serin in absent in the case of CsgB) is faithfully preserved among different variants of CsgA and CsgB. The residual variability in motifs of both proteins does not affect the sequence of other protein. The opposite situation happens in CsgC, where mutations are not limited to specific motifs. On the other hand, CsgA and CsgC keep a similar level of the sequence divergence aside from species of Enterobacter and Salmonella genera, where CsgC accumulates more mutations. **Conclusions:** The interplay of CsgA, CsgB and CsgC suggests that if a mutation occurs in the region responsible for protein interaction, it should be compensated by mutations in other two proteins. For example, the presence of mutations that decrease the aggregation rate of CsgA, allows CsgC to become a less effective inhibitor without affecting the wellbeing of bacteria. Our preliminary study partially confirms this hypothesis by finding out that genera with less efficient CsgA, as Salmonella, can accumulate more mutations in the sequence of CsgC. We have not identified any simultaneous mutations between CsgA and CsgB. This may be due that single mutation in one region is not enough to change the protein function and to cause mutations in another protein. Probably, the compensation of single mutations by the regional structure of mentioned proteins is sufficient.
Virulence Production in *Pseudomonas aeruginosa* is Influenced by Different *In vitro* Culture Conditions and by Treatment with Quorum Sensing Inhibitor Sodium Salicylate

**Background:** In the developed world, approximately 1.5% of the total population will develop a chronic wound during their lifetime [1]. Infection contributes to the delayed healing, and although patients are often treated with antimicrobial agents they may not eradicate persistent biofilm infections [2]. Along with the increasing problem of antimicrobial resistance, there is an urgent need for alternative treatment strategies. Quorum sensing (QS) inhibition represents a potential treatment strategy for infected wounds. Interfering with the bacterial signalling system that regulates pathogenicity may disarm bacteria, by decreasing their toxicity, and increasing bacterial clearance by the host defence. The aims of the present study were: (i) to characterize the production of QS signals and virulence factors in clinical *P. aeruginosa* strains from wound infections, both under serum conditions and using standardized culture media; and (ii) to evaluate the effect of sodium salicylate on QS signal and virulence production.

**Methods:** The production of biofilm, alkaline protease, elastase, pyocyanin, iron siderophores and rhamnolipids were evaluated in 14 clinical wound isolates. The influence of culture conditions, namely solid agar versus liquid broth and the presence of serum, on virulence were also examined. The effect of sodium salicylate on QS inhibition and production of these virulence factors was investigated using a serum-containing wound-like media.

**Results:** The virulence production of the tested clinical isolates differed markedly, both between strains and culture conditions. The presence of serum in solid agar decreases pyocyanin and alkaline protease production, but increases iron siderophore production, after 24 h of culture. However, pyocyanin production seems to increase at later time-points under simulated wound conditions in comparison to general nutrient broth. Preliminary data showed that in the presence of serum, treatment with sub-inhibitory concentrations of sodium salicylate reduced biofilm formation on polystyrene as well as pyocyanin and iron siderophore production. **Conclusion:** These results show that the virulence of clinical isolates is affected by serum, and stress the importance of using relevant culture conditions when evaluating potential treatments for clinical applications. The *in vitro* results of sodium salicylate treatment on bacterial virulence are promising and calls for further work, preferably using *in vivo* infection models, where both the effect on virulence inhibition and the immune system can be investigated.

Session Title: **WEDNESDAY Poster Session 3**

**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 002

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Novel Strategy to Hamper Biofilm Formation on Med. Devices: PHAs-NCO-sP(EO-stat-PO)-Derived Meshes Loaded with Bactericidal and Anti-Adhesive Agents

**Authors:**

S. Piarali¹, J. Salber¹, I. Roy²;

¹Universitaetsklinikum Knappschaftskrankenhaus GmbH, Bochum, GERMANY, ²Faculty of Science and Technology, University of Westminster, London, UNITED KINGDOM.

**Background:** The failure of medical devices, such as implants and endoprostheses, is often linked with the ease to which bacteria can get in contact and adhere to its surface leading to biofilm formation. This, allied with the lack of efficacy of antibiotics, emphasizes the need to develop new locally acting anti-biofilm materials to prevent severe infections. To increase the susceptibility of biofilms to antibacterial treatments, we hypothesized that by combining an enzyme (E), able to hydrolyse exopolysaccharides present in the extracellular matrix of early stage biofilms, with other novel and potent bacteria-killing agents such as antimicrobial peptides (AMPs), one could enhance the anti-adhesion capacities of a material. Based on this, our goal was to develop surface modified polyhydroxyalkanoates (PHAs) combined with isocyanate-terminated star-shaped poly(ethylene oxide-stat-propylene oxide), (NCO-sP(EO-stat-PO),) to covalently link bioactive and anti-adhesive agents, and ultimately produce scaffolds via electrospinning for medical applications.

**Methods and Results:** On a first step, we screened the antibacterial activity of a novel synthetic AMP against *Staphylococcus epidermidis* which not only showed excellent bactericidal properties, but also the capacity to prevent more than 50% of biofilm formation when using a peptide concentration of 13.6 µg/ml. When assessing the enzyme effects against pre-formed biofilms we observed a decrease in the biofilm biomass by 71.3% with an enzyme concentration of only 3 µg/ml. Simultaneously, the cytotoxic effects of both AMP and E were evaluated against L929 murine fibroblasts over a period of 24h showing an IC₅₀ of 117.4 µg/ml and 142.5 µg/ml, respectively. Afterwards, three different meshes were produced via electrospinning, namely, untreated PHAs-NCO-sP(EO-stat-PO), E loaded PHAs-NCO-sP(EO-stat-PO)-E and double loaded PHAs-NCO-sP(EO-stat-PO)-E+AMP. A viability higher than 70% was observed when assessing the cytotoxicity of the meshes against fibroblasts. The anti-adhesion performance of the meshes was tested *in vitro* by CFU counting after sonication for the detachment of the biofilm grown on the neat and loaded PHAs. The modified meshes showed a significant reduction (p<0.05) in the numbers of adhered bacteria in comparison with the untreated mesh. Fluorescent microscopy and SEM imaging confirmed an enhanced action on the double loaded mesh, PHAs-NCO-sP(EO-stat-PO)-E+AMP, by showing an increased reduction in the number and size of bacterial aggregates when compared with the E loaded mesh.

**Conclusions:** These findings suggest that our strategy could be a step change in the current material technology; that the synergistic action of antimicrobial and antibiofilm compounds may be of benefit when targeting the development of anti-adhesive medical devices.
Antibiofilm Strategies

Broad-Spectrum, Long-Term Antibiofilm Features of Metallic Nanoparticles and Antibacterial Monomers on Dental Adhesive and Resin Composite Surfaces

M. Melo¹, L. Cheng², K. Zhang³, M. Weir¹, H. Xu¹;
¹University of Maryland, Baltimore, MD, ²Sichuan University, Sichuan, CHINA, ³Capital Medical University, Beijing, CHINA.

Dental caries, commonly stated as tooth decay, is one of the most prevalent chronic diseases of people worldwide. When dental caries occurs at the margin of an existing dental filling is called secondary caries (SC). SC may occur after initial caries has been removed and replaced by a filling. The replacement of the failed fillings accounts for approx. 60% of all fillings performed in the USA each year at an annual cost of over $5 billion. Tooth colored polymer-based direct placement materials, i.e, resin composites and dental adhesives have become the chosen material for restorative treatment of primary and secondary caries. Biofilm acids contribute to secondary caries, which is the main reason for dental restoration failures. The current resin based dental materials replace the affected tooth structure giving back the form and appearance of the tooth but do not promote biofilm modulation on the surrounding tooth. In fact, this peculiar group of dental materials has been associated with high susceptibility to RC. Therefore, preventing secondary caries involves control of microorganisms and/or the acid produced. An emerging alternative is the use of nanotechnology-based strategies for dental caries management. By releasing high levels of ions related to the small size and high surface area of the nanoparticles, nanoscale strategies such as nanoparticles of metals (i.e., silver-NAg) can impart antibacterial effect and thus help to address negative effects of composites. Another important approach to face this problem involves the synthesis of a new dental composite containing quaternary ammonium dimethylaminohexadecyl methacrylate (DMAHDM). Here we review recent immediate and after 6 months results using silver nanoparticles or DMAHDM against broad-spectrum saliva based inoculum microcosm biofilm model and discuss how their application can improve and facilitate anticaries activity via dental material. A human saliva microcosm model was used to grow biofilms on composites and dental adhesives. Colony-forming unit (CFU) counts, live/dead assay, metabolic activity, and lactic acid production of biofilms were determined. Biofilm response expressed by metabolic activity and lactic acid production of the biofilm were nearly 85% of that on the control and RC (p<0.05). The combined monomers in the new composite decreased the CFU counts of biofilms (12×10⁸) by 3 folds, compared to those on the control (p<0.05). Dental plaque biofilm colony-forming units (CFU) formed on the surface of dental adhesive containing different DMADDM/NAg have shown total microorganisms, total streptococci, and mutans streptococci expressive reductions. After 6 months, DMADDM/NAg reduced the biofilm CFU of the commercial control by two folds. The new formulations represents a promising approach of modulating the biofilm behavior of oral cariogenic biofilm, acid production, and secondary caries.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 004  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** TOL-463 Activity against Mixed-Species Biofilms in an _ex vivo_ Porcine Vaginitis Model  
**Author Block:** C. R. Head, J. Gil, G. D. Glinos, J. Zaias, S. C. Davis, I. Pastar; University of Miami Miller School of Medicine, Miami, FL.

**Background:** Polymicrobial biofilms containing *Gardnerella vaginalis* as the primary bacterial constituent and *Atopobium vaginae* as a secondary pathogen are characteristic of bacterial vaginosis (BV) and may contribute to the resistance associated with standard BV treatments. TOL-463 is a novel boric acid (BA) based vaginal anti-infective enhanced with EDTA in clinical development for the treatment of BV and vulvovaginal candidiasis (VVC), with robust antibacterial/antifungal activity against single species biofilms demonstrated. The objective of this study was to develop a mixed species porcine _ex vivo_ biofilm model closely mimicking human BV and evaluate the impact of TOL-463 against these established biofilms.  

**Methods:** A porcine model was chosen given the morphologic similarities between human and porcine vaginal mucosa. Vaginal tissue explants were sterilized and maintained in air liquid interphase prior to infection. Clinical isolates of _G. vaginalis_ (JCP8151B) and _A. vaginae_ (ATCC BAA-55) were used; the _G. vaginalis_ strain was isolated from a BV patient and previously shown to induce a BV phenotype in mice. Inoculums and infection order were optimized to establish mixed species biofilms at relative compositions consistent with clinical BV. Explants were then divided into 3 treatment groups: TOL-463, BA (50 mg/ml) and vehicle (VEH), and treated every 24 h for 3 doses. _G. vaginalis_ sialidase gene expression, a clinically relevant virulence factor, was also evaluated in the same model. Tissue samples were utilized for CFU assessment, RNA isolation, 16s rDNA quantification and measurement of sialidase expression.  

**Results:** Optimized mixed species biofilms were established with initial _G. vaginalis_ colonization of vaginal epithelium for 12 hours and subsequent infection with _A. vaginae_, supporting growth of both pathogens _ex vivo_ for up to 5 days. TOL-463 treatment resulted in a 6.62 log reduction in mixed biofilm load by the second dose (p<0.0001 vs. VEH) and was below the limits of CFU quantification by the third dose, compared with 4.62 logs CFU/mL of residual biofilm growth with BA (p<0.001 vs. TOL-463). TOL-463 also reduced expression of _G. vaginalis_ sialidase virulence factor below detectable levels whereas BA induced expression relative to TOL-463 and VEH.  

**Conclusions:** TOL-463 effectively inhibits mixed biofilms of _G. vaginalis_ and _A. vaginae_ in the porcine _ex vivo_ vaginitis model superior to BA, with complete eradication by the third dose. TOL-463 also displayed greater antivirulence capacity compared to BA, corresponding to its enhanced antibiofilm efficacy. These results conform with prior TOL-463 biofilm studies against BV and VVC pathogens and support the clinical efficacy of TOL-463 demonstrated in Phase II testing in women with vaginitis. Porcine vaginal model provided a biologically relevant substrate for studying polymicrobial biofilm infections _ex vivo_.
Antibiofilm Strategies

Glycoside Hydrolase DisH from *Desulfovibrio vulgaris* Degrades the N-Acetylgalactosamine Component of Diverse Biofilms

L. Zhu¹, V. Poosarla¹, S. Song¹, T. Wood¹, D. Miller², B. Yin³, T. K. Wood¹;
¹Pennsylvania State Univ., University Park, PA, ²Dow Chemical Company, Collegeville, PA, ³Dow Chemical Company, Collegeville, PA.

The global costs of corrosion are more than $2.5 trillion every year (3.4% of the global gross domestic product), and a large part of corrosion (30%) is microbiologically influenced corrosion (MIC), which affects oil production, drinking water systems, and pipelines. MIC is commonly caused by sulfate-reducing bacteria (SRB) biofilms, and *Desulfovibrio vulgaris* is the model organism. Biofilms of sulfate-reducing bacteria (SRB) produce H₂S, which contributes to corrosion. The biofilm matrix of *D. vulgaris* consists primarily of proteins but we have identified it also contains the polysaccharides mannose, fucose, and N-acetylgalactosamine (GalNAc) (*Environ. Microbiol Reports.* 9:779-787, 2017). However, little is known about how to control its biofilm formation. Although bacterial cells in biofilms are cemented together, they often dissolve their own biofilm to allow the cells to disperse. Using *Desulfovibrio vulgaris* as a model SRB, we sought polysaccharide-degrading enzymes that disperse its biofilm. Using a whole-genome approach, we identified eight enzymes as putative extracellular glycoside hydrolases including DisH (DVU2239, dispersal hexosaminidase), an enzyme that we demonstrated here (DisH was previously unstudied), by utilizing various p-nitrooligosaccharide substrates, to be an N-acetylβ-D-hexosaminidase. For N-acetyl-β-D-galactosamine (GalNAc), $V_{\text{max}}$ was 3.6 µmol of p-nitrophenyl/min/(mg protein) and $K_{\text{m}}$ was 0.8 mM; the specific activity for N-acetyl β-D-glucosamine was 7.8 µmol of p-nitrophenyl/min/(mg protein). Since GalNAc is one of the three exopolysaccharide matrix components of *D. vulgaris*, purified DisH was found to disperse 63 ± 2% biofilm as well as inhibit biofilm formation up to 47 ± 4%. The temperature and pH optima are 60°C and pH 6, respectively; DisH is also inhibited by copper and is secreted. In addition, since polymers of GalNAc and GlcNAc are found in the matrix of diverse bacteria, DisH dispersed biofilms of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. Therefore, DisH has the potential to inhibit and disperse a wide-range of biofilms. DisH is the first glycoside hydrolase that has both GalNAc-ase/GlcNAc-ase degradation activities for diverse biofilms. Hence, we have found a key factor controlling *D. vulgaris* biofilm formation (*Environ Microbiol.* on-line, 2018).
Anti-Biofilm Effects of Auranofin and Auranofin Combination Therapy Using *In vitro* and *In vivo* Model Systems

**Background:** Auranofin is an FDA approved compound originally developed for treatment of severe rheumatoid arthritis. The drug has potent anti-inflammatory properties, and is now known to possess antimicrobial activity as well. The compound’s antibacterial properties appear to be due in part to an inhibition of thioredoxin reductase. Recent studies have suggested that auranofin is effective against mature bacterial biofilms, in contrast to standard antibiotics, which are largely ineffective against preformed communities. The combination of anti-biofilm activity and anti-inflammatory activity indicates that auranofin is a promising candidate for combating infections within wounds, as both the presence of resistant bacteria and aberrant immune responses are primary concerns during treatment. **Methods:** The activity of auranofin was measured *in vitro* against *Staphylococcus aureus* biofilms using plate based assays. Biofilms were grown within polystyrene plates or on MBEC polystyrene pegs. Auranofin treatment was added at various time points during biofilm development. *In vivo* analysis of auranofin biofilm inhibition was performed using a *S. aureus* infected rat segmental defect model with an extended debridement time point to ensure persistent infection. Auranofin was added to infected defect sites via insertion of PUR scaffolds or direct application of powdered treatment. At 6 weeks post infection, animals were humanely euthanized and harvested samples were used to analyze bacterial burden and bone healing. **Results:** *In vitro* data suggests that auranofin is effective at both preventing the initial formation of *S. aureus* biofilms and disrupting mature biofilm communities. The presence of auranofin was associated with a significant reduction of bacteria in multiple models. Analysis of MBEC biofilms treated with auranofin at concentrations of 12-50ug/mL showed a complete or near complete absence of recoverable CFUs following treatment at t=0hours and t=24hours. Crystal Violet based assays also showed a reduction in bacterial biomass when added to nascent biofilms. However, *in vivo* experiments showed that auranofin did not significantly decrease bacterial burden, either alone or in combination therapy. This was consistent between all conditions tested, including various inoculum sizes and delivery methods. **Conclusions:** Auranofin is highly effective at inhibiting and dispersing *S. aureus* biofilms *in vitro* even at relatively low concentrations. However, auranofin does not appear to be an effective therapeutic against *in vivo* biofilms. Furthermore, significant side effects were seen in animals treated with auranofin or a combination of auranofin/vancomycin, suggesting that any potential effects that could be observed by increasing the dosage would likely be poorly tolerated. Therefore, auranofin does not appear to be a viable candidate for treatment of *in vivo* biofilms.
Safety and Efficacy of Using Glycoside Hydrolases to Degrade Biofilms in Chronic Wounds

W. K. Redman, D. F. Fleming, K. P. Rumbaugh; Texas Tech University Health Sciences Center, Lubbock, TX.

Chronic wound infections are an increasing problem within the United States. The treatment and care of these wounds costs millions of dollars annually and infections are associated with a high morbidity rate. Treatment frequently involves repeated debridement, multiple antibiotics, and in some cases limb amputation. Antibiotics often lack the ability to penetrate the biofilms and fight off the infection because the exopolysaccharide (EPS) secreted by bacteria within a biofilm can increase tolerance by up to one thousand percent. Thus, the ability to degrade this protective shield and allow antibiotics to penetrate the biofilm could potentially be a new therapy for chronic wounds. Glycoside hydrolases are enzymes that break down EPS and potentiate the efficacy of antibiotics and antimicrobials. Amylase and cellulase are two promising glycoside hydrolases that hydrolyze α-1,4 and β-1,4 glycosidic linkages respectively, which are common within biofilm EPS. While we have demonstrated in vitro and in vivo efficacy of these enzymes to break down the biofilms in chronic wounds, their safety must be determined in order for this potential therapy to reach clinical trials. In this study we performed long term in vivo experiments, utilizing a murine chronic wound model, to characterize and measure the host response after administration of these enzymes. We assessed antibody production to amylase and cellulase, the inflammatory reaction after administration, and the effect of these glycoside hydrolases on wound healing. Thus far, no adverse effects on wound healing have been observed. As the safety of the administration of these glycoside hydrolases on the host continue, there has been no variation in the host response exhibited between the vehicle control (1xPBS) and treatment. We also extended our preclinical studies to test the efficacy of glycoside hydrolases on clinical strains of bacteria. In order to accomplish this, debridement samples were taken from the chronic wounds of patients and were treated with glycoside hydrolases. These samples were then analyzed for the bacterial community present. Our results clearly demonstrate that as the complexity of the wound population increased, the efficacy of glycoside hydrolase treatment decreased. This indicates that for complex, polymicrobial biofilm infections it will likely be necessary to target more than two of the conserved bonds in EPS, or more than just the EPS component of the biofilm matrix.
Previous studies indicate that bacteria within biofilms are 10- to 1000-fold more resistant to antibiotics, making it challenging but imperative to develop new therapeutics that can disperse biofilms and eradicate persistent microbes. Gram-negative bacteria shed outer membrane vesicles (OMVs) containing outer membrane and periplasmic components including small molecules, proteins, and lipids present on the surface or within the vesicle lumen. OMVs have been shown to play important roles in bacterial interactions with their environment, including interspecies competition. For example, OMVs isolated from *Pseudomonas aeruginosa* were shown to exhibit antimicrobial activity against competitor bacteria due to the presence of peptidoglycan hydrolases. Here, we examined the antimicrobial activity of OMVs derived from *Burkholderia thailandensis* (Bt), a soil saprophyte that is closely related to *P. aeruginosa* but non-pathogenic in animals and humans. We first examined the peptidoglycan hydrolase activity using peptidoglycan degradation assays. Interestingly, Bt OMVs significantly degraded purified peptidoglycan from *Staphylococcus aureus* but not from *Streptococcus mutans*. Nonetheless, Bt OMVs displayed significant antimicrobial activity against *S. mutans*. When *S. aureus* and *S. mutans* were treated with heat-inactivated OMVs, we still found potent antimicrobial activity against both live bacteria whereas the hydrolytic ability of OMVs against *S. aureus* peptidoglycan was abolished. These findings indicate the existence of both heat-stable and heat-labile (i.e. hydrolases) components in Bt OMVs that contribute to the killing of *S. aureus* and *S. mutans*. Additionally, we found that Bt OMVs significantly reduced *S. mutans* planktonic and biofilm cell viability in a time- and dose-dependent manner. Confocal microscopy imaging combined with COMSTAT 2.0 software analyses demonstrated significant reductions in total biofilm biomass, biofilm integrity, and bacterial cell viability in *S. mutans* biofilms after OMV treatment compared to control. Scanning electron microscopy also revealed altered biofilm and cellular morphology in OMV-treated *S. mutans* biofilms. Moreover, we observed a synergistic effect when combining OMV and gentamicin compared to either alone as quantified by minimal biofilm inhibition concentration assay. Based on these data, Bt-derived OMVs represent an untapped resource of novel therapeutics effective against biofilm-forming bacteria. Studies are ongoing to identify and characterize Bt OMV antimicrobial components.
Investigation on the Ability of grZ14s-nvCyc-3PEG-Pal Peptide to Prevent E. coli Biofilm

Background: In recent years, antimicrobial peptides have gained great interest as options to counteract biofilms, thanks to their broad-spectrum of activity and low frequency of resistance. Peptides-coated medical devices can represent a new frontier to avoid biofilm formation, being stable and non-toxic. In this study, the anti-biofilm and anti-adhesive properties of a newly synthesized, 14-AA cyclic peptide, with sequence derived from a human GPCR protein, modified with polyethylene glycol and palmitic acid (grZ14s-nvCyc-3PEG-Pal) have been assessed in static and dynamic conditions against Escherichia coli isolates from catheter-associated urinary tract infections (CAUTIs).

Methods: Nine E. coli clinical isolates were analysed by crystal violet (CV) assay for biofilm formation. Minimum inhibitory concentration and CV assays on 96-well plates were performed for GrZ14s-nvCyc-3PEG-Pal solution (from 10 µM to 0.01 µM) to determine antimicrobial and anti-biofilm activities, respectively. Peptide adsorbed (10µM) onto 96-well plate and 1 cm-long segment of 100% silicone urinary catheter were used to investigate anti-adhesive properties by CV assay and CFUs counts, respectively. Bioflux system was employed to assess the ability of peptide (10 µM)-adsorbed silicone channel to interfere with the adhesion of Ec5-FSL isolate. Results: No antimicrobial and anti-biofilm killing activities of the peptide’s solutions were observed. On the contrary, among all the E.coli isolates, the adhesion of the more strongly adherent Ec5-FSL and Ec7-FSL strains was significantly reduced (P<0.01) on peptide-adsorbed wells. The more stable strong biofilm producer Ec5-FSL was selected to evaluate biofilm reduction when peptide grZ14s-nvCyc-3PEG-Pal was absorbed onto silicone catheter and a 75.6±7.8% CFU/cm² reduction was observed. In Bioflux experiments with microfluidic channels perfused under 0.5 dyn/cm² shear flow, early Ec5-FSL bacterial clusters can be observed in the untreated channel within 2h, while a single cluster arose on the peptide-adsorbed channel after 6h. After 8 h, mature biofilm appeared in the control, while just few macrocolonies occurred in peptide-adsorbed channel. More interesting, by using 1 dyn/cm² shear flow for 14 h, a rich biofilm was observed in the control while no bacteria were detectable inside of peptide-adsorbed channel. Conclusions: The grZ14s-nvCyc-3PEG-Pal peptide showed remarkable anti-fouling properties on strong biofilm-producers E. coli isolates. Interestingly, this peptide, in static conditions, was able to significantly reduce the adhesion of a strongly adherent E. coli strain on silicone urinary catheter and, more important, to effectively delay or definitely counteract bacterial adhesion under very low shear flows (0.5 and 1 dyn/cm², respectively), that are conditions resembling those occurring in catheterized patients.
Introduction: *Staphylococcus epidermidis* is one of the prevalent bacteria involved in orthopaedic device-related infections. A key factor contributing to the virulence of *S. epidermidis* is its ability to form biofilm. In this field, the interest in the development of new approaches for the prevention and treatment of bacterial adhesion and biofilm formation has increased. In a recent paper we reported the purification and the identification of pentadecanal able to impair *S. epidermidis* biofilm formation. One of the aims of this work was the design of some pentadecanal derivatives to enrich the arsenal of weapons to fight biofilm development.

**Materials and Methods:** Pentadecanal derivatives used in this work were: pentadecanoic acid; methyl ester and dimethyl acetal. Biofilm formation of *S. epidermidis* was assessed by crystal violet and CLSM. Biofilm cell viability was also determined by the LIVE/DEAD® Kit. Synergy test of these molecules in combination with vancomycin was also evaluated by using checkerboard assay. Their biocompatibility on eukaryotic cells was investigated by toxicity assay.

**Results:** The synthetized derivatives resulted to have anti-biofilm activity against *S. epidermidis* strains with different capability. This effect was further investigated by a CLSM analysis. The pentadecanal and its synthetic derivatives use in combination with antibiotics on mature biofilm were also explored. Results obtained showed that pentadecanoic acid modulated the antimicrobial activity of the vancomycin. In particular, the MBIC (minimal biofilm inhibition concentration) and MBEC (minimal biofilm eradication concentration) values were reduced by 2-fold in combination with pentadecanoic acid. The biocompatibility of pentadecanal and of its derivatives was investigated on fibroblasts and keratinocytes. All molecules resulted to be fully biocompatible under 50µg/mL.

**Discussion and Conclusions:** All the synthesized molecules resulted to be able to prevent, to a different extent, the biofilm formation of *S. epidermidis*. Their effect on the *S. epidermidis* biofilm structure was evident, as the biofilm formed in their presence is strongly reduced and characterized by a porous structure containing many channels and voids. These results suggested to explore the use of aldehyde and its derivatives in combination with antibiotics to treat biofilm infections. The pentadecanoic acid resulted to be able to modulate the antimicrobial activity of vancomycin it was able to weaken the *S. epidermidis* biofilm structure making it less compact and homogeneous, and thus allowing the penetration of the vancomycin into the structure of the biofilm. This work endorses the pentadecanal and its derivatives as key molecules for the development of innovative approaches for the prevention and, in case of the pentadecanoic acid, for the treatment of *S. epidermidis* biofilm-associated infections.
Managing Environmental Biofilm Formation on Drinking Water Infrastructure using Electrochemically Activated Solutions and Hypochlorous Acid

G. E. Clayton, R. M. Thorn, D. M. Reynolds; University of the West of England, Bristol, UNITED KINGDOM.

Background: Approximately 95% of bacteria in drinking water systems are present in biofilms. Biofilm formation in drinking water treatment systems (DWTS) can lead to biofouling of pipework and filters (i.e. ultrafiltration membranes [UF]), reducing operation time. Therefore, biofilm management on DWTS infrastructure would be beneficial to consumers and operators of drinking water systems. The aim of this study was to determine whether in-situ dosing of electrochemically activated solutions (ECAS) or HOCl managed the formation of environmental biofilms within a model system.

Methods: Biofilms were grown for 48 hours in a Centre for Disease Control (CDC) reactor with polyethersulphone (PES) coupons (to replicate UF membrane material). Feed water was taken directly from a modified artificial water source on the University of the West of England, Bristol campus (UK). ECAS were generated through the electrolysis of weak saline solutions (1% w/v), producing solutions with an oxidation reduction potential (ORP) of >1130 mV. Biofilm systems were dosed in-situ with either HOCl or ECAS to achieve a final free chlorine concentration of 50 mg L\(^{-1}\), or left untreated as a control. Biofilm samples were taken at 24 and 48 hours, whereby PES coupons were disaggregated through vortexing and sonication in 10 mL sterile quarter strength Ringer’s solution. Heterotrophic plate counts (HPC) of the feed water and disaggregated biofilms were plated onto R2A agar in duplicate and incubated at either 22°C or 30°C, for 5 or 3 days, respectively.

Results: The number of heterotrophic bacteria recovered from control PES coupons after 48 hours was 5.3 ± 0.06 (30°C plates) and 4.7 ± 0.28 (22°C plates) log\(_{10}\) CFU coupon\(^{-1}\). The biofilm density after 48 hours when dosed in-situ with ECAS was 2.3 ± 2.45 (30°C plates) and 2.3 ± 2.46 (22°C plates) log\(_{10}\) CFU coupon\(^{-1}\). No biofilm formation was observed when HOCl was dosed at 50 mg L\(^{-1}\) over the 48 hour period. A significantly higher number of bacteria were recovered from the control biofilm (no dosing) compared to ECAS or HOCl treated (P < 0.0001). No significant difference was observed in biofilm formation after 24 hours between ECAS and HOCl dosed systems. After 48 hours, a significantly higher number of bacteria were recovered from the ECAS compared to the HOCl dosed system (p = 0.0079 [22°C] and p = 0.0215 [30°C]).

Conclusions: This study has demonstrated that both ECAS and HOCl had an inhibitory effect upon environmental biofilm formation over a 48 hour period. Although no significant differences between ECAS and HOCl at 24 hours was observed, after 48 hours, biofilm formed in the presence of ECAS dosing was significantly greater than HOCl dosing. Further studies are required to upscale and apply this to water treatment infrastructure such as pipework and UF membranes.
Production of sessile biofilm is an important lifestyle shift for planktonic bacteria in response to environment, nutrition and population behavior. Development of biofilms is often the cause of persistent bacterial infections, with biofilm enmeshed bacteria exhibiting high level of tolerance to host targeted antimicrobial mechanisms and conventional antibiotic therapeutics. The ability to alter the biofilm state of bacteria to successfully treat biofilm infection requires novel compounds that can alter and/or reverse the biofilm state of bacteria as well as successfully inactivate pathogens in the biofilms itself. In our pursuit for identifying novel agents that can alter the biofilm state of bacteria, we optimized a 96-well plate based fluorescence assay to quantify established biofilms. Compared to other pre-established assays this plate reader based approach provides high signal to noise ratio for biofilm detection and quantification. Using a broad host range of opportunistic and biothreat pathogens (Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Burkholderia mallei, Burkholderia pseudomallei, etc.) we also demonstrate that this assay technique is broadly applicable. To further establish the utility of this novel approach for identifying and screening biofilm inhibitors, we assayed several thousand compound library against A. baumannii ATCC19606 and B. pseudomallei Bp82 (ΔpurM, a BSL-2 derivative of B. pseudomallei 1026b) biofilm models. Using this screen we identified one or more lead compounds that are not bactericidal or bacteriostatic at the tested concentrations, but has strong potential to inhibit biofilm production. Employing this easy to adapt assay technique should greatly benefit the biofilm community in screening and evaluating antibiofilm agents.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 015

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** In vitro Activity of Ceftazidime-avibactam for Prevention of Pseudomonas aeruginosa Biofilm Formation

**Author Block:** M. J. Karau, K. E. Greenwood-Quaintance, R. Patel; Mayo Clinic, Rochester, MN.

**Background:** Pseudomonas aeruginosa infections can be challenging to treat due to the propensity of this organism to form biofilms, as well as its often having multiple underlying resistance mechanisms, including β-lactamase production. Ceftazidime is a broad-spectrum β-lactam used to treat P. aeruginosa infections. Avibactam is a β-lactamase inhibitor that when combined with ceftazidime can restore planktonic activity of ceftazidime in the presence of ceftazidime resistance, reducing the minimum inhibitory concentration of P. aeruginosa compared to ceftazidime alone. We hypothesized that ceftazidime-avibactam would be more active in preventing P. aeruginosa biofilm formation than ceftazidime alone. To test our hypothesis, we determined the ceftazidime and ceftazidime-avibactam minimum biofilm inhibitory concentration (MBIC) values of 66 clinical P. aeruginosa isolates.

**Methods:** P. aeruginosa biofilms were grown for 3 hours on Nunc TSP 96 peg plates (Nuclon Delta Surface, Denmark). Pegs were rinsed in sterile PBS and placed into a 96 well plate containing two-fold dilutions of ceftazidime ranging from 512 to 0.5 µg/ml with or without 4 µg/ml of avibactam. Plates were incubated for 18 hours at 37°C and the MBIC determined by assessing the lowest concentration associated with absence of turbidity. MBIC results were reported as the range, the MBIC required to inhibit 50% of the isolates (MBIC$_{50}$), and the MBIC required to inhibit 90% of the isolates (MBIC$_{90}$).

**Results:** Both ceftazidime and ceftazidime-avibactam had MBIC ranges of 1 to >512 µg/ml. The MBIC$_{50}$ of ceftazidime and ceftazidime-avibactam was 256 and 32 µg/ml, respectively. The MBIC$_{90}$ of ceftazidime and ceftazidime-avibactam was >512 and 512 µg/ml, respectively. The MBIC was at least one doubling dilution lower for ceftazidime-avibactam versus ceftazidime alone for 63 of the 66 isolates, and at least two doubling dilutions lower for 42 isolates.

**Conclusion:** Ceftazidime-avibactam is more active in preventing P. aeruginosa biofilm formation in vitro than is ceftazidime alone.
**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** A New Approach to Controlling Biofilms in Catheter-associated Urinary Tract Infections

**Authors:**
- C. J. Jones¹, M. R. Mettetal¹, N. deHerrera¹, B. Xu¹, A. Magyar², V. K. Arthanareeswaran³, L. Soós³, K. Nagy³, A. Dobák³, I. M. Szilágyi³, N. Justh³, A. R. Chandra², B. Köves², P. Tenke², E. E. Mann¹

**Affiliations:**
1. Sharklet, Centennial, CO
2. Jahn Ferenc Dél-pseti Kórház, Department of Urology, Budapest, HUNGARY
3. Corden International, Department of Microbiology, Budapest, HUNGARY
4. University of Technology and Economics, BME, Budapest, HUNGARY

**Background:** Biofilms are a critical component of catheter-associated urinary tract infections (CAUTI), costing the health system over $340MM annually while contributing to poor outcomes. Several approaches have recently emerged to mitigate these effects, however the incidence of CAUTI is rising. Sharklet and Cook Medical have developed and tested micropatterned catheters in a first-in-man clinical trial as a novel approach to reducing these infections. Here, we describe the research that led to the development and deployment of the Radiance® Clear Sharklet® Silicone Foley Catheter.

**Methods:** Initial prototypes were tested for microbial contamination, transfer, and biofilm formation using immersion inoculation followed bacterial recovery and dilution plating from biopsies of the sample to determine bacterial load. Bacterial migration along micropatterned rod segments was determined by a Petri dish migration assay. A single center, open labelled, randomized interventional study compared the Radiance® Clear Sharklet® Silicone Foley Catheter to the standard of care Foley catheter in 50 patients. Outcomes tracked in this study include: Incidence of CAUTI, Surface fouling and biofilm formation, and perceived patient pain. This study is registered in the clinical trial register (NCT02835456).

**Results:** In vitro testing established that Sharklet micropatterned silicone rods significantly reduced initial adhesion of uropathogenic E. coli (UPEC) by 92% compared to smooth silicone rods. Additionally, Sharklet micropattern reduced the migration of both UPEC and P. aeruginosa along the rod by 99.9%. These results suggested that a Sharklet micropatterned catheter might be a viable approach to limit the bacterial colonization, biofilm, and migration that lead to CAUTI. A human clinical trial of 50 catheterized patients (25 Sharklet, 25 smooth) demonstrated that after an average of 8 days, Sharklet catheters accumulated significantly less biofilm than smooth catheters (Tip: P= 0.003, Middle part: P=0.013 and Base: P=0.013). Though no patients in the study developed CAUTI, patients with Sharklet catheters reported significantly less pain compared to the standard of care catheters (p=0.018).

**Conclusions:** Urinary catheters have become an important part of treatment for many patients, however there are several complications that can lead to pain and poor healthcare outcomes. Coupled with the emergence of antimicrobial resistant strains, biofilm formation is a major risk for CAUTI. Innovative approaches are required to combat this issue and limit the risk during catheterization. This study has demonstrated that the Radiance® Clear Sharklet® Silicone Foley Catheter is an effective, safe, and novel approach to mitigating bacterial biofilms that are associated with CAUTI. It highlights the development pathway for new technologies to be implemented in an attempt to mitigate infections.
Abstract Topic: Antibiofilm Strategies

Abstract Title: Heat as a Novel Treatment of Staphylococcus epidermidis Biofilms in an In vitro Flow Cell Model

Author Block: J. K. Beckwith¹, S. Van Aken², J. S. Van Epps³, M. J. Solomon¹;
¹Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, ²Department of Emergency Medicine, University of Michigan, Ann Arbor, MI.

Background: Contamination of implanted medical devices by bacterial biofilms causes significant morbidity and mortality. These biofilm infections are resilient and adaptive, allowing them to resist both host defense and antimicrobial treatment. Currently, the standard of care to treat medical device infections is to surgically remove the device and replace it, which is costly and further increases morbidity and mortality. We have previously demonstrated that modest levels of heat may be a useful treatment in a static culture setting. Understanding biofilm development as the consequence of adsorption, growth and detachment with each effect governed by self-assembly, fluid mechanics and transport phenomena, we translate the use of heat as a potential anti-biofilm therapy from a static culture to a more physiologically relevant flow model.

Methods: We developed an in vitro biofilm reactor system with precise control over flow rate and temperature to mimic the physiologic conditions surrounding a dialysis catheter. Staphylococcus epidermidis, the most commonly isolated species from medical device infections, was seeded and grown under low flow conditions (Reynolds number - Re = 1.6) at 37°C to establish a mature biofilm. Then the flow was increased to Re = 44 and the temperature of the infusate was held at 37°C or increased to 45°C, 50°C, or 60°C. Cell viability was determined by Live/Dead staining and confocal microscopy. Viability was also confirmed by complete dispersal of the biofilms within the flow cell followed by serial dilution, plating and colony enumeration. Biofilms morphology was characterized from confocal microscopy images using Fast Fourier Transform analysis.

Results: The percentage of live cells decreased from 89% ± 2% at 37°C, to 87% ± 2% at 45°C, 64% ± 6% at 50°C and 27% ± 3% at 60°C treatments. There was a tenfold reduction in the number of colony forming units from 37°C to 45°C and a greater than three log reduction for both 50°C and 60°C treatments. We also observed a dramatic increase in the structural heterogeneity with elevated temperature treatment on macro-, meso-, and microscopic scales.

Conclusions: Exposing biofilms to elevated temperatures changes both the morphology and cell viability of the biofilm. Understanding the response of these bacterial cells under thermal stress is a promising step toward the development of an in situ treatment/remediation method for biofilm growth in medical devices.
Modulation of Chickpea (Cicer arietinum L.) Seedlings with Plant Growth Promoting Bacteria and their Anti-biofilm in Pseudomonas aeruginosa PAO1

A. Saral¹, P. D. Rekha², S. S. Bhagyawant¹, K. K. Koul¹;
¹Jiwaji University, Gwalior, INDIA, ²Yenepoya Research Centre, Mangalore, INDIA.

**Background:** Pseudomonas aeruginosa is an omnipresent and opportunistic pathogen causing infections due to their ability to adhere and form biofilm and its distinctive antibiotic resistance mechanisms. The extensive use of antibiotics against biofilm associated infections has lead to the emergence of multi drug resistant strains. Modify the interest in the development of alternative strategies needs to be developed. Plant Growth Promoting Bacteria (PGPRs) have been reported to modulate the synthesis of bioactive phytochemicals such as secondary metabolites, polysaccharides, and proteins. The aim of this study was to evaluate antibiofilm, antibacterial and anti QS activity of chickpea seedling protein.

**Method:** Crude protein extract from 7 day’s old root and seedlings were analyzed for their antibiofilm, antibacterial and anti QS activity against *P. aeruginosa* at sub-MICs. **Results:** Root crude protein extracts and seedling extract showed antibiofilm activity, 33.78 and 43.79 % of inhibition respectively compared to control. A significant antibacterial activity was recorded in the 7 days PGPRs treated root and seedling proteins (71.2 %) in *Bacillus amyloliquifaciens* and *B. subtilis* (85 %) in comparison to non-treated, positive and negative control respectively. The same approach would be helpful to meet the challenges of biofilm associated infections. PGPRs mediated phytochemicals probably displayed inhibitory activities at sub-lethal concentrations that would be further useful for investigation and to establish their potential in clinical application.
Hospital acquired (nosocomial) infections are a serious health concern within the United States and the world at large. Every year there are over 2 million nosocomial infections and over 23,000 resulting deaths in the United States alone. Many of these infections are associated with microbial biofilms that exhibit higher resistance to antibiotics and therefore effective treatment becomes highly challenging. The objective of this study is to assess the efficacy of a gallium (Ga) composite as an antimicrobial agent against planktonic and biofilm forms of *Pseudomonas aeruginosa* PAO1 and *Acinetobacter baumannii* 19606. The Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) of *P.aeruginosa* and *A.baumannii* against planktonic and biofilm cells were determined using broth micro-dilution assay as described in the guidelines of the Clinical and Laboratory Standard Institute (CLSI) and ASTM E-2799 assay. The effect of the Ga composite on preventing biofilm formation was assessed using the traditional crystal violet assay. The MIC values of Ga composite varied with the most effective with a value under 4 µg/mL and comparable to traditional antibiotics. The Ga composite displayed a strong ability to prevent biofilm formation and disrupt established biofilms from both organisms. The cytotoxicity of the Ga composite were tested against multiple cell lines including Human Dermal Fibroblasts (HDF) and Murine macrophages (J774). Results showed that the Ga composite did not exhibit any toxicity against mammalian cell lines until very high concentrations, demonstrating a high therapeutic window. The uptake of Ga into bacteria from the Ga composite was conducted using ICP-MS. Overall, our preliminary studies suggest that our Gallium composite could be potentially used as an alternative to antibiotics for treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections. Further studies are planned to unravel new insights into the currently incomplete picture of gallium uptake and bactericidal mechanisms.
Impact of Deoxyribonucleases on the Structure of Oral Biofilms

N. Rostami1, R. C. Shields2, C. Lawler3, A. Nobbs3, N. Jakubovics1;
1School of Dental Sciences, Newcastle University, Newcastle upon Tyne, UNITED KINGDOM, 2Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, FL, 3School of Oral & Dental Sciences, University of Bristol Dental School, Bristol, UNITED KINGDOM.

Background Bacterial biofilms are able to tolerate high concentrations of antimicrobials and therefore new strategies for biofilm control must be implemented to overcome their global burden. Extracellular DNA (eDNA) is an integral component of many biofilm matrices including dental plaque. Many oral bacteria express deoxyribonuclease (DNase) enzymes to utilise eDNA whereas others require eDNA for attachment and colonization. Previously, we demonstrated that treatment of oral biofilms with a DNA-degrading enzyme, NucB from a marine strain of Bacillus licheniformis, strongly inhibited the accumulation of biofilms. Here, we identified and characterize SsnA, a DNase enzyme produced by the pioneer coloniser Streptococcus gordonii with a view to understanding the role of native extracellular DNases in dental plaque and the potential of exogenous and native DNase enzymes in oral biofilm control. Methods An S. gordonii ssnA null mutant was constructed and complemented by expression of ssnA from a plasmid. A quantitative fluorescence-based assay was employed to determine the activity of DNases under different pH conditions. Inhibition of biofilm formation by S. mutans, a caries pathogen, by SsnA and NucB was assessed by confocal laser scanning microscopy. A microfluidic system (BioFlux dual gas dual inlet) was employed to test SsnA and NucB efficacy in controlling of mixed species oral microcosms under most biologically relevant conditions. Results An S. gordonii ssnA null mutant lacked extracellular DNase activity. Enzyme activity was restored in a genetically complemented strain, confirming that SsnA is the major extracellular DNase in S. gordonii. The presence of glucose, sucrose and maltose, but not galactose, during growth inhibited SsnA expression. Inhibition was alleviated in a ccpA knockout mutant that lacks carbon catabolite repression. Recombinant SsnA was most active at pH 9.5 and was inactive below pH 6. Biofilm formation by S. mutans was inhibited by SsnA and DNase I at pH 6 or 7, but only by DNase I at pH 5. SsnA exhibited a strong antibiofilm activity against oral microcosms grown under flow in natural human saliva. However, presence of sucrose inhibited the antibiofilm activity of SsnA. NucB on the other hand maintained its antibiofilm activity in the presence of sucrose. Conclusions S. gordonii SsnA is an extracellular DNase that inhibits biofilm formation by S. mutans in the absence of sugars and at neutral to high pH. We propose that streptococcal DNases such as SsnA are important for maintaining microbial homeostasis by preventing colonization by pathobions such as S. mutans, and that dietary sugars or the production of acid by S. mutans overcomes this beneficial effect. Addition of exogenous DNases that are active at lower pH values may provide an opportunity for controlling the integration of cariogenic species such as S. mutans into the biofilm.
Structure-based Virtual Screening of Compounds with Potential of Inhibition of Adhesion Antigen I/II of *Streptococcus mutans* Dependent

**Background:** Antigen I/II of *Streptococcus mutans* has been implicated in bacterial adherence to constituents of the salivary pellicle, biofilm formation, collagen-dependent bacterial invasion of dentin and cariogenicity. **Objective:** To identify compounds with inhibitory potential of adhesion through the interaction with antigen I/II of *Streptococcus mutans* using *in silico* methods. **Methodology:** The crystallized structures of the A3VP1 (3IPK: PDB ID) and carboxy-terminus domains of the Ag I/II were selected to predict protein-ligand binding sites using metaPocket 2.0 and COACH meta-servers. Then, the compounds were searched from libraries containing small molecules, drug-like small molecules, and natural product molecules using molecular docking software running on the supercomputer at TACC (Texas Advanced Computing Center). The compounds were selected using two methods: the first one selecting manually the compounds with lower interaction energy and the second one using a script to select which interacted in the highest number of protein-ligand binding sites. Finally, the type of interactions presented between the compounds and the domains were analyzed by BIOVIA Discovery Studio software. **Results:** Structure-based virtual screening of approximately 883,551 compounds was performed resulting in 10 compounds for each 3IPK and 3QE5 domains; showing interaction energies between -9.6 and -12.8 kcal/mol respectively. Additionally, 10 compounds with high affinity were selected for multiple interaction sites of both domains, with an average energy of -8.3 kcal/mol. The most common interactions were hydrogen bonds in aminoacids such as GLY-ALA-LYS-ILE, followed by pi-cation interactions with LYS and pi-pi with the TYR. **Conclusion:** 30 compounds were obtained with inhibitory potential of adhesion of *S. mutans* Ag/I/II dependent, resulting in 10 compounds with high affinity for specific sites at 3IPK domain as well as 3QE5 domain and 10 compounds that interact in both domains.
Background: The increasing prevalence of antibiotic resistance is a primary concern in the health field and the development of novel therapies. The biofilm state of microorganisms is linked with increased antibiotic resistance due to many factors including reduced diffusion of chemotherapeutics through the extracellular polymeric substance barrier. In order to overcome this barrier and disrupt the microorganisms within biofilms, we have investigated the use of magnetic nanoparticles and fields against an in vitro multi-species oral caries-associated biofilm model. Methods: Multi-species biofilms, consisting of a 1:1:0.1 ratio of *Streptococcus gordoni* (DL1.1): *Streptococcus mutans* (UA159): *Candida albicans* (SC5314), were grown in 96 well microtiter plates for 24 h at 37°C and 5% CO₂ prior to dosing. Magnetic nanoparticles with various polymeric coatings were purchased from Chemicell and suspended in 1:1 RPMI:TSBYE 0.6% media at 100 µg/ml concentration. Static magnetic fields were generated using rare earth neodymium magnets of strengths from ±0.01 to 2.70 kG. Preformed biofilms were dosed with magnetic nanoparticles, fields, or combination treatments for 2 minutes to 24 hours. Cell viability was estimated with a PrestoBlue® viability assay and biomass was determined using a crystal violet assay. Results: Preliminary results demonstrate that potential of using magnetic nanoparticles and fields to disrupt orally relevant biofilms. Overall treatment with magnetic nanoparticles was most effective for 1.5-2.5 minute exposures. Nanoparticles with hydrodynamic diameters less than 110 nm were the most effective during this exposure time and comparable to treatment with an antiseptic mouthwash. The application of static magnetic field gradients indicates potential windows of enhanced efficacy against biofilms. Conclusions: The use of magnetic fields and nanoparticles presents an interesting solution to overcoming the barriers that frequently reduce the efficacy of many anti-biofilm treatments. Another important consideration when developing novel anti-biofilm therapies is the exposure time necessary to reduce biofilm viability. Our work with magnetic nanoparticles and fields demonstrates effectiveness within a relevant exposure time for oral cavity applications.
Abstract

Title: In-Vitro Antibacterial and Antibiofilm Effects of Aqueous Extract of *Moringa Oleifera* against *Salmonella* Isolates from Commercial Chickens in Southwest Nigeria

Author: O. I. Olatoye, O. O. Olubodun, O. O. Ishola, M. A. Hambolu, B. J. Olatoye, O. A. Okunlade, M. Adedeji; University of Ibadan, Ibadan, NIGERIA.

Background *Salmonella* is a major food borne pathogen commonly from poultry resulting in large number of outbreaks worldwide. Biofilm is ubiquitous in farms and food processing environment serving as crucial pathogenic and antimicrobial resistance mechanisms of bacteria. The global public health threat of multidrug resistant bacteria has led to the investments in the search for alternative natural (indigenous plants) antibacterial remedies. This study investigated the antimicrobial and biofilm inhibition effects of *Moringa oleifera* leaf extract in *Salmonella* isolates from chicken. Methods Isolation, characterization and enumeration of bacteria were carried out on 334 chicken samples obtained from poultry farms in Lagos, Nigeria. The MIC of aqueous extracts of *Moringa* leaves was obtained followed by in-vitro sensitivity of graded concentrations to the isolates by the agar well diffusion method with ATCC 13311 salmonella as reference strain. The minimum inhibitory concentration (MIC) of these plants for each of the test organisms was evaluated. Biofilm assays of the isolates were performed in triplicate from overnight LB broth culture on Microtiter plate and incubated at 37°C aerobically for 96 hours. Also, activity of the leaf extracts against biofilm formation by *Salmonella* isolates was determined using the crystal violet assay. Biofilm quantification was obtained by O.D. values at 630 nm in microtiter plate reader. Results The prevalence of *Salmonella* spp. in poultry was 16.2% (n=54). The leaf extract inhibited the growth of 85.2% of the isolates with zones of inhibition ranging from 13.6 ±0.7 mm 17.5±0.7 mm. The highest antibacterial spectrum was produced by 100% extract while the MIC was 12.5% concentration. Biofilm formation was exhibited by 38 salmonella strains (O.D. = 0.05-0.12), with 16 strong biofilm producers, 22 strains weak producers and 16 non producers. Biofilm formation by the isolates varied with *Salmonella* strains and concentrations of extract. *M. oleifera* leave extract at 100% concentration significantly inhibited biofilm formation of *Salmonella* spp. (p < 0.05). Conclusions The results of this study indicate that *Moringa oleifera* can protect against chicken Salmonella by inhibiting their growth and biofilm formation. The nutraceutical values of *Moringa oleifera* could be explore in food industry against bacteria food borne bacterial as natural edible additive to improve the quality and safety of poultry products.
Microorganisms can colonize almost all natural and artificial surfaces. If conditions are favorable, the attached cells build complex communities of surface, called biofilms. Initial adhesion is often reversible, so that the cells can break away from a surface if conditions change. The purpose of inhibiting microbial adhesion that prevents the initial steps of biofilm formation should be the most logical choice in infectious processes. The best results were found with the surface treatment or change surface properties with surfactants. Surfactants have potential to prevent bacterial adhesion. Biosurfactants have low toxicity, are biodegradable, exhibit chemical diversity and are more effective in environmental conditions. A major biological for producing biosurfactants sources is through bacterial strains. Furthermore, most studies biosurfactants, only a small part is dedicated to the anti-adhesive properties. Therefore, more studies on biosurfactants are required in order to design strategies to reduce bacterial adhesion that leads to the formation of unwanted biofilms. In the present work, rhamnolipids produced by bacterial strain Rn19a (partially identified as *Pseudomonas*) isolated from soil contaminated with hydrocarbons were evaluated. In the present work, rhamnolipids produced by the bacterial strain Rn19a isolated from soil contaminated with hydrocarbons were evaluated. The Rn19a strain has been partially identified as *Pseudomonas*. The rhamnolipids were used as a biosurfactant. Glass slides treated with 0.4% (w/v) rhamnolipids were used as a substrate. The slides were submerged vertically in sterile nutritive broth and individually inoculated with 6 strains isolated from urinary infections highly biofilm forming, and multiresistant called I3, I7, E26, I27, E30 and I31. Untreated slides were used as control. In order to evaluate the bacterial adhesion, a 36-hour kinetic was followed. Adhesion was evaluated every 6h and stained with 0.1% m / v violet crystal and 0.002% methylene blue (w/v) by optical density measurement. The results of crystal violet and methylene blue were consistent with each other. As a preliminary result, the finding that strains I3 and E26 increased their adhesion was found. Both strains have been identified by biochemical tests such as *Pseudomonas*. The above agrees with the fact that rhamnolipids contribute to the architecture of biofilms. This was expected since the rhamnolipids come from the same *Pseudomonas* family. On the other hand, strains I7, I27, I30 and I31, identified as *E. coli*, *Enterococcus*, *E. coli*, and *E. coli*, respectively, decreased adhesion to at least 1.0 optical density units. In conclusion, the above mentioned suggests that non-*Pseudomonas* strains are susceptible to rhamnolipids to interfere with or retard adhesion to surfaces.
Session Title: **WEDNESDAY Poster Session 3**

**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 025

**Abstract Topic:** Biofilm Antimicrobial Tolerance

**Abstract Title:** Survival of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilms on the Outer Shell of Firefighter Turnout Gear after Sanitation in a Commercial Washing Machine

**Author Block:** D. Farcas, F. Blachere, M. L. Kashon, D. Sbarra, D. Schwegler-Berry, J. D. Noti; Centers for Disease Control and Prevention (CDC), Morgantown, WV.

**Abstract Body:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified on the outer shell of firefighters’ turnout gear jackets, suggesting that contaminated gear may be an indirect transmission source. Using the washing procedures described in the NFPA 1851: Standard on Selection, Care, and Maintenance of Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting, the present study was conducted to develop a quantitative method to assess the antimicrobial efficacy of antimicrobials (Fire Soaps™ Fabric Sanitizer) in the decontamination of *S. aureus* from the outer shell (Gemini™ XT, Pioneer™, and Advance™) of turnout gear. After washing outer shell fabric with sanitizing solution according to specifications described in the ASTM E2274 Protocol for evaluating laundry sanitizers and disinfectants, the efficiency of Fabric Sanitizer was determined using standard microbial techniques and the quantitative real-time polymerase chain reaction (qPCR) to assess for viable and non-viable *S. aureus* that was washed from the fabric. To determine the amount of *S. aureus* that remained attached to the outer shell fabric, the bacteria was recovered by incubation of the fabric for five hours in Dey/Engle Broth (DEB) at 37°C. Using the ASTM E2274 Protocol, disinfection for 10 seconds reduced the viability of *S. aureus* on Gemini™ XT and Advance™ by 73% (0.6 log reduction) and 99% (2.2 log reduction), respectively. The reduction of viability was essentially unchanged after increasing the disinfection time to 10 minutes (68% reduction for Gemini™ XT [0.8 log reduction] and 97% for Advance™ [1.8 log reduction]). Scanning electron microscopy (SEM) was also used to observe the attachment of *S. aureus* to fabrics and characterize the stages of biofilm formation on the outer shell and that result in increased resistance to disinfection. Additional disease transmission risk from cross-contamination of turnout gear by other bacterial species was also revealed through the discovery of a sanitizer-resistant strain of *Pseudomonas aeruginosa* recovered from decommissioned turnout gear jackets. Moreover, the sanitizer-resistant strain of *P. aeruginosa* that was recovered from the decommissioned turnout gear jacket was shown to initiate biofilm formation on Gemini™ XT within 5 hours of incubation in DEB, demonstrating the potential for rapid biofilm formation in the field when worn by firefighters. A simple and inexpensive method that can be used by commercial firefighter laundering facilities to determine the effectiveness of outer shell sanitization in their washing machines is also proposed.
Sewers are not only conduits of wastewater; they are also complex bioreactors. Because wastewater treatment is designed to inactivate pathogenic microbes, fecal matter from patients with infectious diseases is allowed to enter the sewer systems. Here, these cells can get attached to pipes biofilm and exchange, acquire, and spread genetic material including antibiotic resistance genes (ARG). During wet weather, sewer overflows result in the release of these ARG that presents a public health risk. In this study, we used an annular biofilm reactor that simulates sewer conditions to assess the antibiotic resistance genomic composition of biofilms throughout time for two pipe materials: PVC and concrete. Quantification of ARG in biofilm, water, and sediment samples was performed using qPCR. Results demonstrated that two ARG (\textit{sul1} and \textit{blaTEM}) were detected in the biofilm samples on both pipes materials, while tet(G) was only detected on concrete. After 25 days of biofilm development, a disinfection experiment was performed by pumping a solution of 6.25% bleach into the reactor. ARG were quantified after the treatment using qPCR for total DNA and viability PCR that reduces the qPCR signal from cells with compromised membranes. Disinfection resulted in a reduction of ARG in the biofilm and can provide insight into the mechanism of disinfection in sewers (i.e., oxidation of cell membranes in biofilm vs. detachment). Overall these results indicate that sewer biofilms can serve as reservoirs of ARG that can persist after standardized disinfection protocols.
Transcriptomic, metabolomic, physiological, and computational modeling approaches were integrated to gain insight into the mechanisms of antibiotic tolerance in an in vitro biofilm system. *Pseudomonas aeruginosa* biofilms were grown in drip-flow reactors on a medium composed to mimic the exudate from a chronic wound. After three days, the biofilm was 114 μm thick and contained 9.45 log_{10} cfu cm^{-2}. These biofilms exhibited tolerance to subsequent treatment with ciprofloxacin. The biofilm specific growth rate was estimated via elemental balances to be approximately 0.35 h^{-1} or one-third of the planktonic maximum specific growth rate. Global analysis of gene expression indicated decreased anabolic activity in biofilms compared to planktonic cells. A focused transcriptomic analysis revealed the induction of multiple stress responses in biofilm cells including those associated with growth arrest, zinc limitation, hypoxia, and acyl-homoserine lactone quorum sensing. Metabolic pathways for phenazine biosynthesis and denitrification were activated in biofilms. A customized reaction-diffusion model was solved to characterize the distribution of oxygen inside the biofilm. It predicted that steep oxygen concentration gradients form when these biofilms are thicker than about 40 μm. Mutants deficient in Psl polysaccharide synthesis, stringent response, stationary phase response, and membrane stress response exhibited increased ciprofloxacin susceptibility in biofilms while many other mutants had no susceptibility phenotype. These results along with extensive literature supported a generalized conceptual model of biofilm antimicrobial tolerance with the following mechanistic steps: 1) establishment of concentration gradients in metabolic substrates and products through reaction-diffusion interactions, 2) active biological responses to these changes in the local chemical microenvironment through shifts in gene expression or alterations of enzyme activity, 3) entry of biofilm cells into a spectrum of states involving alternative metabolisms, stress responses, slow growth, cessation of growth, or dormancy, and 4) reduced susceptibility of microbial cells to antimicrobial challenges in some of these physiological states.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 028  
**Abstract Topic:** Biofilm Antimicrobial Tolerance  
**Abstract Title:** Correlation between Antifungal Susceptibility & Biofilm Formation in *Candida albicans* Clin. Isolates  
**Author:** S. Hassan¹, A. Ikram², K. Afzal¹, M. Khan¹, A. Hussain¹;  
¹Khyber Medical University, Peshawar, PAKISTAN, ²National Institute Health, Islamabad, PAKISTAN.

**Introduction:** An important contributing factor in *C. albicans* pathologies is their flexibility in adapting to different environmental conditions by virtue of growing preferentially as biofilms. In particular ECM biofilm formation by *C. albicans* carries important clinical repercussions in terms of treatment failure and burden on health-care budget. The purpose of our study is to isolate *C. albicans* from clinical specimens, and to find a correlation between their antifungal susceptibility and biofilm formation.  
**Methods:** A total 300 *Candida albicans* isolated from patients with various clinical problems were included in the study. Confirmation of *Candida albicans* was done through CHROM Agar and API 20 C AUX. Antifungal susceptibility testing of Fluconazole and Voriconazole was undertaken by disc diffusion method recommended by CLSI M44A document. MICs of sessile cells was performed by the CLSI M27-A2 (yeasts) broth microdilution method. Biofilm formation was done in a 96-well flat bottom Antifungal susceptibility testing of Fluconazole and Voriconazole was undertaken by disc diffusion method recommended by CLSI M44A document microtiter plate with standardized cell suspensions in RPMI -1640 (Roswell Park Memorial Institute) that was incubated at 37°C and was observed at different time intervals ranging from 02-48 hours.  
**Results:** The mean age of the patients was 40 years. Male to female ratio was 1:1.2. Total Candida *albicans* isolated were 300. In our recent study fluconazole was susceptible to 116 (39%) and SDD susceptible dose dependent to 36 (12%) and resistant to 148 (49%) by disc diffusion method (Kirby-Bauer). The MIC sessile antifungal susceptibility of fluconazole was 8(29%) sensitive, 25(8%) SDD susceptible dose dependent and 188(63%) were resistant. Susceptibility of *Candida albicans* with respect to voriconazole was analyzed as sensitive in 230 (77%) of patients, it was intermediate in 15(5%) of patients and resistant in 55(18%) patients by disk diffusion. Sessile antifungal susceptibility to voriconazole was 89 (30%) sensitive, 42 (14%) were susceptible dose dependent (SSD) and 169 (56%) were resistant of the total isolates. Biofilm formation was observed in 285(95%) of the isolates and only 15 (5%) were observed as non-biofilm former.  
**Conclusion:** Biofilm-based Candida infections are an emerging problem. High antifungal resistance was observed in biofilm-producing strains in this study. Using the variables affecting biofilm formation, tailored intervention strategies can be implemented to reduce biofilm-based Candidiasis.  
**Key words:** Antifungal drugs, biofilm, Candida, *Candida albicans*,
Characterization of PA2915: A Putative Beta-Lactamase from Pseudomonas aeruginosa

Author: M. C. Goodyear, J. Van Loon, A. M. Berezuk, C. M. Khursigara; University of Guelph, Guelph, ON, CANADA.

**Background:** Pseudomonas aeruginosa is a Gram-negative bacterium that can cause chronic and multidrug resistant infections in immunocompromised individuals. P. aeruginosa possesses a number of intrinsic mechanisms of resistance that allow it to survive antibiotic treatments. These mechanisms include the ability to grow as biofilms and protein-based mechanisms such as antibiotic-degrading β-lactamase enzymes. A previous study in our laboratory compared the proteomes of biofilm and planktonic cultures of the P. aeruginosa laboratory strain PAO1 when grown for 24, 48, or 96h. The proteomics study identified a number of putative β-lactamases that showed biofilm-specific expression. We hypothesize that these uncharacterized β-lactamases contribute to the enhanced resistance of P. aeruginosa biofilms. Our studies aim to confirm the β-lactamase activity of these enzymes both in vitro and in vivo. **Methods:** Current work is focused on PA2915, which showed increased abundance in biofilms at all three time points. Amino acid sequence analysis was used to show that PA2915 is a putative β-lactamase. A 6xHistidine tagged construct of PA2915 was made for over-expression in PAO1. Immobilized metal affinity chromatography and anion exchange chromatography were used to purify PA2915. Purified protein was then tested for β-lactamase activity in vitro using a nitrocefin assay. Nitrocefin is a chromogenic cephalosporin that mimics the structure of β-lactam antibiotics and can be cleaved by β-lactamases. When cleaved, the change in absorbance between substrate and product can be measured and used to confirm β-lactamase activity. To demonstrate the in vivo function of PA2915, a deletion mutant has recently been engineered in PAO1 using two-step allelic exchange. **Results:** The amino acid sequence of PA2915 contains a Hx[DEH]xDH motif typical of metallo-β-lactamase enzymes that require a divalent metal cation cofactor. PA2915 was purified to homogeneity at high quantities (~25mg/mL). In preliminary nitrocefin assays, PA2915 showed zinc-dependent β-lactamase activity. **Conclusions:** Sequence analysis and preliminary nitrocefin assays suggest that PA2915 is a metallo-β-lactamase. Metallo-β-lactamases are an increasing clinical concern as there are currently no inhibitors available for these enzymes. Susceptibility assays are currently underway to determine how the loss of PA2915 affects the resistance of PAO1 when challenged with β-lactam antibiotics in either the planktonic or biofilm mode of growth. The in vitro and in vivo workflows developed here will be used to characterize additional putative β-lactamases that showed biofilm-specific expression in PAO1. Together, these putative β-lactamases may represent a significant and understudied source of resistance in P. aeruginosa biofilms.
Background: Bacterial biofilms have exhibited increased tolerance to antimicrobial agents. Studies using traditional biofilm cultivation devices require larger media volumes and lack spatial control, leading to risk of uneven exposure of disinfectants. This study proposes the use of a multi-channel polydimethylsiloxane (PDMS)-based microfluidic chip to create and maintain microenvironment for biofilm formation and stability. Therefore, the objectives of this study are (i) to evaluate the effect of chlorine on biofilm formation and dispersal in nutrient gradient-mixing channels and (ii) to examine their persistence to changes in chlorine concentration; chlorine levels are commonly increased to remove and suppress potential biofilm growth. **Methods:** The microfluidic chip consists of a PDMS layer mounted atop a cover slip, comprising of 5 parallel channels with diluted nutrient concentrations following from a gradient mixing network. Log phase *P. aeruginosa* cells were incubated for 2 hours prior to 1-day continuous flow of sterile LB at 10μL/min. Channels were exposed to 5mg/L NaOCl for 1 hour and subsequently 2mg/L. The biofilm structures were imaged using Zeiss LSM170 fluorescence microscopy during different conditions; live and dead biomass were calculated by iMaris (Bitplane Inc. Version 9.1.2); average biofilm thickness was measured by ImageJ. **Results:** The initial thinnest biofilm averaged 10μm uniformly, while the thickest biofilm averaged 37.5μm non-uniformly in mushroom-shaped clumps of *P. aeruginosa* biofilms, demonstrating gradient nutrient level induced biofilm thickness. Lower nutrient level prevented the growth of highly stable mushroom-shaped biofilms, possibly accounting for the dislodgement and subsequent 30μm thick aggregation only in the lowest nutrient channel, indicating its higher susceptibility to NaOCl. The thickest biofilm grew up to 77μm thick even when exposed to 5mg/L NaOCl but decreased to below 10μm during subsequent 2mg/L flow. The first exposure might be insufficient a shock to weaken the biofilm strength as the EPS may have neutralized the NaOCl and prevent physical stress, but continuous exposure may eventually compromise the EPS, allowing intrusion of NaOCl deeper into the biofilm. Subsequent LB flow overnight saw a rapid regrowth of biofilms up to 77μm. **Conclusion:** The microfluidic device as a promising platform for bacterial biofilm study was designed to elucidate high resolution observations of combined effects of nutrient levels and disinfectants on biofilm formation. The results have shown that increased nutrients enhanced biofilm’s stability and resistance to dispersal by NaOCl. A high NaOCl dose was ineffective in biofilm dispersal but longer exposure at a lower dose eventually removed more than 50% of the initial biofilm. A lower dose could be applied to weaken and disperse the EPS matrix before using a higher dose to kill the exposed cells.
Abstract Title: Challenging Staphylococcus aureus Biofilms with different Dosing Patterns of Gentamicin in Combination with Rifampicin

Abstract Body:

Background: Orthopaedic device-related biofilm infections (ODRI) caused by Staphylococcus aureus have a high treatment failure rate, despite prolonged systemic (intravenous and oral) and local application of antibiotics (e.g. antibiotic loaded bone cement, ALBC). In this study, we investigated the ability of two commonly applied antibiotics in the treatment of ODRI (gentamicin and rifampicin) to eradicate mature S. aureus biofilm after exposure to three distinct dosing patterns modelled after these different dosing options.

Methods: Biofilms of a clinical S. aureus isolate were formed using the MBEC system for 5 days in TSB supplemented with 1% human plasma. Thereafter, biofilms were exposed to gentamicin alone or in combination with 3 µg/ml rifampicin as follows: 1) constant concentrations of gentamicin for 28 days (2000, 250, 15 µg/ml); 2) pulsed dosing of gentamicin (same concentrations), for two periods of two hours per day over 28 days; 3) burst release: initially high, but rapidly diminishing concentrations over time as may be expected from ALBC (2000 µg/ml on day 1, to 2 µg/ml at day 14). At each timepoint a total viable count (TVC) was performed from a total of 18 pegs per condition. Antibiotic resistance of viable bacteria was assessed by zone of inhibition.

Results: The TVC of unexposed biofilms was ~6x10⁸ CFU throughout the study. In the first test group, constant exposure of biofilms to gentamicin at 2000 µg/ml achieved a mean 7-log reduction in TVC at day 28. Twelve of 18 pegs were culture negative. Adjunctive rifampicin could not decrease TVC further, instead inducing rifampicin resistance by day 14. In the second group, pulsed exposure of 2000 µg/ml gentamicin achieved a mean TVC reduction of only 1 log by day 28 (0 culture-free pegs), but adjunctive rifampicin increased biofilm eradication to 13 out of 18 pegs (p<0.001 compared to control). Mean reduction was only 2 logs due to high numbers of rifampicin resistant bacteria in the culture positive pegs. In the third group, burst release of gentamicin reduced TVC by 1 log at day 7, however, the TVC recovered to control levels at day 14. Interestingly, adjunctive rifampicin reduced TVC 4 logs by day 14 (p<0.001 compared to control), with 6 out of 18 pegs culture negative. Again, culture positive pegs were colonised with rifampicin resistant bacteria.

Conclusion & Discussion: Constant high concentrations of gentamicin were the most effective treatment of S. aureus biofilms; however, these concentrations are not achievable by systemic therapy. Adding rifampicin in this profile was ineffective and induced resistance. Interestingly, pulsed and burst release profiles were effective in achieving reductions in TVC or culture positive pegs when combined with rifampicin, even though total antibiotic exposure was less than the constant exposure profile. This data confirms the opportunities for optimising antibiotic pharmacodynamics against biofilms.
Session Title: **WEDNESDAY Poster Session 3**

**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 032

**Abstract Topic:** Biofilm Antimicrobial Tolerance

**Abstract Title:** Studies on the Effect of Antibiotics on Structural and Molecular Dynamics of *Pseudomonas aeruginosa* Biofilm Formation: Implication in Antibacterial Therapy

**Author Block:** A. Kumar, T. Sengupta;

**Block:** Indian Institute of Science Education and Research - Kolkata, Mohanpur, Nadia, INDIA.

**Background:** Bacteria are continuously evolving new ways as survival strategies to avoid the effect of antibiotics and infections caused by bacteria are becoming more intense. Ability to form biofilm is one of such ways which potentiates the bacteria to establish infection and also to tolerate antibiotic treatment. Thus, bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics and resist phagocytosis, as well as components of the innate- and the adaptive immune systems of a host. The bacteria in biofilms live in a self-produced matrix of extracellular polymeric substances (EPS) that form their immediate environment. EPS are mainly constituted of polysaccharides, proteins, nucleic acids and lipids; they provide the mechanical stability in biofilms and minimize effect of antibiotics. **Methods:** Biofilm formation by *Pseudomonas aeruginosa* isolates KPW.1-S1 and HRW.1-S3 was assayed in 24 well plates by Crystal Violet staining method. Scanning Electron Microscopy (SEM), Confocal laser scanning microscopy (CLSM) were performed for visualization and characterization of KPW.P1 biofilms. Extra-cellular Polymeric Substances (EPS) and generation of reactive oxygen species (ROS) in KPW.1-S1 and HRW.1-S3 biofilms were characterized biochemically. **Results:** *Pseudomonas aeruginosa* isolates KPW.1-S1 and HRW.1-S3, when grown in presence of sub-minimum inhibitory concentrations (sub-MICs) of tetracycline and gentamicin, intense biofilms were formed and the extent of biofilm formation, with respect to compactness and multi-layer structure and EPS formation was observed to be tetracycline and gentamicin concentration dependent. Increased levels of e-DNA and exo-proteins were found in biofilm structures grown in presence of tetracycline and gentamicin as compared to control biofilm structure. Interestingly, Sub-MIC concentration of gentamicin induced the growth of planktonic cells as well as biofilm formation by KPW.1-S1 and HRW.1-S3. **Conclusions:** Our findings show more compact biofilm formation by the isolated *Pseudomonas aeruginosa* strains in presence of antibiotics relative to control and increased biofilm formation was mediated through generation of ROS and increased production of eDNA and exo-proteins.
**Background:** *Enterococcus faecalis* is a commensal of the human gastrointestinal tract; it is also an opportunistic pathogen and one of the leading causes of hospital acquired infections. *E. faecalis* produces biofilms that are highly resistant to antibiotics and have been previously shown to exhibit altered 3D architecture in the presence of subinhibitory antibiotics, daptomycin in particular. A similar biofilm architecture was observed in an *epaOX* deletion mutant, which encodes a glycosyltransferase of the enterococcal polysaccharide antigen (*epa*) gene cluster and was shown to be important in biofilm-associated antibiotic resistance. These data suggested a model in which biofilm growth is associated with cell envelope stress and remodeling of biofilm architecture results from exacerbation of this stress by antibiotics or mutations affecting the cell wall.

**Methods:** The model described above was examined using chemical treatments predicted to alter the cell envelope as well as examining other genes of the *epa* operon. Biofilm production of OG1RF and mutant derivatives was examined in the presence of subinhibitory daptomycin, gentamicin, and sodium cholate (a component of bile) and biofilm architecture was observed after growth on Aclar membranes using fluorescence microscopy.

**Results:** Exposure to both sodium cholate and daptomycin, substances that alter the cell envelope, resulted in altered biofilm architecture in OG1RF. A mutant in *epaQ*, which encodes a hypothetical membrane protein, showed reduced biofilm production in the presence of daptomycin relative to OG1RF. Moreover, Δ*epaQ* exhibited a more extreme altered biofilm architecture than previously observed with Δ*epaOX*. Biofilm production of Δ*epaOX* was reduced in the presence of both sodium cholate and daptomycin. Additionally, altered biofilm architecture was observed upon depletion of *epaOX* expression in a pre-established biofilm. To determine how this stress is sensed, we examined a pathway previously proposed to be involved in cell wall stress response, which includes IreK and the two-component regulatory system CroRS. Preliminary data shows that disruption of this pathway alters the response of OG1RF to sodium cholate and daptomycin, suggesting this pathway is important for modulating changes in biofilm architecture in response to cell wall stress.

**Conclusions:** Our cumulative results indicate that biofilm growth of *E. faecalis* may entail cell envelope stress not encountered in planktonic growth, and the *epa* polysaccharide modulates this stress to allow robust biofilm growth and architecture. *E. faecalis* produces an altered biofilm architecture in response to heightened cell wall stress caused by either chemical exposure or genetic disruption of the normal cell wall structure.
**Background:** To study filamentous fungal biofilms that are representative of those in the built environment, we established a method for engineering biofilms in a controlled reactor under low-shearing force on a glass coverslip. The purpose of this project is to assess cell viability and quantify the biofilm features of *Aspergillus niger* biofilms and those exposed to antimicrobial agents. *A. niger* is being studied because it is ubiquitous and a model organism. **Methods:** Cell viability quantification in *A. niger* biofilms has not been reported, thus we are comparing two different methods to determine which is optimal. One method utilizes the LIVE/DEAD Yeast Viability Kit containing FUN1 cell stain that exhibits orange-red fluorescent intravacuolar structures in metabolically active cells, while dead cells fluorescence green-yellow. The second method involves using the LIVE/DEAD BacLight Bacterial Viability kit containing SYTO9, a green fluorescent stain with a capacity to penetrate the active cell walls, and Propidium Iodide (PI), a red fluorescent stain that penetrates the damaged cell membrane. To determine the efficacy of antimicrobial agents on fungal biofilms, we used sodium hypochlorite solutions and stained with SYTO9 and PI. Confocal Microscopy and the computer program COMSTAT 2.1 are being used to visualize fluorescently labeled cells and quantitating biofilm structures. **Results:** Biofilms were stained using both methods and were compared for reliability. The center portion of the biofilm contained more live cells when compared to the edge portion. Also, the edge portion contained conidiogenous cells and conidiophores. The viability of the edge portion was not homogenous, a majority of the biofilm cell was viable, but a minority of red fluorescent nonviable cells was also noted. This indicated the stage of maturation. In antimicrobial test analysis, we found that it was impossible to distinguish live cells stained with SYTO9 from dead cells due non-fluorescence of dead cells stained with PI. This is because sodium hypochlorite damaged the DNA. If the nucleic acid gets damaged by sodium hypochlorite, it will not be able to get linked with the PI fluorochrome. **Conclusion:** The combination of nucleic acid stains SYTO9 & PI is more reliable for imaging and live-dead cell differentiation. In the case of sodium hypochlorite efficacy test, SYTO9 stained with a capacity to penetrate cell walls with relative ease and stained the cell green regardless of their viability. In comparison, PI cannot stain the cell with nucleic acid damage.
Background. Biofilm forming, antibiotic-resistant strains of bacteria represent an increasingly serious threat to global public health. Bacterial biofilm sensitivity to antibacterial agents may decrease 10-1000 times compared to planktonic forms, leading to multi-drug resistant bacterial spread and ineffective treatment. Therefore new treatment methods are needed against chronic infections caused by bacterial biofilms. With the development of nanotechnology, it is possible to obtain nano-sized particles of different substances, including smaller versions of antibiotics, that differ from its full-length particles by extremely enhanced chemical activity and increased membrane fluidity. The aim of this study was to establish experimental conditions for Staphylococcus aureus biofilm formation and for ultrastructural analysis of such cultured biofilms for future use in studies investigating bactericidal effects of nanoparticles. Methods. Experimental biofilm was obtained from isolated pure Staphylococcus aureus cultures from children’s stool samples and delivered to the Bacteriological Laboratory of the Republican Center of Hygiene and Epidemiology for further processing. The suspension of microorganisms (2x10^8 colony forming units as determined by McFarland turbidity standards) was added onto an aluminum specimen mount soaked in meat-peptone broth for biofilm generation. Results. At day 1, the aluminum-mounted biofilm was fixed with Gentian violet and electron micrographs obtained by scanning electron microscopy (JEOL-40 microscope). Biofilms from the same culture of St. aureus were studied at additional timepoints as well (i.e., days 1, 2, 3, 5 and 10). At early time points (days 1 and 2), the bacterial cells were scattered. Formation of biofilm-specific intercellular primary strands was observed at days 1 and 2 at 20,000x magnification. Subsequently, at days 3 and 5, dense surface colonization, reminiscent of a multilayer frame made of spherical bacterial cells, was revealed. This previously described multilayer frame formation has been shown to hinder recognition of antibiotic targets and could be used in future studies to monitor the effects of nanoparticles on biofilm integrity. Conclusions. The work described here demonstrated a methodology for ultrastructural analysis of aluminum-mounted biofilm samples and follow-up of biofilm formation stages for these infectious agents. This is critical to evaluate nanoparticles as shortened versions of antibiotics in in vitro therapeutic studies or other antibacterial agents on experimentally obtained and native bacterial biofilm. Our in vitro methodology can especially assist in screening nanoparticle-sized antibiotics against biofilm antibiotic resistance and help identify these “shorter antibiotic” compounds showing more fluidic passage through biofilm channels and potentially more efficacious.
Microbial biofilms form complex spatial structures such as wrinkles and fruiting bodies. How do these structures influence the evolutionary fitness of the microbial population and the relative importance of chance in its evolution? Measuring spatially-resolved evolutionary lineages can give us insight into the evolutionary history of these biofilms and an understanding of how it was influenced by these structures. Existing methods of spatial lineage tracing are limited by genetic engineering techniques or to small population sizes (order 1000 individuals), making it challenging to learn about emergent population-level behavior. We have developed a label-free method to infer evolutionary lineage trees in biofilms composed of trillions of cells using population-level time lapse microscopy. We measure local velocity fields from the collective motion of cells and calculate stream lines that are then converted to lineages. This method allows us to show that in wrinkled Bacillus subtilis biofilms, lineages get lost from the population faster at a wrinkle than far away from a wrinkle. This result suggests that wrinkles are local population bottlenecks, where the relative effect of natural selection is reduced in comparison to that of chance.
Microfluidic-based Transcriptomics Reveals Rheosensitive Bacterial Gene Expression

Bacteria must contend with flow to colonize new environments, such as hosts and associated medical devices. However, it was previously unknown if bacteria could actively respond to flow independent of surface sensing. Here we used microfluidic-based transcriptomics to temporally resolve the rheosensitive response of the human pathogen *Pseudomonas aeruginosa*. Flow rapidly induced expression of genes throughout the genome and we show that the flow-induced transcriptome is distinct from the surface-induced transcriptome. Many flow-induced genes are also induced during human infection, including the previously uncharacterized operon *froABCD* (flow responsive operon). Single-cell analysis confirmed that *fro* induction is surface-insensitive and revealed that *fro* induction does not require surface sensors PilY1 or type IV pili. Instead, *fro* induction by flow is mediated by a previously uncharacterized anti-sigma factor FroI and sigma factor FroR. *P. aeruginosa* is capable of dynamically tuning *fro* expression to shear rates of 40-400 sec\(^{-1}\), which closely matches shear rates found in the human host. Thus, *P. aeruginosa* mounts an active response to flow independent of surface sensing that tunes gene expression to environmental shear rate.
Establishment of a Biofilm in a Soil Column is Correlated to Reduced Permeability

Background: The growth and maintenance of a biofilm may be an answer to some civil and environmental engineering problems. Groundwater seepage is associated with failures of dams, levees, and contaminant containment systems. Traditional engineering methods to reduce groundwater seepage in the field can be expensive and may create significant environmental concerns. The use of microbiological processes to modify the properties of in-situ soils has shown significant potential in the laboratory. A biofilm that blocks portions of the void space in soil will reduce the soil permeability and limit seepage. Methods: Our experimental setup consisted of six vertical sand columns confined in acrylic tubes with sampling ports along the length of the column. Nutrients were added to four sand columns stimulating the growth of the extant population of microbes; two columns served as controls and received only de-ionized water. To monitor permeability, the apparatus requires not only a location where the soil is contained and mechanisms that permit flow through the sample, but also allows the measurement of the volume of fluid passing through the sample and the measurement of pressure differences across the soil sample. Nutrients were initially provided daily to the columns; however, clogging occurred above the sample at the location where the nutrients entered the soil and significantly reduced fluid flow. Once the clogging was removed, the nutrients and de-ionized water were provided to the columns on alternate days. Columns were maintained under this schedule for over 11 weeks and permeability was measured daily. Samples of the column fluid were taken through the sampling ports with an 18 Ga needle and syringe, placed on a slide, stained for DNA (with SYTO-9) and EPS (with Con-A conjugated Alexa Fluor 633) and visualized using confocal microscopy. Results: Sustainable permeability reductions of up to one order of magnitude were measured in each experimental column. Although the biofilm was likely disrupted by sampling methods, confocal microscopy confirmed the presence of bacteria and EPS throughout the experimental columns. Conclusions: Biofilm growth in soils may be a low-cost alternative to traditional engineering field methods of permeability reduction.
Candida albicans is a human commensal and opportunistic fungal pathogen. C. albicans biofilms on implanted devices are less susceptible to antimicrobials and can cause systemic infection with substantial mortality rates. Prior works by us and others have shown that micro/nano-scale topographical features influence microbial retention on surfaces. However, a quantitative model describing the effects of the geometry and size of features on microbial retention is lacking. Furthermore, the effect of surface topography on near-surface behavior of C. albicans is largely unexplored. Through synergistic experimental and theoretical investigations, we examined C. albicans adhesion, at single-cell and population-level, to micro/nano-fiber coated surfaces as a function of the geometry (i.e. diameter) and configuration (i.e. inter-fiber spacing) of the surface features. C. albicans interaction with polystyrene (PS) surfaces coated with PS nanofibers of uniform diameter (500 nm-2 µm) and spacing (1-3 µm) was studied using high spatiotemporal resolution optical microscopy to gain insight into the mechanisms involved in response to surface topography in absence of motility. Population-level cell behavior on nanofibers-coated surfaces was characterized using 24-hour dynamic retention assays to quantify cell attachment. A biophysical model was also developed to describe the changes in the total free energy (adhesion energy and stretching energy) of the adherent C. albicans as a function of the diameter and spacing of the fibers, surface energies of the cell and substrate, and cell stretching modulus. Our single-cell experimental studies show that a non-Brownian biologically-driven motion enables C. albicans to adhere in distinctly preferred locations. Through biophysical modeling of cell-surface interactions, we demonstrate that these preferred adhesion locations minimize the total energy of the adherent cells. Comparison of the retention assay and modeling results shows that the cell attachment density trend closely correlates with the theoretically predicted adherent single-cell total energy. The nanofiber coating (1.2 µm diameter, 2 µm spacing) that maximized the total energy of the adherent cell resulted in the lowest microbial retention. Using our biophysical model, relative importance of the surface topography, and cell and surface physicochemical properties were explored and a set of non-dimensionalized curves was developed to cohesively relate all the variables. We show that C. albicans responds to surface topography by adjusting its geometry and relative position to minimize its total free energy. We further demonstrate that the single adherent cell total free energy quantification enables prediction of the population-level cell retention, which can be utilized towards ab initio design of surfaces that resist biofilm growth for medical applications and beyond.
Session Title: **WEDNESDAY Poster Session 3**
Session Date/Time: Wednesday, October 10, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 040
Abstract Topic: Biofilm Metabolism
Abstract Title: Deciphering a Global Role of RNA Chaperone Hfq in the *Pseudomonas aeruginosa* Transcriptome: Comparative CLIP-seq of Planktonic and Biofilm Forms
Author Block: K. Chihara1, T. Bischler2, L. Barquist3, N. Noda4, J. Vogel3, S. Tsuneda1; 1Waseda University, Tokyo, JAPAN, 2Core Unit Systems Medicine, University Hospital of Würzburg, Würzburg, 3Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, GERMANY, 4National Institute of Advanced Industrial Science and Technology, Ibaraki, JAPAN.
Abstract Body:
Small non-coding RNA (sRNA) associated with post-transcriptional regulations in bacteria has attracted attention for its role in the control of bacterial metabolism and adaptation to stressful environments. In *Pseudomonas aeruginosa*, some general sRNAs play an important role in biofilm development and adaptation. For example, RsmA protein titrating sRNAs RsmY/Z indirectly and positively regulate biofilm formations. Generally, RNA chaperone Hfq helps sRNA associate with target RNAs and modulate translation efficiency depending on the physiological state. In a previous report, the comparative RNA-seq of wild type *P. aeruginosa* PAO1 and Δhfq strain revealed that Hfq regulates numerous biological processes. However, since comprehensive Hfq-dependent RNA targets are still elusive, we sought to understand the global role of Hfq in planktonic and biofilm forms in the *P. aeruginosa* PAO1 transcriptome. To reveal Hfq-dependent RNA targets and background RNA expression, in vivo UV crosslinking immunoprecipitation followed by high-throughput sequencing (CLIP-seq) and total RNA-seq were performed in *P. aeruginosa* PAO1 late-exponential cultures and colony biofilms. In the CLIP-seq, UV crosslinking and coimmunoprecipitation were applied to efficiently recover the Hfq-RNA complex. Furthermore, this approach can be used to identify binding sites at high resolution by an RNase treatment process. Next-generation sequencing followed by bioinformatics analysis revealed various genes and biological processes under the control of Hfq in the two physiological states. The difference in known *Pseudomonas* sRNA affinities was estimated by disentangling Hfq peaks from background expression, and the results suggested that sRNA PhrS and PrrF1 have significantly high affinities in planktonic and biofilm forms, respectively. Affinity variations depending on physiological states may be due to target RNA expression, a fact reminiscent of the target-centric perspective in higher eukaryotes. Furthermore, CopaRNA, a tool for sRNA target prediction, was used for five known *Pseudomonas* sRNAs: ErsA, NrsZ, PhrS, PrrF1, and RgsA. The comparison between CopaRNA predictions and Hfq peaks detected by CLIP-seq extracted novel sRNA targets with previously validated ones. Intriguingly, Hfq associates with some tRNAs specifically in the biofilm, despite there being no significant difference in the expression of these tRNAs between the two phenotypic conditions. The global screen thus suggested novel post-transcriptional regulation in *P. aeruginosa* metabolism and supported a critical role of Hfq during the adaptation to biofilm environments. The current work has focused on validating how sRNA–target interactions affect phenotypic properties.
**Abstract Body:**

**Background:** Because current wastewater treatment processes often fail at removing anthropogenic chemicals, chemicals such as antibiotics are frequently discharged into the environment. To improve removal efficiencies, we need to better understand the metabolic capabilities of wastewater biofilms. For example, the role soluble and matrix-bound extracellular enzymes play in the biotransformation of chemicals during biological wastewater treatment is poorly understood. In this study, we aim to explore the roles of different fractions of enzymes in the biotransformation of selected antibiotics. We anticipate that a fast, extracellular transformation of antibiotics reduces the formation of antibiotic resistance in both wastewater treatment plants and environmental systems. **Methods:** We used high-resolution mass spectrometry to measure biotransformation rates and transformation pathways for six amide-containing antibiotics. To assess the contribution of different enzyme fractions to the biotransformation of these antibiotics, we developed a method to separate soluble and matrix-bound enzymes from the entirety of enzymes from wastewater microbial communities derived from full-scale wastewater treatment plants. Furthermore, we used high-throughput fluorescence-based techniques to test for a link between peptidase activity and antibiotic transformation rate. **Results:** We found that the removal rate constants in biological wastewater treatment strongly differed among the tested antibiotics. Interestingly, the observed trends were similar across wastewater treatment plants that were operated under different conditions. Furthermore, we found that while all tested antibiotics were to some extent transformed in intact sludge, only a small subset of the antibiotics was transformed by extracellular enzymes attached to the extracellular matrix of the wastewater biofilm. None of the tested antibiotics were readily transformed by the soluble enzymes. This finding correlated well with the measured peptidase activities, which decreased in the following order: intact sludge, matrix-bound enzymes, soluble enzymes. **Conclusions:** Assuming that the rate of antibiotic resistance formation negatively correlates with the removal rate of the antibiotic during wastewater treatment, the observed difference in transformation rates highlights the importance of considering biotransformation rates when selecting antibiotics for use in human or veterinary medicine. The approach presented here is furthermore key for other metabolomics experiments assessing the transformation potential of extracellular enzymes in biological wastewater treatment plants. Knowledge on the susceptibility of certain functional groups to biotransformation will improve the design of chemicals for a sustainable future and will improve our understanding of this important biofilm system.
Enterococcus faecalis is a gram-positive commensal resident of the gastrointestinal tract that has emerged as a leading healthcare-associated pathogen. E. faecalis clinical isolates are often resistant to antibacterial agents and readily form biofilms, making enterococcal infections difficult to treat. Understanding the genetic and regulatory mechanisms that enable E. faecalis to form and maintain biofilms will be critical for finding novel methods to treat E. faecalis infections. Through the use of recombination-based in vivo expression technology (RIVET) screens, our lab has identified specific E. faecalis promoters that are up-regulated in in vitro biofilm assays and rabbit biofilm infection models (endocarditis and subcutaneous abscess). A putative promoter upstream of the pstB1 ORF was identified in all three screens. This suggests that expression of PstB1, which is annotated as an ATP binding protein in E. faecalis, may be important in enterococcal biofilm formation. pstB1 is located within the pst-phoU locus, a putative operon that encodes a predicted, well-conserved inorganic phosphate (Pi) ABC transport system. The pst-phoU locus is known to play a role in Pi uptake in a variety of bacterial species. Furthermore, phosphate homeostasis is understood to be an important contributor to bacterial virulence, including biofilm formation. However, the roles of Pi and the pst-phoU locus in E. faecalis biology have not been determined. Therefore, we have generated an in-frame ΔpstB1 deletion mutant strain and have initiated phenotypic characterization studies to evaluate the role of pstB1 in E. faecalis membrane stress and biofilm formation conditions. When compared to the wild-type strain (OG1RF), the ΔpstB1 strain exhibited increased susceptibility to bile salts and SDS when plated on solid medium. Unexpectedly, this phenotype was not present when the strains were exposed to bile salts or SDS during growth in broth. The wild-type and ΔpstB1 strains also had identical survival phenotypes in the rabbit subcutaneous abscess infection model, which indicates that pstB1 is not essential for in vivo foreign-body abscess infection. In order to determine the role that Pi plays in E. faecalis biofilm formation, we generated a reduced-phosphate BHI broth. OG1RF and ΔpstB1 grown in the reduced-phosphate BHI did not readily form biofilms compared to bacteria grown in normal BHI. Together these findings demonstrate that Pi plays an important role in the ability of E. faecalis to form biofilms and that deletion of pstB1 contributes to membrane disruption by detergents on solid surfaces.
The health and environmental hazards associated with water body contamination by industrial effluents cannot be over emphasized. This study investigated the bacteriological and physicochemical qualities of effluents from Ebonyi Fertilizer and Chemical Plant and the impact on Azuiyiokwu Creek, located in Abakaliki, Ebonyi State, Nigeria between May, 2010 and March, 2011. Standard microbiological techniques were used for the isolation and identification of microbial isolates. The physicochemical parameters detected from all the samples investigated include pH, Ca, Cu, Pb, Ni, As, Al, Hg, Co, Zn, Fe, Cr, Na, P, Mg, and Mn. The study shows that the concentrations of the metals were constantly higher in fertilizer effluents, than Azuiyiokwu Creek and the control. There was a significant difference (P≤0.05) in concentration of metals between fertilizer effluents, Azuiyiokwu and the Control Creeks. The results also show that metallic contaminations of Azuiyiokwu Creek could be as a result of the infiltration of effluents from Ebonyi Fertilizer and Chemical Plant into Azuiyiokwu Creek. The study revealed higher bacterial load in the Ebonyi Fertilizer and Chemical plant effluents than Azuiyiokwu and Control Creeks. There was a significant difference (P≤ 0.05) in the microbial counts between the Control and Azuiyiokwu Creek. Bacteria genera encountered in this study include: Staphylococcus, Proteus, Klebsiella, Pseudomonas, Bacillus, Escherichia, Salmonella, Vibrio and Aeromonas. This shows that the effluents received by Azuiyiokwu creek have low bacteriological and physicochemical qualities and the range of microorganisms isolated in this study raise more serious concern about the public health implications. This reflects the possible pathetic condition of most water bodies in Nigeria. Therefore, adequate measures should be put in place to help ameliorate the deplorable state of our water bodies. **Keywords**: abakaliki, azuiyiokwu, bacteria, effluents, fertilizer, physicochemical, water, nigeria
Biofilms formed by nontypeable *Haemophilus influenzae* (NTHI) bacteria play an important role in a number of respiratory tract diseases, including chronic otitis media (OM) in children. We aimed to better understand the structure and formation of these biofilms by developing a computational model of NTHI biofilms based on statistical physics. The model was validated by closely comparing computational results to experimental images of *in vivo* NTHI biofilms recovered from the middle ears of the chinchilla model of experimental OM. NTHI biofilms are known to contain extracellular DNA (eDNA); *in vivo* the source of the eDNA includes both the host immune response and the bacteria themselves, and the eDNA network grows denser over the course of the infection. In our simulations, the bacterial growth was modeled with a given eDNA network in place, generated to mimic properties of eDNA seen experimentally, in order to understand the role of the eDNA network on the final biofilm morphology.

The model is based on the following rules hypothesized to describe the behavior of NTHI: (1) biofilm-resident bacteria are resistant to the host’s immune response and do not die on the timescale of the simulation; (2) bacteria attached to the eDNA network can move to adjacent locations with eDNA via a “twitching” mechanism using type IV pili; and (3) bacteria disperse (become planktonic) via a quorum sensing mechanism, and these planktonic bacteria may be either killed by the host or reattach at other sites. Similar to what has been observed experimentally, the morphology was stable on the timescale of weeks. At intermediate times the *in silico* morphologies displayed fractal or self-similar organization of bacterial cells at the surfaces of the bacterial clusters. This was also observed in the analysis of confocal images of biofilms formed by NTHI *in vivo*. We also validated the model further by comparing model predictions regarding biofilm morphology at early stages (e.g., 4 days) of biofilm development. The fractal interfaces can potentially help NTHI survive in the nutrient poor host environment by increasing its exposure to nutrients, however this increased surface area may also increase exposure of biofilm-resident bacteria to antibiotics or immune effectors as well. We expect that simple models that reproduce known bacterial biofilm morphologies can deepen our understanding of bacterial behavior in biofilms, which may lead to improved, targeted treatments for NTHI-related diseases in the future. Funding source: This work was supported in part by the NIGMS grant R01GM103612 to JD.
Anidulafungin Increases the Activity of Tigecycline against Staphylococcus aureus in Polymicrobial Candida albicans-Staphylococcus aureus Device-Associated Peritonitis

O. Rogiers¹, M. Holtappels¹, W. Siala², M. Lamkanfi³, V. François², K. Lagrou⁴, P. Van Dijk¹, S. Kucharikova¹;
¹VIB, K.U. Leuven, Leuven, BELGIUM, ²Université Catholique de Louvain, Woluwe-Brussels, BELGIUM, ³VIB, U Gent, Gent, BELGIUM, ⁴UZ. Leuven, Leuven, BELGIUM.

The emerging use of medical devices encounters an increased occurrence of biofilms-related infections. Both the bacterium Staphylococcus aureus and the yeast Candida albicans are key players in the cause of hospital-acquired infections due to their extreme ability to inhabit diverse host niches especially in immunocompromised individuals. It is crucial to study and understand the behavior of these pathogens when coexisting together and to discover a viable option for treatment of not only single species but also mixed species biofilms. In the present study, we demonstrate for the first time the activity of the antifungal drug anidulafungin and the antibiotic tigecycline against dual species C. albicans · S. aureus biofilms developed in a novel model of intra-abdominal foreign body infection. We provide insight into the pathogenesis of this dual-species biofilm-associated infection. We show that this novel model of mixed biofilm infection is characterized by bacterial dissemination into the vital organs within 24 hours which remains persistent over 21 days. In addition, flow cytometry data reveals significantly greater neutrophil influx upon poly-microbial intra-peritoneal device-associated infection in comparison to single species infection. In search of an effective treatment strategy we demonstrate that tigecycline acts synergistically when combined with anidulafungin against in vivo biofilms. We show that anidulafungin impaired synthesis of poly-(β(1-6))-N-acetylglucosamine (PNAG), a major constituent of S. aureus biofilm matrix. Therefore, we hypothesize that the effect of anidulafungin on fungal and bacterial polysaccharides production may contribute to the synergism between these two drugs.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 046  
**Abstract Topic:** Biofilm: From Nature to Models  
**Abstract Title:** Characterizations of *Pseudomonas aeruginosa* Aggregates in High Viscosity  
**Author Block:** J. Geyer, Y. Irie; University of Dayton, Dayton, OH.  

*Pseudomonas aeruginosa* frequently colonizes the respiratory tract of cystic fibrosis patients where excessive amounts of mucus build-up obstruct the airways. *P. aeruginosa* is known to form aggregates within this mucus plug, causing chronic infections. Previously, *P. aeruginosa* cultured in 0.8% w/v agar was observed to grow as suspended aggregates (Staudinger BJ et al., 2014), which leads us to hypothesize that growth in high viscosity environments induce these aggregations. In this study, we further expanded this observation to better understand the characteristics of these aggregates. Based on our observations, we found that the aggregate sizes are dependent on the concentration of nitrate which serves as an alternative electron acceptor. Aggregates were small in the absence of nitrate and their sizes grew larger as nitrate concentrations were increased. This phenotype is overridden in part by the overexpression of pyocyanin, which may be suggestive of pyocyanin’s role in aiding *P. aeruginosa* anaerobic respiration. Furthermore, the aggregates do not require biofilm extracellular polysaccharides PEL and PSL to form. High c-di-GMP strains, which are well-characterized as hyper-biofilm forming strains due to the overexpression of PEL and PSL, have identical aggregate phenotypes, raising a provocative question of whether suspended aggregates are to be categorically classified as a type of biofilms.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 047  
**Abstract Topic:** Biofilm: From Nature to Models  
**Abstract Title:** Novel *in-vitro* Model for Assessment of Biofilm Formation by Uropathogens  
**Author Block:** B. E. Lam¹, S. Johnson¹, K. Ivanova², T. Tsanov², J. E. Lafleur¹; ¹George Washington University, Washington, DC, ²Universitat Politècnica de Catalunya, Barcelona, SPAIN.

**Background:** Catheter-associated urinary tract infection (CAUTI) is one of the most common nosocomial infections, resulting in over 560,000 infections, 8,000 deaths, and upwards of $1.7 billion in added medical costs each year in the US. Despite several decades of research, a urinary catheter designed to inhibit biofilm formation continues to elude clinical adoption. One reason for this poor track record relates to the *in-vitro* models employed for urinary catheter research, which have mostly relied upon nutrient-rich defined media, and laboratory bacterial strains. These *in-vitro* models poorly mimic *in-vivo* conditions under which CAUTI develops, and lead to failed therapeutic candidates in the clinical domain. To address this problem, we have devised a more clinically relevant *in-vitro* model for assessing biofilm inhibition on non-vital surfaces.

**Methods:** A total of 46 subjects met the clinical criteria for urinary tract infection (UTI) and were enrolled from an urban emergency department. 100 mL of UTI urine was collected and transported to the laboratory. 1 cm² flat silicone surface that was either uncoated or coated with one of two enzymes previously shown to inhibit biofilm formation were individually incubated in 5 ml of fresh uti urine for 4 days at 37°C with rocking. Subsequently, each silicone surface was then removed, stained with a fluorescent nuclear stain and imaged with an epifluorescence microscope. Biofilm images were evaluated with Image J software. Samples were stored and subsequently DNA was extracted for additional biofilm assessment using universal 16S primers in conjunction with ddPCR. Urine culture results were extracted from patient medical records for use in data analysis. Of enrolled subjects, 37 had culture results indicating uropathogens and were included in data analysis. **Results:** Silicone surfaces coated with amylase, (active amylase has previously shown antibiofilm activity), demonstrated a significant increase in biofilm coverage whether all uropathogens were evaluated, or just those urine samples that grew out E. coli. Silicone surfaces coated with acylase, an AI-1 inhibitor, showed a similar trend which, however, did not reach statistical significance. **Conclusion:** We have shown that biofilm formation on silicone surfaces by clinical uropathogens in a clinically relevant medium (UTI urine) can be assessed via image analysis. Further we have shown that engineered enzymatic surface coatings previously shown to inhibit biofilm formation by representative strains of biofilm-forming bacteria, did not inhibit biofilms on silicone surfaces in our model. Work to assess biofilms with greater sensitivity using ddPCR is currently ongoing.
Pathogenic biofilms are detrimentally relevant in over 80% of bacterial infections in the human body. Biofilms are composed of both bacterial cells and a bacterially-secreted extracellular matrix composed of multiple biopolymers. This “extracellular polymeric substance” (EPS) creates a barrier to protect the bacteria from many external threats including antibodies, and cellular arms of the immune system. More importantly, bacteria deep within the biofilm become metabolically quiescent due to nutrient limitation and thus trigger the stringent response. The upregulation of oxidative stress response genes as part of the stringent response makes bacterial biofilm infections resistant to even the strongest antibiotics. Because of this, it is estimated that biofilm infections cost the United States health care system on order of 5 billion dollars per year as they can only be treated through debridement. Developing new drugs against biofilms requires high-throughput methods for growing and analyzing biofilms. Standard methods involve growing bacterial strains in 96-well plates. This method has limitations i.e. bacteria cannot be cultured for an extended period of time. Currently only one system is available on the market which enables the growth of bacterial biofilms for long time periods in the context of a high throughput 96 well plate. Due to the lack of competition that system is expensive to purchase and designed for a single use. Proposed Solution: We have developed a technology, based on individual metal pins, that can be arrayed to fit into 96-well plated and which can be controlled (moved either individually or in full array mode) with magnets because of their ferromagnetic properties. We have shown that bacterial biofilms can be grow robustly and indefinitely on these metal pins, through the serial and facile transfer of the magnetic plate/pin apparatus to fresh media on a daily (or other regular) basis. The biofilms grown on the pins can be quantified by standard biofilm staining methods, confocal and electron microscopy. This technology gives the user the ability pull out and analyze any single pin, or combinations of pins from the 96 well plate without effecting any of the other pins. With current technology it would be necessary to brake and destroy the entire 96 plastic pin assembly just for the analysis of a single pin. In addition, the small metal pins are relatively inexpensive and furthermore they can be washed, sterilized and reused which greatly reduces the total cost of use. The usage of small electromagnets will allow future automation and greater possibility for high throughput screening. The technology was used to evaluate anti-biofilm properties of newly designed compounds (see D.C. Hall Jr. poster) and *E. coli* C biofilm (see J.E Krol poster).
Deep-bed biofiltration technology has been broadly applied in water industry for decades. Biofiltration can effectively remove soluble and particulate organics and nutrients using biofilms and their supporting media (e.g., granular activated carbon) through particle deposition, adsorption and biodegradation. During operation, biofilm growth and particle deposition will increase headloss across the filter, which can negatively impact contaminants removal. Periodic backwash of the biofilter is typically performed in response to headloss buildup. At many full-scale facilities, backwash has been a significant energy and maintenance burden. Thus, there is a need to develop strategies that optimize filter design and operation with respect to backwash requirements. However, the industry still currently lacks a systematic tool for helping biofilter design and optimization. In this study, we developed a biofiltration process kinetic model to quantify contaminant removal while simultaneously predicting headloss development. This model not only considered particle deposition and adsorption on filtration media, but also the biofilm growth during biodegradation of contaminants. The model includes inputs like flowrate, temperature, influent contaminants including organics (e.g., TOC, DOC or COD), nutrients (e.g., N, P), and suspended solids (TSS or turbidity), and can predict the headloss as well as contaminants profile along biofiltration depth and time. Application of the model to full-scale biofilter data was performed. Analyses indicated that the contributions of particle deposition on headloss accumulation were negligible in this system whose biofilter influent had low particulate content. Instead, biofilm growth was the key contributor to headloss accumulation in this system. Moreover, it was found that contaminants breakthrough could be attributed to the reduced hydraulic retention time caused by bed porosity decrease as a result of biofilm growth. The outcome of this study will shed light on prediction and optimization of headloss accumulation as well as contaminant control in deep-bed biofiltration for water and advanced wastewater treatment.
Background: Surgical site infections in orthopaedics are a great problem for both physicians and patients where infection rates as high as 30% lead to high morbidity and mortality. This study focuses on a clinically-relevant visual and quantitative model to understand the development of biofilms, and serve as a platform for testing eradication techniques. Utilizing Confocal Laser Scanning Microscopy and Scanning Electron Microscopy, we assessed the biofilm formation of Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis on Polyether Ether Ketone (PEEK) in a spatio-temporal manner.

Methods: Bacterial Culture and Biofilm Inoculation: P. aeruginosa, S. aureus, and S. epidermidis were propagated in Tryptic Soy Broth overnight at 37°C. Sterile (PEEK) disks were inoculated with a stock concentration of 1x10^8, 2x10^7 and 1x10^7 CFU/mL for S. epidermidis, P. aeruginosa and S. aureus, respectively, with rinses and media replacement at timed intervals. Biofilm Labeling and Confocal Imaging: Biofilms were stained with TOTO-1 Iodide to visualize Extracellular DNA, SYPRO Ruby Biofilm Stain for proteins, and Concanavalin A 635 conjugate for exopolysaccharides. Samples were imaged with an Olympus FV1000 MPE Multiphoton Microscope. Scanning Electron Microscopy (SEM) Imaging: After confocal imaging, samples were imaged on a Hitachi 2700 SEM at both 1,000X and 5,000X magnification. Data Analysis: Images were processed via ImageJ (NIH) for the visual presence of each macromolecule, and quantification of the overall volume at each time point.

Results: The biofilm formation of P. aeruginosa, S. aureus and S. epidermidis on PEEK were observed via the combination of CLSM and SEM. At 4 hours, P. aeruginosa permanently adheres to the substrate, demonstrating stage 2 biofilm. Subsequent time points, show the formation of microcolonies and organized vertical growth and exopolysaccharide secretion. Extracellular DNA, protein, and exopolysaccharide can be observed. SEM analysis of the same structures confirmed the presence of microfibers and matrix components. S.aureus biofilms form at a significantly slower rate than P. aeruginosa, but followed a comparative pattern of biofilm formation.

Conclusions: The combination of CLSM and SEM allowed for informed assessment of a very dynamic biofilm development cycle. This approach allowed for the visualization and quantification of the biofilm composition in three dimensions and detailed topographical information for each stage of biofilm and a very dynamic cycle of biofilm formation on orthopaedic relevant materials, resulting in a platform for which to test irrigation and debridement techniques.

**Background:** Biofilms associated with chronic disease states often exist as consortia of bacteria and other microorganisms reliant on mutualistic relationships to ensure growth and survival. In the oral cavity, biofilms are associated with many disease states. While there are many in vitro models used to study specific aspects of caries-associated biofilms, our study focused on developing a reproducible multispecies model within a 96 well microtiter plate to allow for high-throughput screening of anti-biofilm therapies. **Methods:** The microbial consortia developed in this model consisted of *Streptococcus gordonii* (DL1.1), *Streptococcus mutans* (UA159), and *Candida albicans* (SC5314) grown in 96 well microtiter plates for 24 h at 37°C and 5% CO2. Nine common microbiological media were tested for mono- and multispecies biofilm growth and reproducibility. Additionally, inoculum concentrations and growth kinetics were evaluated for the multi-species biofilms. In addition to biofilm viability and biomass measurements, microenvironment pH and structural properties of the biofilms were assessed. **Results:** Our results indicate that the 1:1 RPMI:TSBYE 0.6% media supported growth of the mono-species and three concentrations of multispecies biofilm best of the nine media tested. This was assessed using both viability measurements and biomass over three independent studies. Inoculum studies confirmed the mutualistic effects of *C. albicans* with both *S. gordonii* and *S. mutans*. Overall the three concentrations of multispecies biofilms tested had increased viability and biomass production compared to the mono-species biofilms. Microenvironment pH measurements showed that *S. mutans* mono-species biofilms on average had the lowest pH over a 48-hour period. Structural assessments using rheology found that multi-species biofilm response to deformation was intermediate of the streptococci mono-species biofilms and *Candida albicans* mono-species biofilms. **Conclusions:** We have successfully developed a reproducible and physiologically-relevant in vitro caries associate biofilm model for high-throughput screening applications. The model uses a defined consortia and laboratory microbiological media to allow for consistent comparison of anti-biofilm therapies.
Biofilms and Infection

Analysis of Biofilm Phenotypes of 350 Clin. Stenotrophomonas maltophilia Reveals High Levels of Phenotypic and Structural Heterogeneity

I. Alio\textsuperscript{1}, M. Gudzuhn\textsuperscript{1}, C. Vollstedt\textsuperscript{1}, U. Manat\textsuperscript{2}, U. Schaible\textsuperscript{1}, S. Niemann\textsuperscript{2}, T. Kohl\textsuperscript{2}, J. Steinmann\textsuperscript{3}, W. Streit\textsuperscript{1};
\textsuperscript{1}University of Hamburg, Hamburg, GERMANY, \textsuperscript{2}Research Center Borstel, Borstel, GERMANY, \textsuperscript{3}Universitätsinstitut der Paracelsus Medizinischen Privatuniversität Klinikum Nürnberg, Nürnberg, GERMANY.

**Background:** The multidrug resistant opportunistic pathogen *Stenotrophomonas maltophilia* is a potent biofilm forming and gram-negative bacterium. It can contribute significantly to disease progression in cystic fibrosis patients, but it is also found in wounds and on catheter surfaces. For an improved understanding of processes and genes involved in the biofilm formation within the genus *Stenotrophomonas*, we are currently analyzing the genomes of over 350 clinical and 40 environmental isolates. In parallel we are investigating the biofilm profile of these isolates. Up to date we have established the genome sequences of over 100 isolates (Steinmann, Front Microbiol. 2018; 9:806) and the genome sequencing of additional 250 isolates is ongoing. The genome data together with the biofilm analysis and other phenotypic and metabolic data will generate the largest data set of *S. maltophilia* and its biofilm formation on a genus and pangenome-wide level.

**Methods:** Biofilm assays were done in microtiter plates and flow cell analyses. Genomes were sequenced using NGS technologies and phylogenetic trees constructed as previously published (Steinmann, Front Microbiol. 2018; 9:806). Transcriptomes were generated using RNAseq and *S. maltophilia* mRNA extraction protocols (Abda, Front. Microbiol. 2015; 6:1373).

**Results:** The microtiter and flow cell analyses of 390 clinical and environmental *S. maltophilia* isolates revealed a strong variation in biofilm forming ability among the isolates. 13.5 % of all isolates formed very strong biofilms, while 10.4 % formed strong, 63.8 % formed moderate and 12.3 % formed rather weak biofilms. Clinical and environmental isolates did not differ in their biofilm formation abilities. Most interestingly, analyses of 3D-structures of biofilms grown in flow cells identified high levels of heterogeneity within the biofilm matrix and appearance independent of the strains and their phylogenetic position within the genus. Furthermore, no correlations between biofilm formation abilities, the 3D-structure and resistance to the antibiotic colistin were observed. Additional transcriptome data for selected isolates are underway to estimate the biofilm formation on a global level. **Conclusions:** *S. maltophilia* isolates both clinical and environmental display a diverse biofilm phenotype. Thus, we speculate that under *in vivo*conditions *S. maltophilia* also reveals varying biofilm architectures on a strain-specific level. The strain specific heterogeneity may just be another strategy to escape antibiotic treatment. The high variation in the 3D biofilm structures of different clinical isolates may correlate with *S. maltophilia* strain specific expression patterns.
Candida albicans Transcription Factor Stp2 Contributes to Virulence in Burn Wound Infection

Background: Skin burn wounds are highly susceptible sites for opportunistic colonization and biofilm formation by bacteria and fungi. About 10-20% of burn wound infections are caused by Candida spp. Although sepsis following failed treatment of such infection is not uncommon and may result in up to 70% mortality of the infected individuals, the factors contributing to burn wound candidiasis are currently unknown. Recent studies show that Candida albicans can rapidly neutralize the extracellular pH in vitro and within the macrophage phagosome. This process is controlled by the transcription factor Stp2 and results in morphogenetic switch from yeast to the more virulent hyphal form. As an acidic pH is critical for wound healing, this study aims to investigate the virulence determinants of burn wound candidiasis, including environmental pH modulation by C. albicans at the wounded site. Methods: C. albicans-optimized luciferase was integrated in the C. albicans wild type (wt) SC5314 and in the pH modulation deficient stp2Δ strain. In order to identify the factors involved in burn wound candidiasis, we established protocols for two skin burn wound infection models: an ex vivo human native skin model and an in vivo rat burn wound model. Results: Our results show that the luciferase expressing C. albicans wt and stp2Δ strains have no phenotypic differences to the strains expressing empty vector. Application of 1x10⁷ fungal cells/ml at the wounded site resulted in persistent infection, since the luminescence signals measured up to six days post infection were about ten times higher than the mock-treated skin. In contrast to the invasive burn wound infection caused by the wt strain, infection with the stp2Δ strain was less aggressive. The pH at the burn site was lower in uninfected wounds and upon infection with the stp2Δ strain compared to wt infected burns, suggesting that C. albicans modulates the wound pH in a Stp2-dependent manner. Conclusion: C. albicans causes Stp2-dependent progressive burn wound infection and can interfere with the wound healing process by increasing the environmental pH.
Susceptibility of *Klebsiella Pneumoniae* Clin. Strains to Antibiotics and Two Quaternary Ammonium Compounds (Benzalkonium Chloride and Deconex) under Biofilm and Planktonic Conditions

M. Shakibaie; Kerman University of Medical Sciences, Kerman, IRAN, ISLAMIC REPUBLIC OF.

The aims of this study were to evaluate the susceptibility of *Klebsiella pneumoniae* clinical isolates to antibiotics and biocides (benzalkonium chloride and deconex) under biofilm and planktonic conditions and also to monitor antibiofilm activities and biofilm eradication time of these two biocides. A total of 85 *K. pneumoniae* were isolated from patients in 4 referral hospitals in Kerman, Iran during six months. We found that, 15% (n=12) of the isolates showing strong, 40% (n=35) moderate, 30% (n=26) weak and 15% (n=12) no biofilm activities. Both the biocides had profound inhibitory activities on planktonic cells (average MIC 0.062±0.4 mg/ml for deconex and average MIC 0.031±0.1 mg/ml for benzalkonium chloride), however, exerted least antibiofilm effect at sub-MIC concentrations (0.015 mg/ml). Nevertheless, biofilm formation reduced considerably in acidic pH and at low temperature (15 °C). Those strains that formed high amounts of biofilm also harbored cepA gene (p≤0.05), although some weak and no-biofilm formers also carried copA gene. Furthermore, we studied Minimum Inhibitory Concentration (MIC) of 12 antibiotics for the cells showing high amounts of biofilm under biofilm and planktonic cells growths, we found that there was considerable increase in MIC to piperacillin/tazobactam, tetracycline, chloramphenicol and cefotaxime for the cells taken from 24 h biofilm as compared to planktonic condition but all these isolates were sensitive to colistin and tigecycline. Most of the biofilms was eradicated from microtiter wells within 30 min exposure to these biocides. From above data, we suggest, benzalkonium chloride and deconex may serve as good hospital disinfectant for removal of planktonic but not biofilms related contaminations at in use concentrations.
**Background:** The DNABII family of DNA-binding proteins serve as critical structural elements to the extracellular DNA scaffold within biofilms formed by all 18 human bacterial pathogens tested to date. Removal of DNABII proteins, including integration host factor (IHF), from biofilms with antibody directed against the DNA-binding ‘tip’ region of this protein induces catastrophic collapse of the biofilm and release of resident bacteria. Biofilms confound the treatment of chronic diseases, including otitis media; therefore, this antibody-mediated biofilm disruption strategy has tremendous clinical utility. A potential adverse outcome of antibody therapy is development of neutralizing anti-antibody; therefore we examined the utility of antibody fragments, specifically the antigen-binding domains of anti-IHF IgG (i.e. ‘Fab’ fragments) to disrupt bacterial biofilms. **Methods:** Nontypeable Haemophilus influenzae (NTHI) biofilms were established in vitro and incubated with 5 μg Fab fragments directed against the DNA-binding tip domain of NTHI IHF, or as negative controls, either Fab fragments against carboxy-terminal (tail)-region of IHF or naive serum. Also, in an experimental model of otitis media, NTHI biofilms were first established in the middle ears of chinchillas, then Fab fragments were delivered directly to this site. Two doses were delivered at 24 hr intervals and animals sacrificed 1 or 7 days later. **Results:** In vitro, incubation of NTHI biofilms with 5 μg tip-directed Fab fragments resulted in a 78% reduction in biomass. In vivo, one day after receipt of the second dose of Fab fragments, a significant 3-log reduction in biofilm-resident NTHI and significantly less biofilm biomass was observed in the middle ears of animals administered tip-directed Fab fragments, compared to Fab fragments against the tail domain or naive serum. This outcome was enhanced over an additional seven days despite no additional treatment, thus residual NTHI within the middle ears of animals that received tip-directed Fab fragments did not re-form a biofilm. Moreover, whereas the middle ear mucosa from animals that received tail-directed Fab fragments exhibited considerable inflammation and middle ear fluids were enriched with pro-inflammatory cytokines, the mucosa from animals administered tip-directed Fab fragments appeared similar to that of a naive animal and the cytokine profile was anti-inflammatory. **Conclusions:** These data demonstrated the efficacy of IHF tip-directed Fab fragments to both resolve NTHI biofilms in vitro and in vivo. Moreover, the sole need of just the antigen binding domain of IgG and not the Fc portion recapitulates our model that binding of the DNABII proteins is the exclusive mechanism of biofilm disruption. As such, these Fab fragments could serve as a powerful biofilm-targeted therapeutic for resolution of recalcitrant diseases. Support: NIH R01 DC011818
Session Title: **WEDNESDAY Poster Session 3**

**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 058

**Abstract Topic:** Biofilms and Infection

**Abstract Title:** Agar Wound Model to Study Killing of _Pseudomonas aeruginosa_ Biofilms by Electrical Current

The Ohio State University, Columbus, OH.

**Background:** Chronic wounds affect over 6.5 million patients with an estimated $25 billion in healthcare costs, annually. Biofilms in chronic wounds significantly hinder wound healing. Recently, there have been several novel approaches to developing electroceutical wound dressings with electric currents to remediate biofilms. These dressings are at various stages of commercialization; however, a significant knowledge gap remains in how electrical current may impact bacterial biofilms in soft tissues, where there is diffusion limitation. In this study, we developed a simple agar-based model to mimic biofilm growth on soft tissue using bioluminescent and antibiotic-resistant strains of _Pseudomonas aeruginosa_.

**Methods:** Two silver (Ag) electrodes were embedded under tryptic soy agar (TSA) polystyrene petri dishes and spread with PA to grow lawn biofilms for 24h. A 6V battery with 1kΩ ballast resistor was used to treat biofilms for 1 or 24h. Colony forming units (CFU) and scanning electron microscopy (SEM) was performed on lawn biofilms at 0h before applying current, when current was stopped at 24h, and after incubation without current until 48h. EDS elemental analysis was performed to determine the distribution of elements within the agar to provide clues as to the nature of the inhibitory compounds. Since hypochlorous acid (HOCl) is produced when current is passed through a saline solution, we looked for the presence of 3-chlorotyrosine, which is formed when hypochlorous acid reacts with proteins by western blot. The amount of killing of PA biofilms was also determined in agar made with 40% human serum (HS) or bovine synovial fluid (BSF) to represent physiologically relevant chemistry. pH and temperature was measured monitored over the electrodes.

**Results:** Loss of bioluminescence with a significant 4-log reduction in CFU was achieved over the anode. Current treatment was also effective in reducing biofilms of antibiotic-resistant strains of PA. SEM showed damaged cells and disrupted biofilm architecture. The antimicrobial activity continued to spread from the anode even 24h after turning off the current. EDS suggested that silver was not responsible for biofilm killing. Chlorotyrosine was detected using western blot analysis suggesting the role of HOCl in the reduction of PA biofilms. Similar killing as on TSA medium was observed with HS or BSF. The initial pH of TSA before applying current was 7.0 ± 0.2. However, at 24 and 48h of current the pH changed to 6.71 ± 0.1 and 6.79 ± 0.1 (anode) and 8.40 ± 0.1 and 8.35 ± 0.1 (cathode) respectively. There was no significant change in temperature after applied current suggesting no role of temperature in killing.

**Conclusions:** The in vitro model could serve as a platform for fundamental studies to explore the effects of electrochemical treatment of biofilms by electroceutical dressings in a diffusion-dominated environments such as found in wounds.
Rapid Interstrain Recombination in *Pseudomonas aeruginosa* generates Adaptations to Biofilm Growth during Chronic Infections

Background: High biofilm forming variants have been associated with pathogen resistance to host immune defenses and antibiotics as well as worse outcomes in chronic infections like cystic fibrosis and wounds. A model microorganism for studying biofilms and their relevance to clinical outcomes is *Pseudomonas aeruginosa*. Loss of function screens and experimental evolution studies of *Pseudomonas* species have elucidated a number of well-characterized SNPs or short indels in genes encoding phosphodiesterase and/or diguanylate cyclase enzymes that increase intracellular cyclic-di-GMP production and confer high biofilm forming phenotypes. However, the contribution of recombination to cyclic-di-GMP-regulating pathways and biofilm regulation is largely unknown. Here, we present evidence for interstrain recombination as an underappreciated evolutionary mechanism for rapid adaptation to the biofilm lifestyle in chronic infections.

Methods: Six strains of *P. aeruginosa*, ranging from environmental to clinical isolates, were co-inoculated into a porcine full-thickness burn wound model. High biofilm producing stable variants were isolated 3, 14, and 28 days post infection and whole genome sequencing was conducted on representative isolates. Genetic targets of selection that conferred the high biofilm producing phenotypic change in the isolated variants were determined by short (Illumina) and long read sequencing (Oxford Nanopore).

Results: Of the six strains inoculated into the burn wound, only strains PA14 and PAO1 evolved high biofilm forming variants over the course of the experiment. Short read genome sequencing revealed a remarkable level of mutational parallelism in the well-characterized *wsp* pathway in PA14. However, no mutations were detected by short read genome sequencing in the high biofilm PAO1 variants. Long read sequencing and de novo assemblies revealed that recombination occurred in PAO1 with exogenous genetic material introduced from the four other *P. aeruginosa* strains that eventually went extinct over the course of the experiment. These recombination events were detected at the earliest sampling time, day 3 after infection, indicating rapid adaptation in the wound. The sites of insertion were most commonly in untranslated regions containing repeat sequences directly upstream of phosphodiesterase genes. These mutations are predicted to inactivate the phosphodiesterase, leading to increased cyclic-di-GMP levels and consequently high biofilm production. Therefore, these recombination events served primarily to disrupt existing genes rather than to provide new genetic material.

Conclusions: We propose that interstrain recombination is an important and often overlooked mechanism of rapid adaptive evolution, and that these mutations can cause drastic shifts in phenotypes associated with poor clinical outcomes.
**Background:** *Pseudomonas aeruginosa* is an opportunistic pathogen and a causative agent of persistent infections due to its capability to form biofilms. The self-produced extracellular matrix enables embedded bacteria to efficiently withstand antimicrobial treatment as well as host immune responses. The ecological success is based on the remarkable capability of *P. aeruginosa* to adapt and to survive in a broad range of diverse and challenging habitats. The large genome and the high proportion of transcriptional regulators enable a versatile lifestyle and flexible changes in bacterial behavior.

**Methods:** In this study, we used confocal microscopy to determine the biofilm phenotypes of >400 clinical isolates. According to their biofilm structure, the isolates were grouped into three major clusters and extensively characterized in respect to various bacterial phenotypes. These included motility and virulence in the *Galleria mellonella* infection model. Furthermore, we analyzed the transcriptional profiles of the clinical isolates of the three distinct biofilm clusters under planktonic and biofilm conditions and performed global comparison studies. **Results:** The distinct biofilm clusters showed a remarkable correlation with an array of virulence-related phenotypes (e.g. *in vivo* virulence in *G. mellonella*, *in vitro* cytotoxicity, elastase secretion, proteolytic activity, pyocyanin production) as well as motility and c-di-GMP levels. Interestingly, transcriptome data recorded under biofilm- but not under planktonic conditions revealed group-specific transcriptional signatures. For each group, unique differentially expressed genes were identified, whereas all three biofilm clusters shared only a small biofilm core transcriptome. **Conclusions:** Our work reveals that discrete *P. aeruginosa* populations adapt specific biofilm phenotypes by following similar evolutionary paths. Most interestingly, no group-specific differences could be observed if the bacteria were cultured under planktonic conditions, indicating that acquired mutations shape the biofilm structure but do not impact growth under rich medium conditions. Distinct biofilm clusters show barely any overlap in the gene expression. Our findings indicate that the biofilm status is not per se selecting for a particular transcriptional profile.
**Background:** Chronic wounds affect approximately 2% of the worldwide population and incur healthcare costs in the billions. Owing to an aging population and a substantial rise in predisposing factors such as obesity, diabetes and cardiovascular disease, chronic wounds have been described as a silent epidemic, the full financial impact of which is immeasurable. Key to their persistence is the formation of microbial biofilms, which are accounted for in nearly 80% of all non-healing wounds. A paradigm shift in wound-care management has resulted in the emergence of smart dressings, which effectively monitor the wound condition without physical intervention. The smart dressing presented herein aims to detect a range of volatile infection protagonists, with a striking colour change that can be visualised with the naked eye, providing 24/7, non-invasive monitoring of biofilm development and antimicrobial treatment efficacy.

**Methods:** A porcine skin wound biofilm model was optimised and validated. A range of coloured indicator films housing dyes responsive to biofilm-derived volatile analytes in the wound headspace were developed and tested against porcine skin inoculated with two common wound pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Digital images of the indicator film were captured regularly over a 16-hour period and the resulting images were aligned and split into red, green and blue (RGB) colour channels to yield semi-quantitative data. Scanning electron microscopy and confocal laser scanning microscopy was used to verify the presence of biofilm on porcine wound explants. **Results:** A CO2-sensing film comprising xylenol blue dye underwent a marked colour change from blue to yellow within 12 hours of inoculation with PAO1, whilst indicators monitoring uninoculated control skin remained blue (no colour change). Studies on indicator films monitoring S. aureus are ongoing. In addition, correlation of biofilm bioburden and indicator sensitivity data indicate a high level of sensitivity to the presence of *P. aeruginosa* in the wound model. GC-MS will be exploited to identify additional volatiles for incorporation into the smart dressing design. **Conclusion:** This *ex vivo* biofilm wound model is suitable for growing and studying pathogenic biofilms. This method is simple, reproducible and the materials used are affordable and easily sourced. The marked colour change exhibited by each indicator film is easily visualised by eye and can be digitally analysed to provide semi-quantitative data. This early warning, point-of-care technology is a promising candidate in combatting biofilm development in wounds.
Background: Pseudomonas aeruginosa and Enterococcus faecium are identified as the most common species isolated from complex polymicrobial wound environments of traumatically injured soldiers, 2011-2016 (TIDOS Study). In addition, at the point of injury, Coagulase-negative Staphylococcus spp. have also been identified. Each of these bacterium are independently known to utilize biofilm formation as a strategy to survive stressful conditions preventing the adequate delivery of antibiotics and promoting resistance. However, the interspecies ability to inhibit or exacerbate biofilm formation, within complex, polymicrobial communities, is unknown. The main objective of this project is to investigate the interspecies effects on biofilm production according to predetermined formula: Gram+ + Gram- + commensal.

Methods: All the isolates used are clinical isolates provided by Multidrug Resistant Organism Surveillance Network. Seven strains of P. aeruginosa and 20 strains of E. faecium and S. epidermidis were tested in a compatibility assay. Strains that showed no antagonistic (i.e. growth inhibition) relationships were chosen to investigate the effects of two and three species polymicrobial biofilms compared to monomicrobial biofilms. Overnight cultures of each strain were grown and inoculum adjusted to ~1.3 x 10^6 colony forming units (CFU)/well. Each individual strain, and all combinations of two species and three species were grown for 24 and 48h in incubator (37°C, 5% CO2). At these time points, biomass was measured (OD600), numbers of surviving bacteria enumerated, and plates were stained with crystal violet to determine biofilm production (OD590). All experiments were performed in triplicate and results expressed as mean ± standard deviation.

Results: S. epidermidis, inoculated singly, produces less biofilm than when grown in the presence of other bacteria. S. epidermidis CFUs significantly increase in P. aeruginosa co-infection, while their CFUs drop to less than half in E. faecium co-infection. When grown individually, P. aeruginosa has similar biofilm and CFU when grown with other species. E. faecium shows significant increase in all measured parameters when grown in the presence of P. aeruginosa. However, when grown with S. epidermidis absorbance values remain similar but CFUs are significantly less.

Conclusions: Data provides evidence that there are varying effects on biofilm production, biomass, adhesion units, and CFU counts when comparing interspecies infections to monoculture and co-culture conditions. When considering two and three species co-cultures, biofilm properties and CFU counts (increase or decrease) based upon the specific species combination of bacteria present in the inoculum. Initial data must be further explored with regards to therapeutic development, clinical therapeutic application, and initial battlefield combat casualty care guidelines.
Abstract Title: Modeling Clostridium difficile Persistence in a Mock Community Generated in Siloxane-based Nanocultures

Abstract Body: Clostridium difficile (C. difficile) is a leading cause in antibiotic-induced diarrhea, particularly in hospitalized patients. Following antibiotic treatment, the native flora (gut microbiome) of the patient is eradicated, leaving C. difficile spores free to colonize the gut and cause persistent infection. The human gut microbiome provides some resistance to C. difficile; however, the specific intestinal bacteria that do this and their mechanisms of resistance are still unclear. Here, we are modeling microbial dynamics between beneficial and pathogenic cells involving C. difficile in artificial microniches. Using a microfluidic-based technique, thousands of nanoliter-sized polydimethylsiloxane (PDMS)-based microspheres are generated to encapsulate bacterial cells including Escherichia coli, Staphylococcus aureus and C. difficile. PDMS provides mechanical and chemical transport properties, which enable the long-term study of microbiome dynamics such as C. difficile persistence in the mock community. After antibiotic treatment of the nanocultures, the nanocultures are imaged for a 24-hour period, capturing the microbial growth dynamics in the presence or absence of antibiotics effective against C. difficile. This platform has potential to investigate microbial pathogenesis by monitoring C. difficile sporulation and growth based on microenvironmental changes as occurring in the human microbiome.

Author: S. Davidson, H. Usman, T. H. Niepa;
Block: 1University of Pittsburgh, Pittsburgh, PA, 2Harry S Truman College, Chicago, IL.
Adhesion is a process that allows bacteria to attach to other cells and surfaces. It is an important step in the colonization and biofilm formation of a new host or environment and can contribute to bacterial pathogenesis. The aims of this *in vitro* study were to investigate a) the impact of bacterial binding on the viable counts in the surrounding suspension, b) the bacterial binding to wound dressing materials at different incubation times and c) the role of the surrounding environment on bacterial binding. The following hydrophobic dressing materials were evaluated for bacterial binding: a silicone film (SF, contact angle (CA) 124°), a polyurethane film (PUF, CA 105°) and a dialkyl carbamoyl chloride (DACC, CA 107°) coated dressing fabric. To evaluate the impact of the test medium, the dressing materials were placed on top of two different suspensions containing 10⁵ colony forming units (CFU) of *Staphylococcus aureus* and *Pseudomonas aeruginosa* either in Phosphate Buffered Saline (PBS) or Simulated Wound Fluid (SWF, 50% fetal calf serum and 50% Maximum Recovery Diluent) and incubated for 1 and 24 hours. Suspensions without any material served as controls. Bacteria adhering to the materials and remaining in suspension were quantified by serial dilution and plating. The results show that the quantity of viable bacteria in the surrounding suspension was barely affected by any of the tested materials, relative to the growth control. The only exception was for *P. aeruginosa* suspended in PBS, where a reduction of about 0.5 log units was observed in the suspension after 1 hour incubation. The reduction of viable counts in the suspension seemed to be affected by the growth dynamics of the bacteria in the suspension, rather than adhesion to the hydrophobic surface of the tested materials. Overall, the bacterial binding to the dressing materials did not significantly reduce the viable counts in the surrounding environment, nor were the materials capable of inhibiting growth over time. Furthermore, the results show that the test medium composition, the incubation time and type of bacteria seem to have a larger impact on the binding and proliferation than the dressing material itself. Proteins, as contained in the SWF, will also bind to the materials and may thereby alter the surface properties leading to a change in hydrophobicity and affinity towards bacterial binding. In addition, larger bacterial adhesion was observed with the longer incubation time, irrespective of the dressing material. This can be explained in part by more bacteria adhering to the surface over time but also by the proliferation of bacteria on the surface. The type of bacteria has very little impact on the binding at 1 hour. However, after 24 hours, *P. aeruginosa* was found to bind significantly more to all materials compared to *S. aureus*, which most probably is a result of the former’s higher capability of growing in the different test media.
**Background:** Biofilms are recognized across many industries as a costly, damaging, and potentially hazardous occurrence whose presence is often difficult to detect and treat. Although the important differences between biofilm-associated bacteria and planktonic bacteria are increasingly acknowledged by both industry and regulatory bodies, there are many applications that rely on monitoring of planktonic bacteria as a measure of the health of the system. This poses a challenge for innovative biofilm prevention or biofilm remediation technologies: how can the efficacy of products targeting biofilm be demonstrated most effectively, both in the lab and in the field?  

**Methods:** We use both (modified) standard lab-scale tests and tests adapted for possible use in the field, including the single-tube method, chlorine demand tests, as well as tests that probe material properties like heat transfer efficiency or flow.  

**Results:** We will discuss the ability of various tests to capture the level of biofilm present on a given surface (reported as CFU/surface area), with particular interest paid to how relevant that particular test may be to industrial applications.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 066

**Abstract**

**Topic:** From Planktonic To Biofilm and Back

**Abstract Title:** Biofilm Formation by the Amphibian Chytrid, *Batrachochytrium Dendrobatidis*: Biofilm Architecture and Resistance

**Author Block:** S. Silva¹, L. Atkins², M. San Francisco¹; ¹Texas Tech University, Lubbock, TX, ²Baylor College of Medicine, Houston, TX.

**Background:** *Batrachochytrium dendrobatidis* (*Bd*) causes chytridiomycosis, a lethal amphibian skin infection. The fungus transitions from the flagellated motile zoospore to a sessile reproductive sporangium with rhizoids and may adhere to surfaces as a film. To study how the fungus survives in the absence of the host and based on our observations in growth media in the laboratory, we hypothesized that the chytrid forms a biofilm. Biofilms formed by microorganisms take multiple forms and serve a variety of purposes. **Methods:** In vitro biofilms were characterized using the crystal violet assays, light and electron microscopy, and gas chromatography/mass spectrometry. Differential expression of genes: zinc-responsive activator, polysaccharide synthase, alcohol dehydrogenase, chitin synthase were carried out using Real-time PCR. The resistance of biofilms, zoospores, and sporangia to a variety of treatments: high temperature, pH, sodium chloride, amphotericin B, and active ingredients of common spices: curcumin, 6-gingerol, and allicin was tested by measuring the cell viability using XTT ((2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) reduction. **Results and Discussion:** Kinetic analyses of biofilms showed the density-dependent biofilm formation and biomass increased over the time. Mature *Bd* biofilms displayed complex, a heterogeneous structure mainly consisting of different developmental stages of sporangia. Microcolonies embedded in a thin layer of extracellular matrix were clear in scanning electron and fluorescence microscopy. The glycosyl composition analysis of exopolymeric material revealed the composition of the matrix consisting of five relatively abundant sugars: ribose, xylose, mannose, galactose, and glucose. Overall, the expression levels of specific genes were higher in zoospores except chitin synthases were upregulated in biofilm and sporangia. This was expected as chitin synthases involved in the cell wall production when wall-less zoospores transition to sporangia and then to biofilms. Overall, biofilm-associated cells and sporangia showed higher resistant to high temperature and pH, and antifungal agents tested. Biofilm-associated cells were significantly highly resistant to temperature (28°C), pH (pH=9), and allicin (3.375 µg/ml). All cell types showed similar susceptibility to sodium chloride. Combination of all three phytochemicals at their MICs and MIC/2 equally affected all three cells types than individual chemicals. **Conclusion:** Our results strongly supported our hypothesis that *Bd* can form a biofilm. We propose that *Bd* biofilm formation may secure the survivability of the fungus in the absence of host by attaching to solid surfaces in ponds and streams while facilitating its group dispersal.
**Abstract Topic:** From Planktonic To Biofilm and Back

**Abstract Title:** Ethanol Induces Biofilm Behaviors in *Pseudomonas aeruginosa*

**Author Block:**
K. A. Lewis, C. E. Harty, S. Kuchma, G. O'Toole, D. A. Hogan; Dartmouth College, Hanover, NH.

*Pseudomonas aeruginosa* causes infections in compromised individuals, burn wounds, and the lungs of individuals with cystic fibrosis (CF). In clinical settings, such as the CF lung, *P. aeruginosa* encounters other species of bacteria and fungi that produce bioactive metabolites including ethanol (EtOH). We show that when exposed to 1% EtOH, *Pseudomonas* induces a rapid and sustained induction of biofilm pathways even in planktonic cells. In medium with EtOH, cells have higher c-di-GMP (cdG) levels (2.5- and 1.9-fold (p<0.0001) at one and 16 hours, respectively) a second messenger known to negative regulate motility and promote biofilm formation. EtOH reduces WT *Pseudomonas* motility in a swim agar assay by 30% (p<0.0001), and a 2-fold increase in flagellar reversal rates is also observed (p<0.01). A genetic screen identified genes involved in the EtOH-induced repression of flagellar motility. We found that two diguanylate cyclases, SadC and GcbA, involved in the initial stages of biofilm formation and surface response, both participated in motility repression by EtOH and a ∆sadC∆gcbA mutant shows a greatly reduced stimulation of cdG in response to EtOH. We also found that the motility repression was dependent on two cdG effector proteins, FlgZ and PilZ. The reduction of swimming motility in response to EtOH not only requires the stator complex, MotAB, but occurs via inhibiting proper flagellar function that rapidly transitions the cells to being immobile instead of motile. We have previously published that EtOH stimulates biofilm matrix production via WspR and we found that SadC, but not GcbA also participates in this response. In addition, we have identified upstream components, and domains within those components, that are necessary for the stimulation of cdG levels and the reduction in motility. Together, these data point to a complex network in which EtOH primes planktonic cells for the switch from a motile to a sessile lifestyle thereby promoting co-colonization of *P. aeruginosa* with EtOH-producing microbes. These data may lead to new insights into how mixed species communities in the CF lung lead to worse patient outcomes. The data may also point to the relevance of EtOH in biofilm initiation and maturation in disease.
Grapping Hooks Involved in Biofilm Development

The Type IV Pilus Assembly ATPase PilB Functions in a Signal Transduction Pathway to Regulate Exopolysaccharide Production in *Myxococcus Xanthus*

W. P. Black, L. Wang, X. Jing, F. D. Schubot, Z. Yang;
Virginia Tech, Biological Sciences, Blacksburg, VA.

*Myxococcus xanthus* possesses a form of surface motility powered by the retraction of the type IV pilus (T4P). Additionally, exopolysaccharide (EPS), the major constituent of bacterial biofilms, is required for this T4P-mediated motility in *M. xanthus* as the putative trigger of T4P retraction. The results here demonstrate that the T4P assembly ATPase PilB functions as an intermediary in the EPS regulatory pathway composed of the T4P upstream of the Dif signaling proteins in *M. xanthus*. A suppressor screen isolated a * pilB* mutation that restored EPS production to a T4P- mutant. An additional PilB mutant variant, which is deficient in ATP hydrolysis and T4P assembly, supports EPS production without the T4P, indicating PilB can regulate EPS production independently of its function in T4P assembly. Further analysis confirms that PilB functions downstream of the T4P filament but upstream of the Dif proteins. *In vitro* studies suggest that the nucleotide-free form of PilB assumes the active signaling conformation in EPS regulation. Since *M. xanthus* PilB possesses conserved motifs with high affinity for c-di-GMP binding, the findings here suggest that c-di-GMP can regulate both motility and biofilm formation through a single effector in this surface-motile bacterium.
The surfaces of bacteria are covered with extracellular appendages helping them explore and physically interact with their surroundings. These thin structures are actively involved in a variety of behaviors such as swimming, cell-to-cell interaction, surface exploration and attachment. Visualization of these nanometric filaments is challenging and relies mainly on labeling. However, labeling protocols are invasive and often require mutations within filament subunits and visualization is subject to bleaching. Therefore, to observe dynamics of unperturbed extracellular structures, there is a need to develop a label free and more sensitive microscopy technique. Here we demonstrate label-free dynamic visualization of these filaments in live cells by interferometric scattering microscopy (iSCAT). iSCAT is more sensitive than any other "brightfield-like" method as it uses interference to highlight the photons scattered by small objects, for example pili. We adapted this method to the visualization of living cells by performing various modification decreasing its phototoxicity. With iSCAT, we could visualize multiple structures involved in Pseudomonas aeruginosa biofilm formation. For example, we obtained high temporal resolution visualization of monotrichous helical flagella, from which we could observe its dimension as well as a translation of the patterns over time due to rotation. Furthermore, we could reveal type IV pili, which are micrometer-long retractile filaments only about 5 nanometers thick. TFP enable P. aeruginosa to explore surfaces through twitching motility during early biofilm formation. We could use iSCAT to visualize the dynamics of these filaments at high spatial and temporal resolutions. Using these visualizations, we could probe how TFP coordinate extension and retraction to optimize displacements onto the surface. Altogether, we demonstrate that iSCAT is a powerful microscopy technique to study extracellular structures of bacteria. We hope to expand the use of iSCAT to the visualization of the many other surface structures of bacteria.
Grapping Hooks Involved in Biofilm Development

Roles, Mechanisms and Regulation of Bacterial Non Fimbrial Adhesins required for Colonisation and Biofilm Formation

J. J. Paxman1, A. W. Lo2, S. Panjikar3, M. Kuiper4, C. Luan5, K. M. Peters6, G. C. Ulett7, T. Wang8, M. A. Schembri9, B. Heras1; 1La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, AUSTRALIA, 2Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, AUSTRALIA, 3Macromolecular Crystallography, Australian Synchrotron, Clayton, VIC, AUSTRALIA, 4Molecular & Materials Modelling group Data61, CSIRO, Melbourne, VIC, AUSTRALIA, 5High Throughput Analysis Laboratory, Northwestern University, Chicago, IL, 6School of Medical Science and Menzies Health Institute, Griffith University, Gold Coast, QLD, AUSTRALIA.

Background: Bacterial surface proteins termed adhesins play essential roles in promoting host colonisation and biofilm associated chronic infections. These adhesins include the well characterized >1 µm fimbrial adhesins along with their more abundant smaller ~10 nm non-fimbrial adhesin counterparts. The largest group of non-fimbrial adhesins include the autotransporters which overall are the most abundant outer membrane proteins in Gram-negative bacteria. Despite their prevalence and clear roles in host colonisation, there remains only one example of a molecular mechanism used by these highly prevalent adhesins to facilitate adherence and biofilm formation. Self-association of the Antigen43a adhesin between neighboring uropathogenic E.coli (UPEC) cells was found to promote bacterial aggregation and biofilm phenotypes.

Methods: We have employed a multidisciplinary approach that combines X-ray crystallography, analytical ultracentrifugation, ligand screening, binding assays, mutagenesis, mass spectrometry along with microbiology techniques, to further uncover new mechanisms of action for diverse autotransporter adhesins.

Results: Our investigation has now revealed a second and completely new molecular function for an autotransporter adhesin. Our first X-ray crystal structure of UpaB from UPEC was found to incorporate unique features that allows it to interact with both human fibronectin and glycosaminoglycans, to promote direct colonisation of UPEC to the urinary tract epithelium. Furthermore, our latest research has finally revealed the molecular mechanism for yet another autotransporter adhesin TibA from enterotoxigenic E. coli (ETEC). Unusually, TibA had been found to be glycosylated by its own glycosyltransferase TibC. Further, TibA is a multifunctional autotransporter, that can promote both bacterial aggregation/biofilm formation in addition to binding/invasion of the intestinal epithelium. Our findings, which include crystal structures of TibA in both its glycosylated and unglycosylated forms, now show how TibA can mediate these two distinct mechanisms of action via regulation by glycosylation.

Discussion: Overall, we have now elucidated in molecular detail two distinct mechanisms used by autotransporter adhesins to promote colonisation and biofilm formation on host surfaces. Furthermore, we have uncovered a new regulation system used to switch the function of autotransporter adhesins at the level of post-translational modification. These mechanisms and forms of regulation are likely to be found throughout other bacteria and provide missing details on bacterial adherence and biofilm formation that could now be used to develop new types of therapeutics.
Background/Purpose: Dental caries is a polymicrobial infectious disease that affects the tooth. The disturbance of host-microbe homeostasis is the central factor initiating caries, there is a shift in microbial balance of the biofilm resulting in increased proportions of acidogenic and aciduric bacteria. It is a costly disease and a major problem to health service providers. The study isolates and identifies some microorganisms found in carious lesions and compares same with caries-free subjects in Enugu, Nigeria. Materials and Methods: The study was carried out in the Microbiology laboratory of University of Nigeria Teaching Hospital Ituku/Ozalla Enugu. A total of 336 samples comprising of 211 patients with caries and 125 caries-free subjects were randomly collected from patients attending some dental clinics in Enugu metropolis. Cheesy portion of carious lesion was excavated with sterile excavator and then swabbed and processed. Standard cultures and biochemical techniques were used for isolation and identification. Standardized questionnaires were used to record the demographic data and other risk factors. Statistical analysis was done using Graph prism Version 6. Results: All samples yielded microorganisms. A total of 635 and 254 microorganisms were isolated from carious lesions and caries-free subjects respectively. Of the 211, 207 (98.1%) were polymicrobial while 4 samples were monomicrobial. Lactobacillus spp (141, 66.8%) was the most predominant pathogen followed by Streptococcus mutans (104, 49.2%) Veillonella spp. 46(21.8%), Candida albicans 38(18.0%), Peptostreptococcus anaerobius 38(18.0%), Actinomyces naeslundii 34(16.1%) . Streptococcus sanguis ranked highest (41, 32.8%) in caries-free subjects. The prevalence of caries was higher in females, though the difference was not statistically significant. Age was found to be a significant factor. A high prevalence occurred within the first 31 years with a cluster of cases between 16-31 years of age with prevalence tapering with advancing age. Conclusion: Finding highlights the polymicrobial nature of carious lesion. There is need to consider the diversity of these microorganism in prevention and treatment of patients. Public awareness, introduction of oral health education in schools and use of good fluoridated tooth paste are advocated. Keywords: Biofilm, Dental caries, Lactobacillus spp, Streptococcus mutans.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 072  
**Abstract Topic:** Host Microbe Biofilms  
**Abstract Title:** The Role of Biofilm and Resident Bacterial Populations of Human Jawbone in Dental Implant Outcomes  
**Author Block:** H. Hu¹, S. Nelson², V. Dzumhur¹, G. Thomas², K. Vickery¹; ¹Faculty of Medicine and Health Sciences, Macquarie University, North Ryde, NSW, AUSTRALIA, ²Department of Obstetrics and Gynaecology, University of Sydney, Camperdown, NSW, AUSTRALIA.  
**Background:** Dental implants are inert titanium fixtures implanted into human jawbone, where the prevailing science insists on sustained bone sterility both before and after the removal of biofilm diseased teeth. This spontaneous bone cure and preserved sterility is based on planktonic bacterial disease aetiology and methodology not suited for biofilms. This study clinically audited the prevailing science of sterile bone theory and spontaneous cure using molecular and biofilm detection method in samples taken from two stage dental implant treatment.  
**Methods:** 222 clinical samples from 68 patients were collected as bone, granulation and serous effusion from extraction sockets, non-debrided and surgically debrided apparently healed bone, failed fixtures and pristine sites of congenital tooth absence. Scanning electron microscopy (SEM) was used to visually confirm bacterial biofilm presence. Bacterial population was determined by DNA extraction and 16s rRNA amplicon pyrosequencing.  
**Results:** SEM confirmed deeply invasive pathogenic bacterial biofilms persisting in diffuse osteosclerotic periapical bone 9 months post extraction and in long term osteolytic and osteosclerotic bone. There was universal bacterial presence independent of health or disease status or healing time after disturbance. We report the curation and multivariate statistical ordination of a 20 phyla human jawbone microbiome. Multivariate statistical ordination revealed ecological separation of different clinical groups. Native, pristine sites of congenital tooth absence provided benchmarked homeostasis as a health control. Surgical debridement beyond sclerosis progresses the clinical healing closer to the health controls. Surgically debrided groups ordinate closer to ecological health control with increased community diversity, suggesting surgical debridement beyond sclerosis may be responsible for microbial ecological shift and provides a mechanism for restoration of pre-disturbance condition - ecological resilience. It creates a "cleaner" healthy balanced microbial ecosystem, improves bone quality and osseointegration outcome, contributes to implant success. Non-debrided ordinated groups have significantly different genera in their compositional structure to the health microbiota. These groups ordinate closer to disease with lower community diversity, ecosystem stability and osteolytic/osteosclerotic bone quality correlating with biofilm impaired healing.  
**Conclusions:** Dental implant failures are biofilm infections of the biomedical surface accompanied by chronic osteomyelitis. Sterile bone theory is invalid and spontaneous cure does not happen. Pathology in the dental implant bone bed is consistent with the expectations of biofilm science where a commensal (resident) bacterial biofilm population is subject to colonisation following disturbance and population shift.
Abstract Title: Influence of Bacterial Biofilm on the Corrosive Processes in Electronic Equipment

Author Block: I. Dzieciuch, M. Putnam; SPAWAR, San Diego, CA.

Humidity is known to degrade Navy ship electronic equipment, especially in hot moist environments. If left untreated, it can cause significant and permanent damage. Even rigorous inspection and frequent clean-up would not prevent further equipment contamination and degradation because of the constant presence of favorable growth conditions for many microorganisms.

Generally, relative humidity levels of less than 60% will inhibit corrosion in electronic equipment, but because NAVY electronics often operate in hot and humid environments, prevention via dehumidification is not always possible. Currently, there is no defined research that fully describes key mechanisms which cause electronics and its coating degradation. The corrosive action of most bacteria is mainly developed through (i) mycelium adherence to the metal plates, (ii) facilitation the formation of pitting areas, (iii) production of organic acids such as citric, iso-citric, cis-aconitic, alpha-ketoglutaric, which are corrosive to electronic equipment and its components. Our approach studies corrosive action in electronic equipment: circuit-board, wires and connections that are exposed in the humid environment that gets worse during condensation. In our new approach the technical task is built on work with the bacterial communities in public areas, bacterial genetics, bioinformatics, biostatistics and Scanning Electron Microscopy (SEM) of corroded circuit boards. Based on these methods, we collect and examine environmental samples from biofilms of the corroded and non-corroded sites, where bacterial contamination of electronic equipment, such as machine racks and shore boats, is an ongoing concern. Sample collection and sample analysis is focused on addressing the key questions identified above through the following tasks: (1) laboratory sample processing and evaluation under scanning electron microscopy (2) initial sequencing and data evaluation; (d) bioinformatics and data analysis. Preliminary results from scanning electron microscopy (SEM) have revealed that metal particulates and alloys in corroded samples consists mostly of Tin (< 40%), Silicon (< 4%), Sulfur (< 1%), Aluminum (< 2%), Magnesium (< 2%), Copper (< 1%), Bromine (< 2%), Barium (< 1%) and Iron (< 2%) elements. We have also performed X 12000 magnification of the same sites and that proved existence of undisrupted biofilm organelles and crystal structures (Fig 1). Non-corrosion sites have revealed high presence of copper (< 47%); other metals remain at the comparable level as on the samples with corrosion. We have performed X 1000 magnification on the non-corroded at the sites and have documented formation of copper crystals.
Persistent polymicrobial infections are common in individuals with chronic respiratory disease, including chronic rhinosinusitis (CRS), chronic obstructive pulmonary disease and Cystic Fibrosis (CF). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most frequently isolated bacterial pathogens from CF children and adults, respectively. *P. aeruginosa* acquisition correlates with seasonal respiratory virus infections, and CF patients experience severe exacerbations during viral coinfection. We observed *P. aeruginosa* biofilm growth on CF airway epithelial cells (AECs) is enhanced during respiratory virus coinfection and hypothesized virus infection also alters microbial community dynamics in the CF airways. Using co-culture systems, we observed that cultured individually with CF AECs, *P. aeruginosa* and *S. aureus* each exhibit enhanced biofilm growth on RSV-infected cells. However, when *S. aureus* and *P. aeruginosa* are co-cultured, RSV co-infection leads to a dramatic reduction in *S. aureus*. We observed *P. aeruginosa* exhibits enhanced production of the antimicrobial pyocyanin (PYO) during RSV co-infection. Exogenous PYO addition at concentrations observed during *P. aeruginosa*-virus co-infection decreases *S. aureus* biofilms on CF AECs. When co-cultured with a *P. aeruginosa* PYO mutant, *S. aureus* populations did not decrease during virus co-infection. We are currently investigating if this outcome is mediated by specific *P. aeruginosa* and host interferon-mediated antimicrobial mechanisms activated during respiratory virus infection. We observe RSV infection increases expression of the host interferon stimulated gene *IDO1*, resulting in elevated levels of the secondary metabolite kynurenine. *IDO1* controls tryptophan metabolism during infection via the kynurenine pathway and has antimicrobial effects towards a range of pathogens, and *P. aeruginosa* utilizes kynurenine to produce the quorum-sensing signal PQS, which regulates PYO production. Over-expressing *IDO1* in CF AECs increases apical kynurenine, and co-culture on *IDO1* over-expressing cells leads to enhanced PYO production and a decline in *S. aureus* populations, as observed during virus co-infection. Dual host-pathogen RNA sequencing to evaluate transcriptomic changes in CF AECs, *P. aeruginosa* and *S. aureus* during virus co-infection confirmed that in CF AECs, expression of *IDO1* and SLC6A14, encoding the kynurenine transporter hATB, is increased during co-infection with all three pathogens. *P. aeruginosa* genes *pqsABCD* and *pceM* also show increased expression, supporting our hypothesis that these pathways mediate antimicrobial activity during polymicrobial infections. This work will further our understanding of how virus coinfection alters interactions between bacterial pathogens and shapes host-associated polymicrobial communities.
**Abstract Topic:** Host Microbe Biofilms

**Abstract Title:** Examining the Effect of *Pseudomonas aeruginosa* Biofilms in Cystic Fibrosis

**Author:** D. Brown, V. V. Phelan;

**Block:** University of Colorado at Denver, Aurora, CO.

**Background:** In Cystic Fibrosis (CF), the lung microbiome becomes dysbiotic because of a mutation in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a chloride channel, leading to bronchiectasis, inflammation and oxidative stress. A hallmark of CF disease pathology is chronic *Pseudomonas aeruginosa* infections characterized by bacterial growth in biofilms. Biofilms provide protection from innate immune defense, antibiotics, and facilitate production of high titer virulence factors. Virulence factors can be directly toxic to the host or can act indirectly to influence innate immunity. For example, virulence factors such as Cif protein can exacerbate CFTR trafficking issues and modulate host-derived inflammatory mediators (oxylipins), leading to chronic inflammation. The objective of the present study was to determine the effect of *P. aeruginosa* biofilms on activation of innate immunity in CF host bronchial epithelium (CFBE).

**Methods:** CFBE41o− cells, homozygous for the ΔF508 mutation, and CFBE41o− pCEP-WT cells, complemented with WT-CFTR, were provided by Dr. Dieter Gruenert at the University of California San Francisco. *P. aeruginosa* PA14 and a Cif deletion mutant (ΔCif) were provided by Dr. George O’Toole at Dartmouth University. In all studies, CFBE were grown as monolayers using standard cell culture techniques. Separately, *P. aeruginosa* biofilms were grown in artificial sputum media (SCFM2) and cell-free supernatant was collected. Then, CFBE monolayers were treated with biofilm supernatants. Following co-culture, culture medium and cell lysates were collected and analyzed. Non-targeted and targeted metabolomics were performed on biofilm supernatants and culture medium, respectively, to reveal the suite of virulence factors produced and their subsequent effect on pro-inflammatory oxylipin profiles. qRT-PCR and Western blotting were used to determine effect of co-culture on oxylipin metabolic gene and enzyme expression. Finally, Redox Western blots were performed to look at the effect of biofilm supernatant treatment on compartmental redox balance. **Results:** CFBE41o− CFBE challenged with *P. aeruginosa* PA14 biofilm supernatants display lower oxylipin metabolic gene expression, protein expression, and a lower titer of pro-inflammatory oxylipins compared to CFBE41o− pCEP/WT CFBE. Additionally, increases in mitochondrial, cytosolic, and membrane oxidation were observed following treatment. When CFBE41o− CFBE was challenged with ΔCif-PA14 biofilm supernatants, levels of innate immune activation were increased and oxidative stress was decreased compared to WT-PA14 biofilm supernatant treatment. **Conclusions:** Cif protein and other enzymatic virulence factors represent a key link between chronic infections and the damaging inflammatory and oxidative environment observed in cystic fibrosis.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 077  
**Abstract Topic:** Regulation of Biofilm Development  
**Abstract Title:** Incorporation of *Vitreoscilla* Hemoglobin Gene (*vgb*) Mitigates Biofilm Formation in *Bacillus subtilis* DK1042  
**Author Block:** R. Vyas, M. Pandya, N. Gattupalli; The M S University of Baroda, Vadodara, INDIA.

**Background:** *Bacillus subtilis* has been considered a model organism to study formation of complex multicellular structures called biofilm. Impaired respiration is one of the environmental signals triggering biofilm formation in *B. subtilis*. *Vitreoscilla* hemoglobin (VHb) is known to supply oxygen to respiratory chain and hence improves aerobic growth of variety of bacteria including *Bacillus* spp. Although VHb improves respiration, very little efforts have been made in elucidating its effect on biofilm formation. Here, we report the effect of incorporation of VHb on biofilm formation in *B. subtilis* DK1042 in different conditions. **Methods:** *B. subtilis* DK1042 was genetically modified to develop two Integrants NRM1113 and NRM1114 containing *vgb-gfp* operon under 2 and 5 copies of P43 promoters, respectively, at *amyE* locus by double cross over events. Promoter strength was characterized by measuring the GFP fluorescence of both the integrants. Effect of VHb on biofilm formation by integrants and wildtype (WT) was assessed on both solid and pellicle biofilm in lysogeny broth (LB) and LB supplemented with 1% glyceol and 0.1 mM manganese (LBGM). Pellicle biofilms were assayed by crystal violet staining method. Solid biofilms were monitored for the development of complex architecture. Sporulation efficiency was determined by plate count method. Effect of VHb was also monitored by exposing the cells to 6% salt stress in solid LB medium. **Results: Time dependent analysis of cell growth and fluorescence of *B. subtilis* integrants revealed that integrant NRM1114 having higher copies of P43 promoter upstream of *vgb-gfp* operon showed two fold increase in fluorescence/OD600 as compared to NRM1113 in both minimal medium and Luria Bertani medium. Moreover, production of brown pigment upon sporulation was reduced in integrants as compared to WT in minimal medium. Biofilm formation was significantly reduced on LB and LBGM in integrants as compared to WT. Integration of *vgb* also decreased biofilm-associated sporulation in both the integrants as compared to WT on LB and LBGM agar as well as LB agar supplemented with 6% NaCl. **Conclusion:** Biofilm formation is an adaptation by microorganisms to withstand variety of environmental insults. Reduced biofilm formation and sporulation by both the integrants harboring *vgb* suggests better capacity to survive adverse environment. VHb is known to improve antioxidant status of the host cells. Thus, improved aerobic metabolism and detoxification of ROS by constitutive expression of VHb together contribute to maintaining the vegetative state longer than the WT. Incorporation of VHb in other *Bacillus* spp. may have similar beneficial effects during their sessile lifestyle in rhizosphere that may enhance their performance as Plant Growth Promoting Rhizobacteria (PGPR). It would be interesting to understand the molecular processes involved in VHb modulated biofilm formation.
**Regulation of Biofilm Development**

**Potential of Marine Actinomycetes for the Reduction of Biofilm Formation**

**R. M. Magsino**, G. R. Dedees; 1Far Eastern University, Manila, PHILIPPINES, 2University of Santo Tomas, Manila, PHILIPPINES.

**Background:** Biofilms are complex communities of microorganisms embedded in extrapolymeric substances (EPS) matrix. Due to their inherent resistance to antimicrobial agents and their ability to form on a variety of surfaces, biofilm formation poses a serious problem to the industry, marine transportation, public health, and medicine. Although majority of clinically useful drugs have been obtained from terrestrial natural sources, recently marine actinomycetes are being tapped as the new emerging and underdeveloped source of novel compounds with promising pharmaceutical potentials. Marine bacteria produce antibacterial compounds that may inhibit human pathogens and detrimental biofilm formation. Up to date, few studies have been reported on the isolation of actinomycetes from marine environments most especially in mangrove ecosystem. **Material/methods:** In this study, nine isolates of actinomycetes were isolated from soil and sediment samples in a mangrove swamp. Morphological and biochemical characterizations have been performed resulting to five different actinomycete isolates coded as BA01, BB02, CB02, CC03, and CG07, respectively. Each isolate was tested for antibacterial activity against the known biofilm forming bacteria; *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Salmonella typhimurium* (clinical isolate), and *Klebsiella pneumoniae* (clinical isolate). **Results:** Isolates BA01 and BB02 exhibited activity against *B. subtilis* and *P. aeruginosa*. The rest of the isolates (CB02, CC03, and CG07) did not manifest any effects on the test bacteria. Each of these 5 isolates was mass produced in starch casein broth, using ethyl acetate as the extracting solvent and then further tested for antibacterial activity using the disc diffusion assay. Ethyl acetate extract showed partially active to active activity against *B. subtilis* and *P. aeruginosa*. Biofilm inhibition assay were done using the 96-well microtitre plate. All of the isolates were able to inhibit the biofilm formation of the test organisms but greater inhibitions were observed in *E. coli*, and *B. subtilis* most especially *P. aeruginosa*. **Conclusions:** These five marine Actinomycetes can be utilized further for the discovery of pharmaceutically important compounds.
Under nutrient-deprived conditions bacteria can grow as biofilms. These structures provide protection against physical stresses and allow slowed growth and metabolism, which extends survival. In open environment such as water, *E. coli* O157:H7 respond to the phosphate (Pi) starvation by inducing the Pho regulon controlled by PhoBR. The Pst (phosphate specific transport) system serves as a sensor of the extracellular Pi concentration. In the *pst* mutant, the regulator PhoB is constitutively activated and the expression of genes from the Pho regulon is modulated. O157:H7 *E. coli* strain EDL933 grown in low Pi condition and its *pst* mutant grown in excess of Pi displayed an increased ability to auto-agglutinate and to form biofilm. Analysis of double *pst* phoB mutant indicated that the increased biofilm and auto-agglutination phenotype were dependent on *phoB*. The goal was to identify specific *E. coli* factors that are involved in the increase of biofilm formation in response to phosphate starvation. 5118 transposon mutants derived from the *pst* mutant of EDL933 were screened to isolate 30 mutants defective in auto-agglutination and biofilm formation. Transposon insertion sites were identified by high-throughput sequencing. The transcriptome profiles of the *pst* mutant and its parental strain were also compared to each other. O157 LPS modifications were analysed by SDS-PAGE and MALDI-TOF. For several auto-agglutination and biofilm defective mutants, the transposon was inserted in genes *waaF*, *waaC*, *waaD*, *waaE*, *waaG* and *waaQ* that are involved in the synthesis of the lipopolysaccharide (LPS) core. Global transcriptomic studies of *pst* mutant revealed that genes involved in the biosynthesis and export of LPS were down regulated. Some of these genes contain Pho box binding sites. LPS analysis by SDS PAGE had shown that the O chain was absent and lower bands corresponding to rough types LPS were observed. Absence of O antigen units was confirmed by immunoblotting and MALDI analysis. The main LPS molecular species corresponded to R3 core. Thus the LPS core of *pst* mutant is truncated and lacks the O157 antigen. Our results suggest that truncated LPS core plays a role in the biofilm and auto-agglutination phenotype of *pst* mutant. Although *waaH* gene, responsible of glucuronic acid (GlcUA) core modification of *E. coli* K12 strain, was highly upregulated in *pst* mutant, addition of GlcUA on LPS was not detected. Nonetheless, *waaH* mutant in low Pi and *pst waaH* double mutant displayed a decreased ability to form biofilm suggesting a role for this glycosyltransferase in biofilm formation in response to Pi starvation. In response to low Pi environment, *E. coli* O157 outer membrane undergoes several modifications controlled by the Pho regulon leading to increase the potential of biofilm formation. The biofilm ability of *E. coli* O157 may play a role in its transmission, persistence and virulence.
Abstract

**Topic:** Regulation of Biofilm Development

**Title:** PPAD Modulates Porphyromonas gingivalis Biofilm Formation via Citrullination of T9SS-Secreted Proteins

**Author Block:** D. M. Vermilyea, G. K. Ottenberg, M. E. Davey;
University of Florida, Gainesville, FL.

**Background:** Porphyromonas gingivalis is a Gram-negative anaerobe that persists within the subgingival biofilm adjacent to the epithelium in the human oral cavity. This bacterium is asaccharolytic, but highly proteolytic; it utilizes protein substrates as a main source for energy production and growth. A type IX secretion system (T9SS) has been shown to be essential for the secretion of a variety of proteases and protein modifying enzymes. Included in this repertoire are the arginine and lysine gingipains as well as a unique peptidylarginine deiminase (PPAD). PPAD is an enzyme that converts positively charged arginine residues within proteins to neutral citrulline residues, which can subsequently affect the conformation and function of proteins. Although the removal of free L-arginine from the environment has been shown to alter P. gingivalis physiology and inhibit biofilm formation, the impact of removing peptidylarginine via citrullination is not known. Here we report that the increased presence of peptidylarginine in a PPAD deletion mutant (Δ8820) enhances biofilm formation.

**Methods:** Biofilm formation by the wild type (strain 381) and Δ8820 was measured by staining with safranin and with the LIVE/DEAD BacLight Bacterial Viability Kit. PPAD and gingipain enzymatic activities were measured using colorimetric assays. Fimbriae and adhesin protein levels were assessed under different growth conditions using western blot analysis. Mass spectrometry was performed on cell lysates to identify citrullinated proteins. 381 and Δ8820 cells from colony biofilms grown on plates were observed by transmission electron microscopy (TEM).

**Results:** During early stages of biofilm development, more Δ8820 cells attached to surfaces compared to the parent strain 381. Mature Δ8820 biofilms were comprised of a higher density of cells, larger microcolonies, and less void space. Gingipain-derived adhesin proteins were predicted by mass spectrometry to be citrullinated and citrullination of these proteins by strain 381 was confirmed in vitro. Furthermore, Δ8820 biofilms contained more gingipain-derived adhesin proteins than 381 biofilms. Surprisingly, Δ8820 colony biofilms produced extracellular fibers that looked to form a matrix when observed by TEM.

**Conclusions:** The enhanced biofilm phenotype of Δ8820 is due, in part, to the lack of citrullination or retention of arginine residues within T9SS-secreted proteins known to play a role in attachment and biofilm formation, including gingipain-derived adhesin proteins. Overall, our data indicates that citrullination of T9SS cargo proteins modulates attachment and biofilm development in P. gingivalis biofilm.
Regulation of Biofilm Development

Serine Hydroxymethyltransferase ShrA (PA2444) Controls Rugose Small-Colony Variant Formation in *Pseudomonas aeruginosa*

T. K. Wood, M. Pu, L. Sheng, S. Song, T. Gong;
Pennsylvania State Univ., University Park, PA.

*Pseudomonas aeruginosa* causes many biofilm infections, and the rugose small-colony variants (RSCVs) of this bacterium are important for infection. We found here that inactivation of PA2444, which we determined to be a serine hydroxymethyltransferase (SHMT), leads to the RSCV phenotype of *P. aeruginosa* PA14. In addition, loss of PA2444 increases biofilm formation by two orders of magnitude, increases exopolysaccharide by 45-fold, and abolishes swarming. The RSCV phenotype is related to higher cyclic diguanylate concentrations due to increased activity of the Wsp chemosensory system, including diguanulate cyclase WspR. By characterizing the PA2444 enzyme *in vitro*, we determined the physiological function of PA2444 protein by relating it to S-adenosylmethionine (SAM) concentrations and methylation of a membrane bound methyl-accepting chemotaxis protein WspA. A transcriptome analysis also revealed PA2444 is related to the redox state of the cells, and the altered redox state was demonstrated by an increase in the intracellular NADH/NAD⁺ ratio. Hence, we provide a mechanism for how an enzyme of central metabolism controls the community behavior of the bacterium, and suggest PA2444 protein should be named ShrA for serine hydroxymethyltransferase related to rugose colony formation (Frontiers Microbiol. 9:315, 2018).
Pseudomonas aeruginosa is an opportunistic pathogen that can form robust biofilms in environments as varied as domestic showerheads and human airways. Biofilm development involves discrete steps: initial surface attachment and commitment, followed by microcolony growth, biofilm maturation and dispersion. While the early regulatory steps of biofilm development have been explored, relatively little is known about the mechanisms required for P. aeruginosa to sustain a mature biofilm, a state associated with antimicrobial tolerance and environmental persistence. We have identified three genes that play putative roles in biofilm maturation - all three of these genes encode proteins that impact the level of the bacterial second messenger, c-di-GMP. Using 96-well and flow-cell based biofilm assays, we found that inactivation of these genes - PA14_7500 and morA, encoding proteins with motifs associated with both diguanylate cyclase (DGC) and phosphodiesterase activity (PDE), and PA14_10820, encoding a HD-GYP domain typically associated with phosphodiesterase activities - compromises biofilm architecture, resulting in the strains being unable to sustain a mature biofilm. Additionally, loss of these genes uncovered a non-canonical relationship between c-di-GMP signaling and biofilm formation whereby gene inactivation increased concentrations of c-di-GMP prior to the disintegration of the mature biofilm. These findings provide new insights into the genetic mechanisms through which mature biofilms are regulated and suggest a previously unknown role for these c-di-GMP signaling proteins in this process.
Hfq-Assisted RsmA Regulation is Central to Pseudomonas aeruginosa Biofilm and Motility

Y. Irie¹, J. Geyer¹, V. Shingler²;
¹University of Dayton, Dayton, OH, ²Umeå University, Umeå, SWEDEN.

Expression of biofilm and motility genes is controlled by multiple regulatory elements, allowing bacteria to appropriately adapt a sessile or motile lifestyle. In Pseudomonas aeruginosa, the post-transcriptional regulator RsmA has been implicated in the control of various biofilm- and motility-associated genes, but much of the evidence for these links is limited to transcriptomic and phenotypic studies. RsmA binds to target mRNAs to modulate translation by affecting ribosomal access and/or mRNA stability.

Here we trace the global regulatory role of RsmA to the inhibition of Vfr - a key transcription factor required for efficient production of two other transcriptional regulators, namely FleQ and AlgR. FleQ and AlgR, in turn, directly control flagella and pili genes, respectively. FleQ also controls biofilm-associated genes that encode the PEL polysaccharide biosynthesis machinery. Furthermore, we show that RsmA cannot bind vfr mRNA alone, but requires the RNA chaperone protein Hfq. This is the first example where a RsmA protein family member is demonstrated to require another protein for RNA binding.
Feedback Regulation of Caulobacter crescentus Holdfast Synthesis by Flagellum Assembly via the Holdfast Inhibitor HfiA

C. Berne¹, C. K. Ellison¹, G. B. Severin², A. Fiebig³, C. M. Waters², Y. V. Brun¹;
¹Indiana University, Bloomington, IN, ²Michigan State University, East Lansing, MI, ³University of Chicago, Chicago, IL.

To permanently attach to surfaces and form robust biofilms, Caulobacter crescentus produces a strong adhesive, the holdfast. The timing of holdfast synthesis is developmentally regulated by cell cycle cues. When C. crescentus is grown in a complex medium, holdfast synthesis can also be stimulated by surface sensing, in which swarmer cells rapidly synthesize holdfast in direct response to surface contact. In contrast to growth in complex medium, here we show that surface contact does not trigger holdfast synthesis when cells are grown in a defined medium. In this study, we investigate the role of the flagellum and its rotation in adhesion and holdfast synthesis in C. crescentus under different nutrient conditions. We compare mutants lacking the flagellum (Fla−) or the stator required for flagellum rotation (Mot−) to wild-type cells. Though both mutants exhibit non-motile phenotypes, their adhesion phenotypes reveal several differences that are conditional upon media composition. Both Fla− and Mot−mutants display similar attachment deficiencies in complex medium, while Fla−mutants form more robust biofilms over time when grown in defined medium, despite impaired initial adhesion. We find that the overall enhanced adhesion phenotype in Fla−mutants in defined medium is due to an increase in the number of cells that synthesize holdfast, and to a premature production of holdfast during the cell cycle. Our results show that the status of flagellum synthesis regulates holdfast production via transcriptional control of the holdfast inhibitor HfiA. hfiA transcription is reduced in Fla−mutants, and this reduction is modulated by the diguanylate cyclase developmental regulator PleD. Our data support a model in which flagellum assembly feeds back to control holdfast synthesis via HfiA expression in a c-di-GMP dependent manner under defined nutrient conditions.
Protein Lysine Acetylation Plays a Regulatory Role in *Bacillus subtilis* Biofilm Formation

**Author Block:**
A. Reverdy\(^1\), Y. Chen\(^2\), E. Hunter\(^1\), Y. Chai\(^1\);
\(^1\)Department of Biology, Northeastern University, Boston, MA, \(^2\)Institute of Biotechnology, Zhejiang University, Hangzhou, CHINA.

Protein lysine acetylation is a post-translational modification (PTM) that alters the charge, conformation, and stability of proteins. Published lists of acetylated proteins, or acetylomes, in bacteria have demonstrated that protein lysine acetylation occurs on proteins with diverse function, including central metabolism, DNA transcription and binding, chemotaxis, and cell size. *Bacillus subtilis* is a non-pathogenic soil bacterium, and model organism for sporulation, motility, biofilm formation, and multicellular development. The genetic regulation of biofilm formation has been extensively studied, however, regulation by PTM has not yet been explored. To further investigate protein acetylation, we generated an acetylome under biofilm-inducing conditions and identified acetylated proteins, many of which are involved in biofilm development. Various single and double mutants of genes known to encode enzymes involved in global protein lysine acetylation in *B. subtilis* demonstrated that lack of acetylation impacts biofilm development. Deletion of *acuA* and *pta*, the genes that encode an acetyltransferase and a phosphotransacetylase, respectively, showed a decrease in biofilm matrix production based on visible phenotype and quantitative β-galactosidase assay of matrix gene expression. Lastly, we targeted three known acetylated biofilm proteins; SinR, the biofilm master transcriptional regulator, YmcA, a biofilm regulatory protein, and GtaB, a UTP-glucose-1-phosphate uridylyltransferase that synthesizes a nucleotide sugar precursor for exopolysaccharide synthesis. To determine the importance of acetylated lysine residues for the function of these proteins in biofilm development, we performed site-directed mutagenesis of the acetylated lysine codons and assayed the cells containing those point mutants for biofilm formation. Results demonstrated severe biofilm defects upon mutation of lysine residues SinR Lys7 and Lys13, YmcA Lys64, and GtaB Lys89 and Lys191 suggesting the importance of these residues for protein function in biofilm formation. Here, protein lysine acetylation is presented as a potential regulatory mechanism for bacterial protein function during biofilm development.
How SpeG Polyamine Acetyltransferases from Different Pathogens Acetylate Polyamines: A Kinetic Comparison

M. L. Kuhn, V. T. Le, A. Shornikov, P. Boeck, R. Renolo, J. Dang; San Francisco State University, San Francisco, CA.

**Background:** Polyamines are polycationic molecules that are important in a variety of cellular processes, including formation of bacterial biofilms. One enzyme that is present in a diverse set of bacteria, called SpeG, catalyzes the acetylation of these polyamines. We previously showed that SpeG is an allosteric protein that binds polyamines in both allosteric and active sites. While the speG gene has been shown to be important for pathogenicity for some bacteria, to our knowledge the role of the SpeG protein and its products are still unclear. It currently appears that different pathogens may use speG and SpeG in different ways.

**Methods:** To gain a greater understanding of how the recombinant SpeG proteins from different pathogenic bacteria catalyze their reactions, we performed a kinetic characterization of the recombinant proteins and compared their kinetic parameters.

**Results:** We found that turnover, catalytic efficiency, and sigmoidicity of SpeG from different pathogens varied even though they are homologs and catalyze the same reaction. Additionally, we found that pH affected these kinetic parameters.

**Conclusions:** Our kinetic analysis of SpeGs from different pathogens show that the enzyme behaves differently across organisms and should be further studied to understand how these variances may contribute to polyamine levels and biofilm formation within each organism.
Background: Gradually increasing antibiotic resistance is a phenomenon observed worldwide in the past few decades that threatens to reverse the advancements of anti-infective therapies, a keystone of modern medicine in general. In addition, antibiotic treatment regularly fails to cure patients suffering from infections caused by adaptively resistant microbial communities, referred to as biofilms. Even though at least two thirds of all clinical infections are associated with biofilms, there are no biofilm-specific therapies on the market or in clinical trials. *Pseudomonas aeruginosa* is a remarkably antibiotic resistant, nosocomial pathogen and biofilm-former that causes morbidity and mortality especially in cystic fibrosis, nosocomial pneumonia and immunocompromised patients. This project aims to identify the genes associated with drug resistance in *P. aeruginosa* biofilms and to provide novel biofilm-specific targets for the design of potent drugs.

Methods: Genome-wide screens using transposon-sequencing (Tn-Seq) pools of *P. aeruginosa* strain PA14 and the cystic fibrosis epidemic isolate LESB58 were performed to identify genes involved in adaptive antibiotic resistance associated with biofilms. Tn-Seq mutants growing under planktonic but not under biofilm conditions will have lost the ability to form biofilms; thus the inactivated genes in these mutants are potential candidates required for biofilm formation. Treatment of biofilms with sub-inhibitory concentrations of antimicrobial compounds allowed only the survival of mutants retaining adaptive resistance, whereas more susceptible mutants survived within untreated biofilms. I will discuss regulatory genes involved in both processes as well as genes corresponding to the known resistome of antimicrobials.

Results: Random insertion of promiscuous, mariner-based transposons into the genomes of *P. aeruginosa* strains PAO1, PA14 and LESB58 resulted in the generation of Tn-Seq pools that each contained more than 200,000 mutants. To identify and quantify transposon mutants present in the pools, we developed a method combining the amplification of transposon-genome junctions with high-throughput sequencing. Preliminary analysis of the three generated Tn-Seq pools suggested that ~90% of all genes were successfully mutagenized in each respective genome. Furthermore, biofilm growth conditions were established for an *in vitro* Tn-Seq screen of *P. aeruginosa* PA14 in synthetic cystic fibrosis medium on hydroxyapatite discs, a substrate mimicking bone tissue and teeth, and sequencing suggested nearly 600 genes were essential for biofilm formation including dozens of regulatory proteins.

Conclusion: The *P. aeruginosa*Tn-Seq pools are enabling us to screen the whole genome for genes associated with biofilm growth and adaptive antibiotic resistance.
c-di-GMP Receptor PlzC Affects Diverse Cellular Processes in *Vibrio cholerae*

**Abstract Body:**
A bacterial global second messenger cyclic dimeric guanosine monophosphate (c-di-GMP), play important roles in the physiology of many bacterial pathogens. c-di-GMP is produced by diguanylate cyclase (DGC) proteins containing the GGDEF domain and degraded by phosphodiesterase (PDE) proteins bearing the EAL or HD-GYP domains. Receptor proteins or RNAs sense c-di-GMP, and then interact with a downstream protein to affect a particular cellular function. PilZ domain proteins are a class of c-di-GMP receptors, which have RxxxR, and D/NxSxxG consensus motifs required for c-di-GMP binding. *Vibrio cholerae*, the causative agent of the disease cholera, has five PilZ domain proteins (PlzA-E); we do not yet know how PilZ domain containing c-di-GMP receptors controls specific downstream biological processes. In this study, we analyzed the role of PlzC in c-di-GMP-regulated cellular processes and found that ΔplzC mutant exhibit altered motility, biofilm formation, and whole genome expression profile compared with wild type. Additionally, we found that intracellular c-di-GMP levels of the ΔplzC mutant decreased compared to the wild type suggesting that PlzC can function not only as a c-di-GMP receptor but also as a modulator of cellular c-di-GMP levels. To investigate how PlzC regulates diverse cellular processes, we evaluated interactions between PlzC and a set of proteins involved in motility/chemotaxis and c-di-GMP synthesis/degradation using bacterial two-hybrid system. We found that PlzC interacts with specific c-di-GMP metabolizing enzymes. These results suggest that PlzC controls diverse cellular processes by modulating intracellular c-di-GMP levels and by participating in protein-protein interactions with DGCs, PDEs.
**Abstract**

Glucose Induces Biofilm Formation in Insecticidal Protein (cry) Producing *Bacillus thuringiensis* KPW.P1

**Author**
S. Jha, T. Sengupta; IISER KOLKATA, Kolkata, INDIA.

**Background:** *Bacillus thuringiensis* are ubiquitous Gram-positive bacteria that are agriculturally and medically important as they produce insecticidal Cry proteins, thus have bio-control applications. Previous studies reported that the ubiquitous carbon source glucose could induce restricted motility and fractal pattern formation in the growing colonies of the isolated pH, salt and arsenate tolerant *Bacillus thuringiensis* KPW.P1. As bacteria are evolved with the ability to exhibit multicellular behaviour and biofilm formation under harsh or limiting conditions as survival strategies, our present study was focused on exploring the effect of glucose in biofilm formation by *Bacillus thuringiensis* KPW.P1.

**Methods:** Biofilm formation by *Bacillus thuringiensis* KPW.P1 was assayed in 24 well plates by Crystal Violet staining method. Scanning Electron Microscopy (SEM), Confocal laser scanning microscopy (CLSM) and cell surface hydrophobicity assay (MATH assay) were performed for visualization and characterization of KPW.P1 biofilms. Extra-cellular Polymeric Substances (EPS) in KPW1.P1 biofilms were characterized biochemically.

**Results:** With respect to control, six times more biofilm load was marked for *Bacillus thuringiensis* KPW.P1 in presence of 2% of glucose. Interestingly, it was observed that the effect was glucose specific as other sugars could not induce any significant increase in KPW.P1 biofilm load, although all sugars tested, could increase the planktonic growth of KPW.P1 in a dose-dependent manner. Scanning Electron- and Confocal Laser Scanning microscopic studies revealed increased densely packed microcolonies of KPW.P1 inside of exopolymeric substances (EPS) in presence of higher concentrations of glucose. It was also observed that increased glucose contributed increased EPS production by KPW.P1 and increased hydrophobicity and adherence properties in KPW.P1 cells.

**Conclusions:** The present study, clearly states that presence of glucose in growth media induces biofilm formation by *Bacillus thuringiensis* KPW.P1 and such effect is glucose specific as the presence of other sugars in growth media could not result in the induction of biofilm formation by KPW.P1. The observed glucose induced increased biofilm formation is most likely mediated through the adherence property of the bacteria and more EPS production resulting in more biofilm formation. These outcomes can give a major premise to a more exact investigation of the biofilm formation of this Cry producing bacterium *Bacillus thuringiensis* on plant surfaces in response to glucose.
Conserved Genes Contribute to Similar Biofilm Phenotypes in *Pseudomonas aeruginosa* and *Escherichia coli*

M. Kaleta, R. Goetz, M. Gowett, C. Light, K. Sauer; Binghamton University, Binghamton, NY.

Biofilms represent the predominant mode of growth of most, if not all, bacterial species. The ubiquity of the biofilm trait, however, raises the question of whether there are genes that contribute to biofilm formation in a similar manner across species boundaries. If such genes exist, we surmised that these genes should be increased in transcript abundance under biofilm growth conditions, and conserved in sequence. To identify biofilm-specific genes, we first determined the transcriptomic profile of *Pseudomonas aeruginosa* planktonic and biofilm cells by RNA-seq. The analysis revealed several genes that were highly expressed in *P. aeruginosa* biofilms that were conserved in other bacterial species including *Escherichia coli*. Three pairs of *Pseudomonas* and *E. coli* homologs, PA0918 and *yodB*, PA2184 and *yciE*, and PA3915 and *moaB*, were selected for further study. To determine if these genes are conserved in function we evaluated the role these genes in attachment, biofilm formation, and swarming in two species, *P. aeruginosa* (PAO1) and *E. coli* (BW25113). Inactivation of PA0918 or *yodB* had no effect on attachment and swarming, but resulted in decreased biofilm biomass accumulation. Inactivation of PA2184 or *yciE* coincided with reduced attachment and mutant biofilms demonstrating an altered biofilm architecture relative to wild type. Moreover, ∆PA3915 and ∆*moaB* mutants were characterized by reduced attachment, biofilm biomass accumulation, and increased swarming relative to wild type. Moreover, biofilms formed by *P. aeruginosa* mutants ∆PA2184 and ∆PA3915 were impaired in their tolerance to tobramycin. Given the similarity in function, we also determined whether the *E. coli* homologs are capable of complementing the *P. aeruginosa* mutant phenotypes associated with biofilm formation and tolerance. Expression of either the *P. aeruginosa* or *E. coli* version of the genes restored the respective mutant phenotypes including tolerance to wild-type levels. Overall, our findings suggest that homologs of biofilm-specific genes that contribute to biofilm phenotypes by *P. aeruginosa* carry out similar functions in other biofilm-forming bacteria. Moreover, our findings suggest the likely existence of genes that contribute to the biofilm trait that are conserved in sequence and function.
Background: The ability to form biofilms is an important virulence trait of oral bacteria. These oral biofilms are diverse, multispecies structures that are regulated by bacterial communication processes. Oral biofilms are enriched in outer membrane vesicles (OMVs), which carry biomolecules including toxins and other virulence factors, peptidoglycan, and nucleic acids. The ability of OMVs to transport these molecules to host cells and the role of this process in bacterial virulence has been well-studied, but the process of OMV delivery to bacterial cells within a biofilm and the impact of this process on biofilm formation has not yet been studied. To investigate this process, we looked at the ability of Aggregatibacter actinomycetemcomitans OMVs to alter biofilm production by Streptococcus mutans, the etiologic agent of dental caries. A. actinomycetemcomitans is a Gram negative organism associated with localized aggressive periodontitis. Strains that produce more leukotoxin (LtxA), a secreted protein that specifically kills human immune cells, are more closely associated with disease than those that do not produce this toxin. Because competitive interactions between these two organisms have been reported, we hypothesized that A. actinomycetemcomitans OMVs would inhibit the ability of S. mutans to form biofilms.

Methods: We collected OMVs from two strains of A. actinomycetemcomitans: JP2, a highly pathogenic strain that produces LtxA, and JP2-1704, an isogenic ltxA mutant. Purified A. actinomycetemcomitans OMVs were then added to S. mutans Clarke (ATCC 25175) cultures at various time points, before biofilm production was initiated and after the biofilm had been established, and biofilm production was quantified using crystal violet staining. We also investigated interactions between these OMVs and planktonic S. mutans cells using flow cytometry. Results: We determined that A. actinomycetemcomitans JP2 OMVs associated with S. mutans cells in culture, but this association had no effect on planktonic growth. However, when S. mutans was grown in the presence of JP2 OMVs, biofilm production was inhibited in a dose-dependent manner. These JP2 OMVs also disrupted established S. mutans biofilms. Interestingly, JP2-1704 OMVs, which do not contain LtxA, increased rather than decreased biofilm production by S. mutans, suggesting that this toxin may play a role in the competitive interactions between these organisms. Purified LtxA, however, did not affect S. mutans biofilm production, likely due to transport limitations through the biofilm.

Conclusions: This work presents a new understanding of the ability of A. actinomycetemcomitans to regulate the virulence of S. mutans by altering its ability to form biofilms. The importance of LtxA in this process suggests a new role for this toxin that is dependent on its packaging in these bacterial vesicles.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 092  
**Abstract Topic:** Regulation of Biofilm Development  
**Abstract Title:** Arginine Uptake Impacts Porphyromonas gingivalis Biofilm Development  
**Author Block:** S. Ghods, F. Moradali, M. Davey; University of Florida, College of Dentistry, Department of Oral Biology, Gainesville, FL.

**Background:** Many chronic inflammatory diseases, including periodontal infections, are biofilm-based pathologies mediated by commensal microbiota persisting within complex host-associated microbial communities. The central hypothesis for this project is that the availability of L-arginine is a key signal that directs colonization and expression of virulence determinants by the periodontal pathobiont *P. gingivalis*. Our studies have shown that L-arginine removal or addition are fundamental signals that impact colonization and expression of key surface structures (fimbriae) known to be required for biofilm development and pathogenicity. Yet, how *P. gingivalis* senses L-arginine and the regulatory mechanisms that transmit and control the response have not been identified. Our current goal is to determine how *P. gingivalis* senses L-arginine in its environment and how it responds in regards to its metabolism and biofilm formation. **Methods:** For detecting arginine-interacting protein(s), we employed arginine affinity chromatography followed by SDS-PAGE electrophoresis and mass spectrometry. **In silico** analysis was used to identify arginine transport systems. By generating mutants and *in trans* complementation, the function of relevant genes were studied with regard to biofilm formation. Moreover, the impact of the availability of free arginine on generated mutants and wild-type were studied via biofilm assessment and growth rates. In addition, the metabolic status of the mutants and wild-type were analyzed via untargeted global metabolomic analysis. **Results:** Our results show that under certain growth conditions free arginine can inhibit biofilm establishment and development. We show that the major outer membrane protein RagA, which is predicted to be a macromolecular transporter binds arginine. We also show that a putative ATPase/kinase (PGN_1641) predicted to be involved in the transport of arginine, and the response regulator RprY (PGN_1186), which has been shown to regulate PGN_1641; both negatively regulate biofilm formation. The degree of inhibition by the availability of free arginine was found to be dependent on the physiological state of cells before and after establishment of biofilm. Lastly, metabolomic analysis discovered that the production of polyamines is significantly altered when arginine is available. **Conclusions:** Our data indicate that RagA, RprY, and a predicted ATPase/kinase (PGN_1641) mediate or control arginine sensing. In the context of pathogenesis, arginine levels can directly impact the expression of virulence determinants and survival.
Many bacteria use 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, 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 to perceive temperature. The archetype of these proteins is the thermosensory diguanylate cyclase (TdcA), identified in a human isolate of *Pseudomonas aeruginosa*. TdcA thermostats c-di-GMP biosynthesis with catalytic rates that change up to 10-fold per 10 °C. These enzyme kinetics enable rapid physiological changes over narrow temperature ranges, and challenge long-standing theory for universal enzymatic rate-temperature dependencies. Domain-swapping analyses indicate that heat-sensing is mediated by a cofactorless Per-Arnt-SIM (PAS) type III domain, which is a previously undescribed function for this widespread protein domain family. Using intravital imaging, we demonstrate that *tdcA* allows *P. aeruginosa* to suppress early innate immunity in the murine lung. This immune evasion depends on the c-di-GMP-regulated extracellular polysaccharides PEL and PSL. TdcA orthologues are widespread in bacteria, and putative heat-sensing PAS domains are linked with hundreds of predicted diguanylate cyclases and c-di-GMP-specific phosphodiesterases in the PFAM database. We propose, therefore, that thermotransduction is a prevalent function of bacterial c-di-GMP signaling networks.
Background: The oral microbiome contains highly diverse microorganisms with certain subsets predominating at different habitats. It has been shown to play a crucial role in maintaining oral and systemic health. Aside from causing diseases in the oral cavity, certain oral commensals can be implicated in systemic diseases, including cardiovascular diseases, aspiration pneumonia, stroke and diabetes. Although these oral commensals can be found in healthy and disease-affected subjects, the portion of certain bacteria, e.g. lactic acid bacteria, appears to substantially increase in oral disease patients, suggesting an imbalance among oral ecosystems. Moreover, it has been recognized that caries, periodontitis and other dental infections are caused by consortia of organisms in a biofilm rather than a single pathogen. Therefore, it is essential to understand the ecology of the oral commensals not only in oral diseases but also in the healthy oral cavity. Bacterial commensal Corynebacterium spp. has been shown to play a protective role by, at least in part, utilizing human triacylglycerols and releasing antimicrobial free fatty acids, resulting in altering the environment and inhibiting the growth of the pathogenic strains, such as Streptococcus pneumoniae. To our knowledge, the inter-species interactions of oral commensals, in particularly, Corynebacterium spp. and Streptococcus spp., and the effect of such interactions on dental diseases have never been investigated. In this study, we aim to understand the molecular mechanism(s) of different taxa interactions and the implications on oral diseases. Corynebacterium durum (Cd) and Streptococcus sanguinis (Ss), the two common commensals found in the oral cavity and dental plaque, have been used. Methods and Results: So far, we have discovered that, when treating Ss with culture supernatant collected from Cd, Ss chain length was substantially elongated. We additionally tested other oral streptococci but none of them showed any morphological alterations, suggesting species-specific interaction between Cd and Ss. By using real-time PCR, an alteration of genes predicted to be involved in glycerol and lipid metabolism, and cell division has been observed in Cd-supernatant treated Ss compared to the untreated Ss. An increase in cell aggregation of Cd-supernatant treated Ss was also observed. Interestingly, an ability to phagocytose of monocyte/macrophage-like RAW 264.7 cells was decreased when challenging with co-cultured Ss with Cd in comparison to Ss. Conclusions: Our findings so far have suggested that there is inter-species interactions between both dental commensals Cd and Ss, at least in part, for the potential benefits of oral commensal colonization which can prevent colonization of pathogenic strains.
The opportunistic pathogen P. aeruginosa, employs a hierarchical quorum-sensing network to regulate virulence factor production which cooperatively benefit the population at a cost to the individual. Quorum-sensing suppression was therefore proposed as an attractive anti-virulence target. Furthermore, it was argued that the evolution of a cooperative mutant in a quorum-sensing-suppressed population would be hampered through its exploitation by neighboring non-mutant cells. It remains unclear whether mechanisms which overcome this exploitation exist. We investigated the regain of quorum-sensing cooperation by combining rational design of candidate strains and directed evolution of a mutant of the lasR master quorum-sensing regulator. We find that the most effective mutations pleiotropically combined regain of cooperation and private benefit. We find one such mutation which led to significant conflict between regain of cooperation and antibiotic resistance. Reference: Oshri et al, ISME, 2018, "Selection for increased quorum-sensing cooperation in Pseudomonas aeruginosa through the shut-down of a drug resistance pump"
Communication between Two *Bacillus subtilis* Biofilm Colonies in Proximity

P. Murugan, M. Saravanan; Indian Institute of Technology Kanpur, Kanpur, INDIA.

Different soil bacteria are known to produce small chemical signals that can regulate itself and also the organisms around it. These chemical signals are also known to regulate biofilm formation. Bacteria in the environment are predominantly found as biofilms. *Bacillus subtilis*, a gram positive model bacterium for studying biofilm. They differentiate into different subpopulations with response to environmental cues but are genetically identical cells. This indicates the existence of various signaling molecules which can regulate its metabolism when in a biofilm. We aim to identify and study such signals of chemical nature in and between *Bacillus subtilis* biofilms. To investigate this we isolated a new strain of *Bacillus subtilis* confirmed by 16s rDNA sequencing and whole genome sequencing. Two *Bacillus subtilis* biofilm communities were grown nearby to check for any chemical communication molecules. Using chemical indicators and ion chromatography we were able to confirm ammonia as a communicator molecule between two biofilm communities that are placed nearby. Ammonia is already known to be correlated to the metabolic state of a *Bacillus* biofilm but not known to be a communicator molecule between two communities of biofilms. Ammonia gas was also seen to induce fruiting body formation in the newly isolated *Bacillus subtilis* strain. We also observed interstrain communication with ammonia which is reported here. Here we suggest the *Bacillus subtilis* colonies produce ammonia which creates a temporary alkaline gradient which is perceived as a signal by a neighboring colony. The colony growth in the direction of amplified signals is inhibited. Hence colonies orient themselves towards the area which minimize the competition for nutrients.

A. Bleem¹, D. J. Madsen², G. Christiansen², H. Maric³, K. Stromgaard³, R. L. Meyer², D. E. Otzen², V. Daggett¹, J. D. Bryers¹;
¹University of Washington, Seattle, WA, ²Aarhus University, Aarhus, DENMARK, ³University of Copenhagen, Copenhagen, DENMARK.

Background: When bacteria dwell in biofilms on the surface of an implanted medical device or surgical site, they produce an extracellular matrix (EM) that encases the cells and acts as a layer of protection against antibiotic infiltration. Recent evidence suggests that certain biofilm-associated bacteria utilize β-sheet-rich amyloid proteins as a scaffold to reinforce the EM. Amyloids are typically associated with mammalian neurodegenerative disease, but they play a functional role in the EM to help biofilms resist dispersion by chemical or mechanical agents. Consequently, these functional amyloids represent a novel target to interrupt medical biofilm formation. We hypothesize that the pathway to amyloid formation is characterized by specific physicochemical motifs; therefore, peptides engineered to bind these motifs should inhibit fibril formation and destabilize the biofilm. Methods: We applied a combination of computational predictions and biophysical assays to identify regions of high aggregation propensity in functional bacterial amyloids. We also used molecular dynamics (MD) simulations to characterize conformational changes in several amyloid proteins. These data were then applied to design peptides that inhibit amyloid formation by targeting unique secondary structures. Results: In amyloid proteins from P. aeruginosa, mutation of amyloidogenic motifs to a designed, non-amyloidogenic motif suppresses fibrillation. Further, MD simulations suggest that a variety of amyloids adopt a non-standard secondary structure, termed “α-sheet”, in the early stages of aggregation. Accordingly, peptides designed to incorporate complementary α-sheet structure suppress amyloid formation in vitro. These peptides also inhibit biofilm formation in S. aureus, P. aeruginosa, and E. coli by targeting functional amyloids in the EM. Conclusions: The results reported here expand our understanding of the mechanisms of bacterial amyloid formation, particularly the role of α-sheet structure in this process. Designed anti-α-sheet peptides represent a novel, non-killing strategy to combat medical biofilms.
Changes in the cell wall compositions of *Staphylococcus aureus* and *Enterococcus faecalis* were characterized using combined solid-state NMR and liquid chromatography-mass spectrometry (LC-MS). Solid-state NMR was used to characterize whole cells and cell walls of *S. aureus* during planktonic, immature biofilms (formed after 24 h after stationary phase), and mature biofilms (after 60 h after stationary phase) labeled either by (i) \([15N]\)glycine and l-[1-13C]threonine, or in separate experiments, by (ii) l-[2-13C,15N]leucine. We then measured \(^{13C,15N}\) direct bonds by C\{N\} rotational-echo double resonance (REDOR). The increase in peptidoglycan stems that have bridges connected to a surface protein was determined directly by a cell-wall double REDOR difference. For both planktonic cells and the mature biofilm, 20% of pentaglycyl bridges are not cross-linked and are potential sortase-mediated surface-protein attachment sites. None of these sites has a surface protein attached in the planktonic cells, but one-fourth have a surface protein attached in the mature biofilm. Thus, a primary event in establishing a *S. aureus* biofilm is extensive decoration of the cell surface with surface proteins that are linked covalently to the cell wall and promote cell-cell adhesion. We also examined changes in peptidoglycan (PG) composition of *Enterococcus faecalis* associated with the biofilm formation. For accurate analysis, we developed “Stable Isotope Labeling by Amino Acids in Bacterial Culture” (SILAB) optimized for bacterial cultures with incomplete amino acid labeling. The analysis was carried out by labeling *E. faecalis* in biofilm with heavy-Lys (l-[13C6, 2D9, 15N2]Lys) and planktonic bacteria with natural abundance l-Lys, then mixing the equal amount of bacteria from each condition and carrying out cell-wall isolation and mutanolysin digestion for LC-MS. SILAB analysis of 47 pairs of PG fragment ions from isolated cell walls of planktonic and biofilm samples show increased PG cross-linking, increased N-deacetylation of GlcNAc, decreased O-acetylation of MurNAc, and increased stem modifications by d,d- and l,d-carboxypeptidases in *E. faecalis* biofilm.
Session Title: **WEDNESDAY Poster Session 3**
Session Date/Time: Wednesday, October 10, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 101

Abstract Topic: Synthesis and Assembly and Function of the Biofilm Matrix

Abstract Title: A Biofilm Matrix-Associated Protease Inhibitor Protects *Pseudomonas aeruginosa* from proteolytic Attack

Author Block: B. Tseng¹, C. Reichhardt², G. E. Merrihew³, S. A. Araujo-Hernandez¹, J. J. Harrison¹, M. J. MacCoss², M. R. Parsek²;
¹University of Nevada Las Vegas, Las Vegas, NV, ²University of Washington, Seattle, WA, ³University of Calgary, Calgary, AB, CANADA.

*Pseudomonas aeruginosa* produces an extracellular biofilm matrix that consists of nucleic acids, exopolysaccharides, lipid vesicles, and proteins. In general, the protein component of the biofilm matrix is poorly defined and understudied relative to the other major matrix constituents. While matrix proteins have been suggested to provide many functions to the biofilm, only proteins that play a structural role have been characterized to date. Here we identify proteins enriched in the matrix of *P. aeruginosa* biofilms. We then focused on a candidate matrix protein, the serine protease inhibitor ecotin (PA2755). This protein is able to inhibit neutrophil elastase, a bactericidal enzyme produced by the host immune system during *P. aeruginosa* biofilm infections. We show that ecotin binds to the key biofilm matrix exopolysaccharide Psl and that it can inhibit neutrophil elastase while matrix-associated. Finally, we show that ecotin protects both planktonic and biofilm *P. aeruginosa* cells from neutrophil elastase-mediated killing. This may represent a novel mechanism of protection for biofilms to increase their tolerance against the innate immune response.
**Session Title:** WEDNESDAY Poster Session 3  
**Session Date/Time:** Wednesday, October 10, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 102  
**Abstract Topic:** Synthesis and Assembly and Function of the Biofilm Matrix  
**Abstract Title:** Extracellular Z-DNA and not B-DNA is the Important Extracellular DNA Configuration that maintains the Structural Integrity of Mature Biofilms  
**Author Block:** S. D. Goodman, J. Buzzo, A. Devaraj, J. A. Melvin, L. O. Bakaletz; Nationwide Children's Hospital, Columbus, OH.  

**Background:** Biofilms consist of bacteria embedded in a self-produced extracellular polymeric substance (EPS) that is comprised of often species-specific polysaccharides and proteins, as well as extracellular DNA (eDNA). We have shown that eDNA and the ubiquitous DNABII family of DNA-binding proteins are universal components of biofilm EPS, and together provide essential structural support for biofilm development and maintenance. While DNA nucleases can prevent efficient biofilm formation, mature biofilms are typically resistant to nucleases. This phenomenon has been attributed to a diminished structural role for eDNA in mature biofilms. However, abundant eDNA is present in mature biofilms, and removal of DNABII proteins disrupts mature biofilms. Thus, eDNA provides essential structural support to mature biofilms, and must instead have adopted a nuclease-resistant form. DNA structure is in equilibrium among interconvertible configurations. B-form is the canonical and dominant configuration that consists of a nuclease sensitive right-handed double helix, whereas the Z-form consists of a left-handed helix with distinct geometry that is nuclease resistant. While not abundant intracellularly due to the high intrinsic energy of this state, Z-form DNA can exist under defined conditions such as in the presence of certain biogenic amines, e.g. spermidine. Since spermidine is available at high concentrations extracellularly, we hypothesized that eDNA adopts the Z-form as biofilms mature, which provides resistance to nuclease-mediated disruption. **Methods:** DNABII protein stabilization of Z-DNA was assessed by circular dichroism spectroscopy. To assay the role of biofilm matrix components in Z-DNA formation and its correlation to biofilm stabilization, we grew bacteria in chambered coverglass and detected matrix components and eDNA structures in the resultant biofilms by immunofluorescence microscopy. To quantify biofilm formation, we stained biofilms grown in chambered coverglass with Live/Dead™ stain, imaged biofilms by confocal laser scanning microscopy, and analyzed images with COMSTAT software. **Results:** DNABII proteins facilitated conversion of DNA to the Z-form through DNA-binding. Further, as bacterial biofilms matured, extracellular spermidine concentrations increased concomitantly with the appearance of Z-form eDNA, albeit at consistently lower levels than B-form eDNA. Remarkably however, nuclease digestion of mature biofilms that fails to disrupt biofilm structure completely eliminated B-DNA and predictably preserved eDNA in the Z-form. Finally, conditions that stabilize eDNA in the Z-form facilitated biofilm formation whereas conditions that favor B-form DNA impeded bacterial biofilm maturation and growth. **Conclusions:** We contend that eDNA in the Z-form is the critical configuration for structural stability of a mature bacterial biofilm EPS.
Abstract

Extracellular Polymeric Substances from Biofilm Forming Marine Streptomyces sp and their Radionuclide Bioremediation Application

Author

K. Kamala, P. Sivaperumal, R. Tilagaraj;

SRM Institute of Science and Technology, Kattankulathur, INDIA.

Extracellular Polymeric Substances (EPS) producing marine actinobacterium has been isolated from a marine natural biofilm matrix and identified as Streptomyces sp. CuOff24 through 16S rDNA sequencing. Approximately 450 mg L$^{-1}$ EPS were produced and major content of carbohydrate followed by protein, nucleic acid and unidentified compounds (68.6%, 12.3%, 10.4% and 8.7% respectively) were quantified. The spectroscopy study also confirmed the presence of carbohydrate functional groups on the EPS surface and GCMS showed that the EPS comprised of mannose (45.2%), glucose (28.8%) and arabinose (26%). The 1g EPS could remove 86.7±0.61% of Cs$^+$ ion from test solution having 10mM CsCl$_2$ with KCl. The biosorption of Cs$^+$ ion in marine actinobacterial EPS with different environmental factors (pH, temperature, time interval) were also tested. SEM coupled with EDS also confirmed the Cs$^+$ ion adsorption by EPS obtained from Streptomyces sp. CuOff24. Maximum of the cesium ion was found to be matrix with EPS and it could suggest that, the radionuclide ions were interact with surface active groups. The high Carbohydrate compounds in marine actinobacterial EPS could be used for the large scale remediation of radionuclides contaminated environment.
Session Title: **WEDNESDAY Poster Session 3**

Session Date/Time: Wednesday, October 10, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 104

Abstract Topic: Synthesis and Assembly and Function of the Biofilm Matrix

Abstract Title: Structural Changes and Matrix Composition of Biofilms Formed on Meat by Psychrotrophic Pseudomonads

Author Block: N. Wickramasinghe¹, M. M. Hlaing², J. T. Ravensdale¹, R. Cooray¹, G. A. Dykes¹, P. S. Chandy²;
¹School of Public Health, Curtin University, Bentley, WA, AUSTRALIA; ²CSIRO, Agriculture & Food, Werribee, Victoria, AUSTRALIA.

**Background:** Psychrotrophic pseudomonad species are the dominant bacterial component on aerobically stored chilled meat. Biofilm formation by pseudomonads during chilled storage and transportation results in slime formation which is a major spoilage characteristic. Limited *in-situ* characterization of the structure and matrix composition of biofilms, which may aid in understanding their dominance on chilled meat, has been undertaken. We studied the structural changes of biofilms formed on meat with time and analyzed the matrix composition of *Pseudomonas fragi* (n=2) and *Pseudomonas lundensis* (n=2) using confocal laser scanning microscopic (CLSM) imaging, cell counts and confocal Raman spectroscopy.

**Methods:** Sterilized beef samples were inoculated separately with each of the species at cell numbers similar to those on retail meat (10⁴ CFU/cm²) and incubated aerobically at 10⁰C and 25⁰C for 7 days. On each day, biofilms were stained with Live/Dead fluorescent stains and imaged with CLSM. At the same time, numbers of bacteria in biofilms formed under identical conditions were determined by total plate counts from disrupted and sonicated biofilms. To study the matrix composition, biofilms of same meat isolates were grown at the same temperatures on sterile nitrocellulose membranes placed on sterile beef cuts to minimize the contamination from the meat. Raman spectra were obtained and principal component analysis was conducted to identify differences between matrix components based on temperature and species level.

**Results:** Bacterial numbers in the meat grown biofilms increased rapidly in all the strains and after reaching a population maximum of around 10¹¹ CFU/cm² began to decline. The changes in cell count data correlated well with CLSM images which showed that all the strains produced thick biofilms at both temperatures. After populations reached maximum levels, biofilm structures began to degrade and live/dead ratio declined with time. Preliminary Raman spectroscopic data showed differences in matrix composition between biofilms grown at 10⁰C and 25⁰C in both species. Analysis confirmed that the spectral changes are associated with protein, polysaccharide and DNA/RNA contents obtained in different biofilm samples.

**Conclusion:** Despite the abundant access to nutrients from raw meat, biofilms formed by the proteolytic *Pseudomonas* species dispersed with time. The signal for dispersal could be the depletion of meat muscle with glucose and/or production on nitrogenous compounds as a result of proteolysis during the latter stages of biofilm growth. The differences between Raman spectra of biofilms grown at 10⁰C and 25⁰C suggest that selected psychrotrophic spoilage pseudomonads respond to cold stress by modifying the matrix composition. **Key words:** Biofilm dispersal, biofilm matrix, Chilled meat, *Pseudomonas fragi, Pseudomonas lundensis*
Aspergillus fumigatus (AF) is a ubiquitous saprophytic fungus and the second most common causative agent of fungal infections in hospitals. Its ability to resist currently available antifungal drugs and evade the immune system is facilitated by its ability to form a biofilm. A key component of the AF biofilm is the exopolysaccharide adhesin galactosaminogalactan (GAG), a heteropolysaccharide composed of α-1,4-linked galactose, N-acetylgalactosamine, and galactosamine. In A. fumigatus, GAG biosynthesis and export across the cell membrane is facilitated by the protein products of a five-gene cluster. One of these genes, ega3, encodes a glycoside hydrolase (GH) belonging to the GH114 superfamily. To understand the role of Ega3 in GAG export and its enzymatic mechanism, structural and functional studies were performed. Recombinant Ega3 was expressed and purified using Pichia pastoris and crystallization screens conducted. Ega3 crystals diffracted to 1.76 Å, and the structure was solved with a distant homolog using the ARCIMBOLDO-SHREDDER software. The atomic structure reveals a characteristic (β/α)8 fold with an electronegative cleft that binds the sugar polymer. In addition, co-crystallization with galactosamine revealed a flexible loop that folds over to coordinate the sugar monomer. 

In vitro activity assays performed with purified Ega3 support that it is an endo-acting hydrolase that can disrupt A. fumigatus biofilms. Mutation of the conserved residues in the putative binding cleft supports a central aspartic acid (D189) and glutamic acid (E247) as the catalytic residues. Our combined results suggest that Ega3 is involved in GAG processing during export and can be repurposed as a novel therapeutic for biofilm related infections.
**Abstract**

**Background:** In the United States alone, more than two million people become infected by antibiotic-resistant bacteria each year, leading to at least 23,000 deaths as a result. Biofilms, a matrix of bacterial cells, act to further protect bacterial infection from any kind of antimicrobial treatment. These protective structures serve as a physical means of antibiotic resistant. MreB, an essential protein for proper morphological development of all gram-negative bacteria, plays a key role in forming biofilms. In healthy *E. coli*, the protein causes cells to hold a rod-like shape rather than the circular shape they hold without it. Another important protein is ftsZ, although it plays a different role than MreB. FtsZ is a necessary protein for the cell division process, establishing where exactly the cell will be divided during binary fission. Together, these two proteins serve to properly form biofilms. **Methods:** The present study seeks to compare the effects of the inhibition of the two proteins on K-12 *E. coli*. This inhibition will come through the application of two inhibitory chemicals; A22 Hydrochloride inhibiting MreB and PC190723 inhibiting ftsZ. The efficacy of the chemicals will be measured through biofilm density, quantified using a crystal violet staining assay on 24-well plates. The stains will be put into solutions which will then read be read a spectrophotometer. **Results:** After experimentation, it was found that a mid-level dosage of A22 significantly decreased biofilm growth while there was no dose response to PC190732. This signifies that the inhibition of MreB was more effective than that of ftsZ. **Conclusions:** To further study biofilms and their antibiotic-resistant abilities, testing should be done on the dual inhibition of MreB and ftsZ. This conjunct inhibition would allow for any synergistic or additive effect to become present. Additionally, testing should be done to determine if the protein inhibition will increase antibiotic susceptibility to Amoxicillin. This future experiment would allow insight into the effectiveness of antimicrobial treatment after the biofilm density has been decreased, truly showing the practical application.
**Background:** According to the World Health Organization, nearly fifteen percent of all hospitalized patients worldwide acquire nosocomial infections. A particular area of concern for bacterial build up in hospitals is sink drains. The moist, microbiologically active environment of drains promotes the formation of biofilms that are difficult to target with standard chemical disinfectants. Bacteriophage, however, show potential to be used as a disinfecting agent in hospital drains. Not only do bacteriophage increase in titer as they infect, spreading to hard-to-reach surfaces, numerous phage have been shown to degrade the extracellular polymeric substances of biofilms and gain access to underlying bacteria. Water channels in the extracellular matrix of various biofilm, such as *E. coli* biofilms, allow entry of phage T4. This research explores the potential of a bacteriophage-based disinfectant to eradicate biofilms in an environment modeling a sink drain by comparing the efficacy, thoroughness, and durability of the phage disinfectant to a chemical disinfectant. **Methods:** *E. coli* biofilms were grown in M9 minimal media placed in sink P-traps. The P-traps were divided into three treatment groups: one group was treated with bacteriophage T4, a second group with a chemical disinfectant, and a control group with deionized water. To compare the efficacy of treatments, the biofilms were quantified at five time points - 1 hour, 12 hours, 24 hours, 72 hours, and 1 week. This process involved washing to remove planktonic cells, applying crystal violet to stain biofilms, adding 30% acetic acid to solubilize biofilms, and using a spectrophotometer wavelength 570 to measure optical density. **Results:** Crystal violet staining revealed that one hour after application of treatment, both the chemical disinfectant and bacteriophage were able to reduce biofilm growth compared to the negative control, with the chemical disinfectant being slightly more effective. With heavier biofilm growth at longer time intervals after treatment - such as forty-eight hours to 1 week -- the bacteriophage began exhibiting the same effectiveness at removing biofilm as the chemical disinfectant. Upon visual examination, sink P-traps treated with bacteriophage showed a tendency for more uniform destruction of biofilm across the drain compared to P-traps treated with chemical disinfectant. **Conclusion:** Overall, this work highlights the potential of bacteriophage as an alternative to conventional chemical disinfectants for long term biofilm control in settings such as hospital drains. Future work will be done to quantify the distribution of biofilm elimination across the P-trap.
Efficacy of Melittin Against *E. coli* Biofilms When Used with DNase I

Background: Biofilms are aggregations of bacteria living together surrounded by a matrix and connected to a surface. They are highly antibiotic resistant, and this comes at a great cost to the public as many infections are potentially biofilm-related; this is seen particularly in nosocomial infections, with 34.7% of health-care-associated infections in 2014 being surgical-site or urinary tract related, two areas where biofilm formation is common. This high antibiotic resistance is coupled with an increased chance of the bacteria developing resistance to whatever treatment used due to factors such as a higher rate of mutagenesis and plasmid transfer compared to planktonic bacteria. One field of treatment that has been explored is the use of antimicrobial peptides, including a peptide derived from bee venom, melittin. Melittin has been demonstrated to have antimicrobial effects on both bacteria in a planktonic state and bacteria in a biofilm state. On biofilms, however, melittin has been demonstrated to have a minimum inhibitory concentration that is too high for it to be applicable in a clinical setting. The use of an agent to break down the biofilm matrix has been previously demonstrated to improve the efficacy of antimicrobial peptides when used in combination with them. DNase I is one such agent, as eDNA is a structural component of many biofilm matrices, including that of *E. coli*. 

Methods: Whether DNase I could be used to increase the efficacy of melittin and make it a clinically possible treatment was investigated, as well as the best way to use them in combination. Melittin and DNase I were applied to *E. coli* biofilms in 24-well plates, both individually and in combination with each other. The combination treatments were melittin and DNase I applied simultaneously and DNase I applied 7 hours before the melittin was. The amount of biofilm in each well was analyzed using a crystal violet assay and 24 and 48 hours, with the optical density of the crystal violet dissolved in ethanol being determined using a spectrophotometer. 

Results: Through comparison of the treatment wells to wells that had only the buffers of melittin and DNase I applied to them, preliminary results show that DNase I was the most effective treatment. Ongoing research is investigating the propensity of *E. coli* biofilms to become resistant to DNase I treatment. 

Conclusions: Data collected indicates that DNase I and melittin used in combination is not an effective biofilm treatment, but that DNase I may be a good option for clinical use despite concerns about the possibility of it increasing biofilm dispersal and recolonization.
Effect of pH on Pairwise Interactions in a Model Cheese Rind Community

B. Anderson¹, R. Dutton²

¹Biological Sciences, University of California, San Diego, La Jolla, CA. ²University of California, San Diego, La Jolla, CA.

The formation of a model cheese-rind biofilm involves a reproducible succession of bacteria and fungi. As the growing biofilm breaks down cheese proteins and releases ammonium, the pH of the cheese increases drastically, from pH 5 to pH 8. This dynamic abiotic environment can modulate the nature of interactions between biofilm residents in two possible ways: either by removing or imposing a reliance on another species, or by altering the mechanism by which two species interact. As a multi-species biofilm undergoes such abiotic and biotic dynamics, it can be expected that the structure of the biofilm will reflect and further impact these dynamics. Using a 7-member in vitro cheese rind community as a model, growth assays of all pairwise combinations reveal species pairs that interact in a pH-dependent versus pH-independent manner. To understand how these changing interactions are reflected in the spatial organization of species pairs and whether these patterns are maintained in a complete community, fluorescence in situ hybridization methods have been optimized to visualize in vitro cheese rind biofilms consisting of two- to seven-microbe biofilms. These results may have implications for the resultant pattern of succession observed in the complete 7-member community.
### THURSDAY Poster Session 4 - Abstracts

**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 001

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Deciphering the Biofilm Eradication Mechanism of Thymol against *Cryptococcus neoformans* Using Multiomics Approach

**Author Block:**  
P. Kumari¹, N. Arora¹, A. Chatrath¹, R. Gangwar¹, D. Kumar², K. M. Poluri¹, R. Prasad¹;  
¹Indian Institute of Technology Roorkee, Roorkee, INDIA, ²Centre of Biomedical Research, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, INDIA.

Cryptococcosis is a multifaceted and potentially fatal systemic fungal infection, globally responsible for ≈1 million cases/year mostly occurring in immunodeficient patients. This infection is further augmented due to the ability of *Cryptococcus neoformans* to form biofilm. Considering the enhanced biofilm resistance and toxicity concerns of synthetic antifungal drugs, search for efficient and non-toxic natural therapeutics have received a major boost in recent times. Hence, in the present study the anti-biofilm potential of thymol, a monoterpenoid phenol of thyme oil and its mechanism of action (MOA) against *C. neoformans* were evaluated. To test the potency of thymol the minimum inhibitory concentration (MIC) and the biofilm inhibitory/eradicating concentration (BIC/BEC) were determined. The morphological and physiological changes in response to thymol were analyzed using biophysical techniques. NMR based metabolomic and Mass spectrometry based proteomic studies were performed to assess the MOA of thymol. Real time qPCR was conducted to substantiate the multiomics results. Finally, the toxic nature of thymol against human cell-lines was evaluated using co-culture assays. Thymol showed an antifungal and anti-biofilm activity of MIC 16 µg/mL and BIC/BEC 32/128 µg/mL respectively lower than fluconazole. Microscopic analysis revealed absence of extracellular polymeric matrix, reduction in capsule size, and disruption of surface/ultrastructure of biofilm cells. The metabolomic analysis evidenced for a decrease in TCA cycle intermediates and amino acid metabolism while an increase in the energy metabolites suggesting thymol interference with the key metabolic pathways. These results were in line with the proteomics data as it showed upregulation of stress proteins while downregulation of carbohydrate metabolism and cell wall integrity related proteins. RT-PCR studies established that stress responsive gene calcineurin, *CNA1* is overexpressed and cell wall integrity gene ergosterol, *ERG11* is under expressed, thus substantiating the above omics results. Furthermore, we observed that thymol is minimally toxic as it showed selective killing of *C. neoformans* without affecting keratinocytes in co-cultures. This study provided mechanistic insights depicting how thymol can modulate the membrane permeability and regulatory pathways to invade *C. neoformans*; and demonstrated its therapeutic potential to be formulated as next-generation antifungal agent.  

**Keywords:** *C. neoformans*, biofilm, metabolomics, proteomics, NMR, *ERG11*
Abstract

Topic: Antibiofilm Strategies

Title: Molecules Affecting *Listeria Monocytogenes* Motility and Biofilm Formation

Author: I. Doghri¹, T. Cherifi¹, C. Goetz¹, F. Malouin², M. Jacques¹, P. Fravalo¹;
¹Université de Montréal, St-Hyacinthe, QC, CANADA, ²Université de Sherbrooke, Sherbrooke, QC, CANADA.

**Background:** *Listeria monocytogenes* is an ubiquitous Gram-positive foodborne pathogen, and the agent of listeriosis. Its mode of growth in biofilm represents a source of resistance and contamination at all stages of the food-processing chain. These stable structures are composed of sessile bacterial communities surrounded by a self-secreted extracellular polymeric matrix, by which microorganisms aggregate and interact with each other. The aim of current study is to explore *in vitro* antibiofilm efficacy of molecules of various origins against *L. monocytogenes*. 

**Methods:** Four strains of *L. monocytogenes* were used in the present study. These strains were isolated from pork slaughterhouses and cutting facilities after sanitation procedures. Antibiofilm, dose-dependent activity was screened in a microtiter plate biofilm assay to determine active concentrations of tomatidine (plant compound), zinc chloride and EDTA (chemical compounds), and staphylococci exoproducts (bacterial compounds). Inhibition of *L. monocytogenes* biofilm formation was also evidenced using a microfluidic system (BioFlux 200) and confocal microscopy. Additional experiments were subsequently carried out in order to target the mode of action of these different antibiofilm molecules, such as bactericidal or bacteriostatic effects, autoaggregation, and bacterial motility. 

**Results:** All molecules were effective to inhibit *L. monocytogenes* biofilm formation under static (microtiter plates) and dynamic (microfluidic system) conditions. Active antibiofilm concentrations were found to be 144 µM for tomatidine, 1000 µM and 100 µM respectively for zinc chloride and EDTA and, 50% V/V for staphylococci preparations. Further experiments indicated that these molecules were affecting an early stage of biofilms' development (adhesion phase) but failed to disperse mature biofilms. This adhesion defect seems to be mainly due to an autoaggregation and motility disruption. 

**Conclusions:** Our study highlights the critical role of motility for *L. monocytogenes* initial surface attachment in the steps leading to biofilm formation. Overall, our results indicate that flagellum-mediated motility is a promising molecular target to develop new strategies against *L. monocytogenes* colonisation and biofilm formation in the food processing environment.
**Abstract Title:** Antibiofilm Strategies

**Poster Board Number:** 003

**Abstract Topic:** Herring Oil and Omega Fatty Acids Inhibit *Staphylococcus aureus* Biofilm Formation and Virulence

**Author:** J. Lee, C. Raorane, Y. Kim, J. Lee; Yeungnam University, Kyeongsan, KOREA, REPUBLIC OF.

*Staphylococcus aureus* is notorious for its ability to become resistant to antibiotics and biofilms play a critical role in antibiotic tolerance. *S. aureus* is also capable of secreting several exotoxins associated with the pathogenesis of sepsis and pneumonia. Thus, the objectives of the study were to examine *S. aureus* biofilm formation *in vitro*, and the effects of herring oil and its main components, omega fatty acids (cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) and cis-5,8,11,14,17-eicosapentaenoic acid (EPA)), on virulence factor production and transcriptional changes in *S. aureus*. Herring oil decreased biofilm formation by two *S. aureus* strains. GC-MS analysis revealed the presence of several polyunsaturated fatty acids in herring oil, and of these, two omega-3 fatty acids, DHA and EPA, significantly inhibited *S. aureus* biofilm formation. In addition, herring oil, DHA, and EPA at 20 μg/ml significantly decreased the hemolytic effect of *S. aureus* on human red blood cells, and when pre-treated to *S. aureus*, the bacterium was more easily killed by human whole blood. Transcriptional analysis showed that herring oil, DHA, and EPA repressed the expression of the α-hemolysin *hla* gene. Furthermore, in a *C. elegans* nematode model, all three prolonged nematode survival in the presence of *S. aureus*. These findings suggest that herring oil, DHA, and EPA are potentially useful for controlling persistent *S. aureus* infection.
Designing Rechargeable N-Halamine Nanoparticles for Biofilm Prevention

Biofilm formation is a serious problem in medical and industrial settings due to the increased resistance of these communities to killing compared to free-living bacteria. This has prompted the search for agents that can inhibit both bacterial growth and biofilm formation. In this study, N-halamine rechargeable nanoparticles (NPs) were synthesized by co-polymerization of the monomer methacrylamide and the cross-linker monomer N,N-methylenebisacrylamide, and were subsequently loaded with Cl\(^-\), using bleach. The chlorinated NPs demonstrated remarkable stability and durability to organic reagents and to repetitive bacterial loading cycles. The antibacterial mechanism of the P(MAA-MBAA)-Cl NPs involved generation of reactive oxygen species (ROS) only upon exposure to organic media, but not upon suspension in water, revealing that the mode of action is target-specific. Further, a unique and specific interaction of the chlorinated NPs with Staphylococcus aureus bacteria but not with human cells was discovered, whereby these microorganisms were all specifically targeted and marked for destruction. Finally, in collaboration with Netafim Ltd. irrigation drippers containing the P(MAA-MBAA)-Cl were incubated in the field and were shown to prevent fouling on them for 5 months compared with the control, hence providing the drippers with 'self-cleaning' and 'self-sterilizing' properties. Further, the NPs offer recharging to the surface, thus providing long-lasting protection that does not exist in the products available today. In summary, our findings underscore the potential of developing sustainable P(MAA-MBAA)-Cl NPs-based devices for inhibiting bacterial colonization and growth.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 007  
**Abstract Topic:** Antibiofilm Strategies  
**Abstract Title:** Towards Improved Measurements of Bacteria Viability in Biofilms  
**Author Block:** B. H. Jones¹, H. Iyer², S. J. Stranick¹, N. J. Lin³, J. P. Dunkers³;  
¹Materials Measurement Science Division, National Institute of Standards and Technology, Gaithersburg, MD; ²Statistical Engineering Division, National Institute of Standards and Technology, Gaithersburg, MD; ³Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD.

**Background:** Current methods for measuring bacteria viability in biofilms have challenges beyond what is encountered for planktonic cultures. The majority of live bacteria in biofilms are sessile, and current viability assays based on metabolism or membrane integrity often provide unclear results. Membrane voltage probes partition across the cell membrane in proportion to the membrane voltage strength and have been used as indicators of bacteria viability. In this work, we evaluate whether bis(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)), an anionic membrane voltage probe, can differentiate dead from sessile bacteria using fluorescence lifetime microscopy (FLIM). FLIM is very sensitive to fluorophore local environment and much less susceptible to photobleaching that affects intensity measurements. Here we use a stationary phase (SP) planktonic culture of Streptococcus mutans in buffer as a starting model system. The SP is our sessile component which is compared against heat killed S. mutans. We extract the FLIM variables for individual bacteria in both conditions and use statistical visualization and machine learning to evaluate the effectiveness of this approach to quantify bacteria viability.  

**Methods:** S. mutans (UA159, ATCC) were grown overnight in Todd Hewitt Broth (THB) at 37 °C, 5% CO₂ to SP and resuspended in phosphate buffered saline (PBS). For the dead control, the SP was exposed to 65 °C for 1 h. S. mutans (SP or dead) were incubated with 1 μM DiBAC4(3) at 25 °C for 20 min, washed, and resuspended in PBS. 5 μl of culture was sealed between a microscope slide and coverslip. Fluorescence lifetime decay images of S. mutans incubated with DiBAC4(3) were collected using a two-photon laser operating at 900 nm, 1 mW and time correlated single photon counting. The fluorescence lifetime response exhibited a double exponential decay. The effectiveness of fluorescence intensity, lifetime variables (t₁, t₂, p₁) and phasor values (G,S) to differentiate SP and dead bacteria was evaluated using statistical visualization and machine learning. Each data point is the average pixel value over an individual bacteria object. **Results:** Notch box plots of selected variables reveal that the median value for intensity is significantly different for individual SP and dead bacteria. The same is true for phasor variable G. For both variables, the distributions for SP and dead overlap, leading to possible uncertainty in assigning viability to any individual. Machine learning based on a random forest model using multiple variables provided a much more robust approach to classifying the viability of individual bacteria. **Conclusions:** We show that FLIM response of a membrane voltage probe has merit to distinguish heat killed from SP S. mutans in planktonic culture. This foundational study paves the way for improved methods for evaluating the effectiveness of antibiotics and antimicrobials for biofilms.
Background: Biofilm is a dynamic, heterogeneous cluster of cells encased in an extracellular matrix. Biofilm cells are encountered in almost all wet surfaces and cause serious problems in the food sector, medical devices etc. Biofilms are resistant to stress conditions as they form a layer of polymeric matrix (extracellular polymeric substances) around them which is difficult to penetrate for destroying the microbial cell. It has been reported that enzymes isolated from microbes can degrade the extracellular matrix of biofilm. Enzymes use environmental friendly approach for complex chemical transformations. They are highly selective biocatalyst which reacts with the specific components in the biofilm. The property of enzymes makes it a suitable candidate for biofilm removal, but free enzymes cannot be easily isolated from the reaction mixture, further, poor stability and reusability limit its applications. Therefore, enzymes need to be immobilized for a better performance. Magnetic CLEA (cross-linked enzyme aggregate) is a new technique for immobilization of enzyme which improves its stability, easy isolation from the reaction mixture, and it can be reused. In the present study, magnetic CLEA of pectinase (MCP) was prepared which is used for biofilm removal of gram-positive and gram-negative bacteria. Methods: Preparation of magnetic CLEA of pectinase MCP was prepared by incubating pectinase enzyme, with amino-functionalized magnetic nanoparticles. The mixture is then incubated at 30°C for 15 minutes at 150rpm in a shaking incubator. Then, ethanol and glutaraldehyde were added for precipitation and cross-linking. m-CLEA was separated using a magnet and used for further studies. Biofilm preparation and degradation Escherichia coli and Staphylococcus aureus were grown in a flask containing Luria broth medium and incubated at 37°C, 120 rpm for 24 hours. 200 µl of the microbial suspension containing 2.5 x 10^6 cells of E. coli and S. aureus were inoculated separately in 96 well plate and incubated without shaking at 27°C for 48 hours as performed earlier by Pitts et.al 2003. Different concentration of enzymes and MCP were added to the wells. Well without any treatment was taken as blank. The absorbance was taken at 595nm by microtiter plate reader. Results: MCP was prepared which shows thermal stability at 70°C and no loss of enzyme activity was observed in magnetic CLEA until the sixth cycle. The percentage degradation of E.coli biofilm by free pectinase and MCP at the concentration of 200U was observed to be 61.8% and 51.52% respectively. Similarly, percentage degradation of biofilm of S. aureus by free pectinase and MCP was observed to be 52.46% and 40.14% respectively. Conclusions: MCP is successful in degrading biofilm of E. coli and S.aureus up to most extent, therefore it can be helpful in removing biofilms in storage tanks in industries, medical devices, etc and can be commercialized.
Background: Biofilms have raised significant public health concerns and economic losses. For example, pathogenic biofilms are believed to result in hospital acquired infections and foodborne diseases. Compared with conventional disinfection strategies, photocatalysis holds promise for a biofilm control because of broad spectrum effectiveness under ambient conditions, low cost, easy operation and maintenance. We develop graphitic carbon nitride (g-C₃N₄), an emerging visible-light-responsive photocatalyst, for biofilm inhibition and eradication, and understand the mechanism of photocatalytic biofilm control. Methods: g-C₃N₄ powder was synthesized through thermal polycondensation of melamine, cyanuric acid, and barbituric acid and g-C₃N₄ coupons were fabricated via hydraulic press of the powder. Staphylococcus epidermidis (S. epidermidis), a Gram-positive bacterium, was selected as a model pathogen to grow biofilms on the coupons. Biofilms were grown in the dark and under visible light irradiation to understand biofilm inhibition by photocatalysis, and mature biofilms were also explored for eradication under visible light exposure. Optical coherent topography (OCT), confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM) were used to characterize the thickness, coverage, morphology, and viability of the biofilms. Atomic force microscopy (AFM) was used to characterize the mechanical properties of the biofilms. In addition, a fluorescent lectin, polysaccharide intercellular adhesin (PIA), was utilized to evaluate the presence and content of extracellular polymeric substances (EPS) of the biofilms. Reactive oxygen species (ROS) generated were quantified using radical probes to explore biofilm control mechanisms. Results: OCT and CLSM suggested that g-C₃N₄ coupons inhibited biofilm development and eradicated mature biofilms under the irradiation of white LED light. Compared to mature biofilms developed in the dark, photocatalysis reduced the EPS content based on SEM and PIA staining. In addition, biofilms after photocatalysis became stiffer, which could also be explained with the loss of EPS. H₂O₂ and ¹O₂ were identified as the main ROS that control biofilm development and eradication, not only inactivated bacterial cells but also weakened chemical bonds within the EPS matrix. A mechanism of ROS diffusion into and inactivation of the biofilms was proposed based on a biofilm eradication kinetics study. Conclusions: g-C₃N₄ holds promise for anti-biofilm applications including both biofilm inhibition and eradication under visible light irradiation. Photocatalysis controls biofilms via simultaneous bacterial inactivation and EPS decomposition under ambient conditions, and it can potentially use indoor visible lighting to reduce the cost, energy consumption, and chemical footprint for sustainable antimicrobial applications.
Quorum sensing (QS) is a crucial mechanism involved in pathogenesis of numerous bacterial infections. Emergence of multi drug resistance is increasing every day and hence it is essential to develop novel strategies against these super bugs. LasR, a transcriptional regulator that plays a vital role in regulation of QS and pathogenesis of *Pseudomonas aeruginosa*. The present study reports a novel urea tailed Mannich base (1-(phenyl (o-tolylamino) methyl) urea with enhanced quorum sensing inhibition. The synthetic compound revealed prolific interactions with LasR quorum sensing receptor and exhibit LasR mediated antagonistic activities in *P. aeruginosa*. In-vitro LasR-based inhibitory activities were further confirmed by biofilm and pyocyanin inhibition assays. In-silico and structure activity relationship studies confirm that the urea moiety present in the Mannich base plays a vital role in antagonizing the LasR receptor by forming a discrete H-bond with Tyr47 residue in the active site and also the presence of carbonyl group in the Mannich base is a discerning advantage. Overall, the findings will be useful in the development of urea-based drugs against *P. aeruginosa* infections in the future.
**Background:** Periprosthetic joint infections (PJI) and chronic pain remain challenging post-arthroplasty complications, significantly affecting patients’ quality of life. While the prevalence rate of PJI is low, the mortality rate associated with these infections is estimated at 25%. Post-arthroplasty chronic pain is more common, and is often addressed with systemic opioids, which have serious side effects. We propose the local delivery of analgesics from ultra-high molecular weight polyethylene (UHMWPE) bearing surfaces to address post-arthroplasty pain more effectively with minimal side effects. Given that various analgesics were shown to possess antimicrobial activity against *Staphylococci* – species commonly causing PJI – we hypothesize here that an analgesic-loaded UHMWPE can also provide antibacterial properties to mitigate biofilm formation relevant to multimodal therapy for preventing PJI after an arthroplasty.

**Methods:** Minimum inhibitory concentrations (MIC) of bupivacaine, lidocaine, and ropivacaine were determined using the micro-dilution method. Bupivacaine was incorporated into medical grade UHMWPE at 10, 15, and 20 wt% via phase-separated compression molding. Drug elution studies were conducted in deionized water for up to 3 days. The agar diffusion test was used for the preliminary assessment of antimicrobial properties of analgesic-loaded UHMWPE against methicillin-sensitive *S. aureus* (MSSA). Anticolonizing activity of the tested materials was initially assessed using crystal violet (CV) assay. Further, the dynamics of bacteria attachment over the 48-hour period was evaluated. The obtained biofilms were additionally visualized using scanning electron microscopy (SEM).

**Results:** Bupivacaine was shown to possess the most pronounced antimicrobial activity against MSSA, and thus, it was chosen for incorporation into UHMWPE matrix to render anticolonization properties. The obtained drug release kinetics suggested that the effective daily dose of bupivacaine delivered from the UHMWPE matrix (30 – 300 mg) falls above its MIC value for at least 3 days. The agar diffusion test confirmed that bupivacaine-loaded UHMWPEs possesses dose-dependent antibacterial properties against MSSA. The CV assay showed antibiofilm activity of the anesthetic-loaded samples, which significantly increases with the increase of bupivacaine content (p < 0.05, n = 5). Further, we showed that drug-loaded UHMWPEs can significantly mitigate bacterial adhesion and biofilm development, which was additionally supported by the SEM images.

**Conclusions:** To the best of our knowledge, this is the first study showing that bupivacaine-loaded UHMWPE possess anticolonizing activity. Therefore, anesthetic-loaded UHMWPE bearing surface is a good example of a multifunctional approach addressing post-operative pain and providing antibacterial prophylaxis after an arthroplasty.
Abstract

Development of an Antibiofilm High Content Screening Assay Against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Author Block: C. H. Franco, B. Wenyi, C. B. Moraes, L. Freitas-Junior; Institut of Biomedical Sciences from University of São Paulo, São Paulo, BRAZIL.

Abstract Body: Nosocomial infections are a major public health issue worldwide, leading to high mortality ratio and increased hospital costs. These infections are caused by a range of microbes, mostly bacteria, among which those in the ESKAPE group (Enterococcus faecium, *Staphylococcus aureus*, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) are responsible for the majority of hospital-acquired infections and strongly associated with high tolerance to most antibiotics available. Despite antibiotics resistance and tolerance pose arguably one of the greatest threats to global health, the R&D pipeline for new antibiotics remains extremely limited. Therefore, the discovery and development of new antibiotics is critically needed. A major complication of bacterial infections is the formation of bacterial communities known as biofilms. Biofilm infections can either cause or contribute to common and complicated infections such as chronic otitis media, recurrent tonsilitis and sinusitis, burns and skin infections, periodontal disease, ear infections, endocarditis, and pneumonia in intensive care unit or in cystic fibrosis patients. Biofilms are also associated with several device-related infections, given their capability of adhering and growing in abiotic surfaces (such as on catheters, bone and joint prosthetics, intrauterine devices, among others). Formation of biofilms increases the bacterial population survival despite the individual bacterial genotypes present in the population (resistant or sensitive to the drug), and in some cases antibiotics can even prompt the survival of bacterial biofilms due to drug tolerance, a phenomenon that is not yet fully understood. The project aims at developing an *in vitro* screening assay for anti-biofilm compounds against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The assay will be developed using high content technology and can be used to determine compound activity and classify them as biofilm-formation inhibitors or biofilm disruption agents (or both). Once developed, the anti-biofilm high content assay will be used for compound triage from Brazilian pharmaceutical companies synthetic libraries. Also, the prospected hit compounds will be characterized and evaluated for human cells cytotoxicity, mechanism of action and resistance induction.
**Background:** Periodontitis is a chronic biofilm-mediated disease caused by dysbiosis of the oral community from a healthy to pathogenic state by pathogens such as *Porphyromonas gingivalis* (*Pg*), which over time can cause systemic disease and bone loss. *Pg* itself cannot establish an independent biofilm *in vivo*, but instead requires an established biofilm community with specific partners e.g. *Streptococcus gordonii* (*Sg*). Biofilms universally contain extracellular DNA that is stabilized by the ubiquitous DNABII family of proteins. Antibodies directed against DNABII proteins sequester free DNABII proteins which causes catastrophic collapse of the biofilm. We have found that antibodies directed against one member of the DNABII family have high avidity to other members of the DNABII family. Indeed, *Sg* comprises a single highly conserved DNABII protein, HU*Sg*, that, similar to most tested DNABII family members, is recognized by an extensive panel of DNABII antisera assayed to date. In contrast, *Pg* expresses an antigenically unique DNABII protein, HUβ*Pg* that is only recognized by antibodies raised against it. 

**Methods:** Western analysis, immunofluorescence and confocal laser scanning microscopy was used to characterize the roles of DNABII proteins in mixed species biofilms of *Pg* and *Sg* *in vitro* where *Pg* is added to established *Sg* biofilms to mimic *Pg* entry into an extant biofilm in the oral cavity. Furthermore we introduce a novel rat model to study periodontal disease, using a dual species biofilm of induced osteolytic infection. *Pg*, *Sg*, and *Pg*-*Sg* dual species biofilms (*Pg* added to extant *Sg* biofilms) were grown on the heads of titanium implant screws (sterile screws served as negative controls) and surgically implanted into the maxilla alveolar bone. 

**Results:** We demonstrated that both extracellular HUβ*Pg* and HU*Sg* are increased in dual species biofilms compared to mono-species biofilms *in vitro*. Strikingly, HUβ*Pg* is more abundant than HU*Sg* (>3 fold), even when *Pg* represents only 5% of the biofilm population. Addition of HUβ*Pg* antibodies to *Pg*-*Sg* biofilms resulted in a significant reduction of *Pg* within the dual species biofilm with minimal disturbance of the underlying *Sg* biofilm. In *vivo*, 14 days post implantation, micro computed tomography was used to measure bone loss surrounding the screws, where only rats that harbored *Pg* as both mono and dual species biofilms showed bone loss. qPCR from DNA extracted from the screws as well as the tissue and bone surrounding the screws revealed that *Pg* and *Sg* were present 14 days post screw implantation. 

**Conclusions:** The *in vitro* outcomes suggest that HUβ*Pg* antibodies could be used for selective treatment to target *Pg* for elimination, with minimal disturbance of healthy commensals (*Sg*). Our *in vivo* studies validated our animal model for the native sequential events that lead to *Pg* entry into an extant *Sg* biofilm and a means to test our selective anti-*Pg* therapeutic *in vivo*. 
Session Title: THURSDAY Poster Session 4
Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 015
Abstract Topic: Antibiofilm Strategies
Abstract Title: Interactions of Gold Nanoparticles, Chitosan and their Combination with Bacterial Biofilms
Author Block: O. Chlumsky, V. Fuchsova, H. Michova, K. Demnerova;
University of Chemistry and Technology Prague, Prague 6, CZECH REPUBLIC.

Biofilms are currently the most emerging issue regarding food safety. The most significant is the ability of microorganisms to adhere and grow up on foods and surface which they are in contact with. Biofilm formation usually occurs when microorganisms are not completely removed from the surface. Biofilm matrix provides higher protection against negative impact of external conditions in comparison with planktonic growth. Cells in biofilm have more than thousand times higher resistance against antimicrobial substances. To avert biofilm formation, it is necessary to apply different preventive and control strategies. This study was aimed on selection and testing of gold nanoparticles, chitosan and their combination to effectively suppress the adhesion of microbial cells, and thus eliminate the biofilm formation. Staphylococcus aureus 816, 1241; Listeria monocytogenes 149, 164; Escherichia coli 683/17, 693/17 and Salmonella spp. S13, S59 strains isolated from food processing facilities were used for testing. The ability of planktonic cells to adhere and create biofilm were studied after treatment with gold nanoparticles, chitosan and their combination. Biofilm development after pre-cultivation and its eradication were also tested. Biofilm of S. aureus 816, 1241; L. monocytogenes 149, 164 and E. coli 683/17, 693/17 were cultured at 37°C in tryptone soya broth. Salmonella spp. S13, S59 were cultivated at 25°C in the brain heart infusion broth. Cultivation was performed in a pre-sterilized, polystyrene, 96-well, flat-bottomed microtiter plate. Optical density and absorbance were measured spectrophotometrically. Optical cell density was assased at 620 nm. The biofilm quantity after crystal violet staining and metabolic activity of cells were observed at 595 nm. From the performed analyzes, minimum inhibitory concentration (MICₘ) and minimum biofilm eradication concentration (MBECₘ) of gold nanoparticles, chitosan and their combination were established. We determined the MICₘ of gold nanoparticles to planktonic cultures of S. aureus 816, 1241, and of chitosan to S. aureus 1241; L. monocytogenes 149, 164 and Salmonella spp. S13, S59. The MICₘ of gold nanoparticles modified by chitosan were defined for planktonic S. aureus 816 and Salmonella spp. S13, S59 strains. The MBECₘ of gold nanoparticles was not yet established to any of the tested strains. The MBECₘ of chitosan was determined for S. aureus 816, 1241 and L. monocytogenes 149, 164, and of modified gold nanoparticles to S. aureus 1241. Our results indicate higher effects of chitosan than gold nanoparticles on planktonic cultures and biofilms. No effect was observed for gold nanoparticles modified by chitosan. This study has been supported by GACR project 17-15936S and specific university research (MSMT No 21-SVV/2018).
**Abstract Body:**

**Background:** The formation of biofilms by bacterial pathogens presents a major obstacle in the treatment of a wide variety of infectious diseases, as biofilm formation confers increased resistance to harsh environmental conditions, host immune effectors, and antibiotic therapies relative to the planktonic phenotype. *Salmonella enterica* serovar Typhi, the etiological agent of Typhoid fever, is able to establish asymptomatic chronic infection in the human gallbladder by forming biofilms that are recalcitrant to conventional antibiotic therapy. These chronic carriers serve as the major reservoir for the bacteria and aid in typhoidal transmission via fecal shedding. The identification of novel strategies to inhibit S. Typhi biofilms is therefore of the utmost importance towards the prevention of chronic S. Typhi infections. **Methods:** In order to identify compounds capable of inhibiting S. Typhi biofilms, we screened 90 kinase inhibitor derivatives for their ability to inhibit biofilm formation in the related *S. enterica* serovar Typhimurium, resulting in the identification of the small molecule T315. EC\textsubscript{50} concentrations for inhibition of both *S*. Typhimurium and *S*. Typhi biofilms by T315 were determined, and inhibitory activity against additional Gram-negative biofilms was tested. T315 anti-biofilm activity against *S*. Typhimurium and *S*. Typhi was further examined by testing the compound in the presence of sub-MIC doses of ciprofloxacin. In an effort to identify the bacterial target(s) of T315, biotinylated T315 probes were synthesized and used to pull down putative targets from *S*. Typhimurium lysate. **Results:** Biofilm inhibitor T315 EC\textsubscript{50} values were 21.0 and 7.4\(\mu\)M for *S*. Typhi and *S*. Typhimurium, respectively. T315 also exhibited anti-biofilm activity against the nosocomial pathogen *Acinetobacter baumanii* but did not reduce biofilm formation in *Pseudomonas aeruginosa*, suggesting some level of specificity. Additionally, exposure of *Salmonella* to a combination of T315 and sub-MIC doses of ciprofloxacin resulted in an augmented reduction in biofilm formation. Proteomic analysis of pull-down fractions identified the flavin mononucleotide-dependent NADH:quinone oxidoreductase WrbA as a bacterial target of T315. Using a \(\Delta\)wrbA mutant of *S*. Typhimurium, we showed that this protein is involved in *S*. Typhimurium biofilm formation and that it is required for maximum T315 anti-biofilm activity. **Conclusions:** We describe for the small molecule T315 a novel application as a *S. enterica* biofilm inhibitor and demonstrate that the use of such anti-biofilm compounds in combination with sub-MIC doses of antibiotics is a promising strategy to combat biofilm-mediated infections such as the chronic carriage of typhoidal *Salmonella*.
Developing a Small Molecule Inhibitor of *Pseudomonas aeruginosa* biofilm Exopolysaccharide Deacetylase Pela

**Background:** *Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen and a leading cause of pulmonary and nosocomial infections. The success of *P. aeruginosa* as a pathogen stems, in part, from its ability to grow in a biofilm. One of the exopolysaccharides utilized by *P. aeruginosa* to form a biofilm is Pel, a β-(1,4)-linked polymer composed of partially deacetylated N-acetylglucosamine and N-acetylgalactosamine. This cationic polysaccharide binds to eDNA in the base of the biofilm forming a structural core. Pel is crucial for maintaining cell-to-cell interactions, and providing protection against aminoglycoside antibiotics. Each of the proteins encoded by the *pel* operon, *pelABCDEFG*, are required for the biosynthesis and export of the polysaccharide. During biosynthesis Pel is chemically modified by PelA; a multi-domain periplasmic protein that contains a carbohydrate esterase family 4 (CE4) deacetylase domain. De-N-acetylation of the hexosamine sugars in Pel imparts the positive charge to the polymer, and mutation of deacetylase catalytic residues abrogates biofilm formation. This suggests that de-N-acetylation is an important process in Pel biosynthesis, and a potential therapeutic target to prevent Pel-dependent biofilm formation.

**Methods:** To identify inhibitors of PelA deacetylase activity, a high-throughput targeted enzyme assay screen of 68,666 compounds was completed. This screen identified 56 compounds that were classified as hits capable of significant inhibitory activity *in vitro*. These compounds have been assessed for biofilm inhibition using a secondary *ex vivo* biofilm assay.

**Results:** Four compounds were found to prevent biofilm formation in a Pel overexpression strain. One of the compounds was able to inhibit biofilm formation in several Pel-dependent strains in a dose-response manner. A small library synthesized to explore structure-activity relationships (SAR) of this compound identified an analog that inhibits Pel-dependent biofilm formation and has low cytotoxicity to human lung fibroblast cells. **Conclusions:** The high-throughput screen and subsequent SAR study has identified a low cytotoxic compound able to inhibit Pel-biofilms. Continued SAR will identify the pharmacophore and allow further optimization. Steady-state enzyme kinetics are in progress to determine the mode of inhibition. Structure determination of PelA will advance our understanding of the mode of inhibition and aid in the development of second generation inhibitors.
Abstract Topic: Antibiofilm Strategies
Abstract Title: Membrane Integrity Based Viability Staining with DNA-binding Propidium Iodide Underestimates Viability of Sessile Bacterial Cells on Glass

Author Block: M. Rosenberg¹, N. F. Azevedo², A. Ivask³; ¹National Institute of Chemical Physics and Biophysics, Tallinn, ESTONIA, ²University of Porto, Porto, PORTUGAL, ³Ministry of Social Affairs, Tallinn, ESTONIA.

Background: Intact membrane impermeable DNA-binding stain propidium iodide (PI) is widely used for bacterial viability staining in combination with membrane-permeable DNA-binding counterstains. Although some criticism to the method has arisen concerning for example counterstain fluorescence intensity differences for viable and dead cells and energy transfer during co-staining, the principle of such co-staining has proven to be quick, reliable, commercially available, and widely used for planktonic cultures. PI-based viability staining has also been used for sessile bacteria in biofilms where staining offers a valuable tool as culture-based methods for viability assessment in biofilms, especially in multispecies or environmental settings, are limited. Methods: Monolayer aggregates of 24 h old Staphylococcus epidermidis DSM-20044 or Escherichia coli MG1655 biofilms on glass in phosphate buffered saline (PBS) were either rinsed, stained with 30 µM PI and 5 µM Syto 9 in situ or cells were harvested via sonication, stained and filtered. Samples were incubated in dark for 15 minutes prior to epifluorescence microscopy. Staining with 25 µg/ml fluorescein diacetate and plate counts were used as controls. Results: In situ stained 24 h biofilms consist of 75.69±18.44% to 96.35±5.3% PI-positive red cells for S. epidermidis and E. coli respectively even though 68% the cells of either species in these aggregates are metabolically active when stained with fluorescein diacetate (FDA) while 99% or more planktonic cells above the surface stain green when co-stained with PI and Syto 9. Higher biofilm viability estimates with 19.56±8.93% to 43.50±5.30% PI-positive red cells for S. epidermidis and E. coli respectively were achieved after harvesting adherent cells via sonication probably due to extracellular matrix being removed during the process but the results still underestimated viability compared to 82% of harvested E. coli and 89% of S. epidermidis being cultivable. Confocal laser microscopy reveals that this false dead layer of red cells consists of cells that have green interiors under red coating layer which hints at extracellular DNA being stained outside intact membranes. Conclusions: In this study we show that PI-based viability staining significantly overestimates dead cell counts in 24 h E. coli and S. epidermidis biofilms formed on glass in PBS. We conclude that extracellular DNA that is needed for biofilm formation and attachment to surfaces and its possible impact on PI-based viability staining outcome must be considered and controlled for to avoid significant overestimation of dead cells in biofilms. This finding has critical impact on estimation of biofilm viability especially in antimicrobial surface efficiency testing as well as assessment of biofilm viability in general.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 019

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Estuarine Suspended Sediment Biofilm as a Microbial Refuge

**Author Block:** J. Calomiris, Sotiria Science, Arnold, MD.

**Abstract Body:**

**Background:** Aquatic biofilm can function as a refuge microcosm enabling pathogens to survive austere conditions and evade control measures. Biofilm of water systems such as drinking water distribution networks and cooling towers typically predominate as surface-associated structures. Alternately, estuarine biofilm can exist as loose, untethered migratory masses that deposit upon sediment beds, resuspend into water columns, and travel with tidal currents to reach distant sites. In this study, estuarine suspended sediment biofilm parameters (architecture, diffusivity, and disinfectant reactivity) were examined to develop strategies for effective control of microbes of complex waters to address recent national and global regulations for ship ballast management.

**Methods:** Samples of estuarine water with suspended sediment were collected from a recreational beach of a Chesapeake Bay tributary (Magothy River). Suspended sediment biofilm and extracellular polymeric substance (EPS) structures were characterized by brightfield and polarized light microscopy. Intact suspended sediment and sample dispersed by physical and chemical treatments were compared to elucidate biofilm properties. Dispersion was quantified by increases in microbe-size particles (MSP), heterotrophic plate count (HPC) bacteria (R2A medium), UV-absorbing substances (UVAS), and turbidity. Rates of solute diffusion and disinfectant reactivity by intact and dispersed biofilm were derived by uptake of a cationic compound (methylene blue) and consumption of free available chlorine (FAC), respectively.

**Results:** Suspended sediment consisted primarily of floccular biofilm masses harboring large numbers of MSP and HPC bacteria. EPS association with suspended sediment was suggested by birefringence of MSP and diffuse material within and at the periphery of the floccular masses. Effective dispersion of suspended sediment was demonstrated by microscopic examination and significant release of MSP, HPC bacteria, and soluble UVAS. Rates of methylene blue uptake and FAC consumption were much greater with dispersed suspended sediment than with intact suspended sediment. Dye uptake displayed first-order kinetics before reaching equilibrium. FAC consumption appeared as first-order kinetics initially (first min) followed by a mixed-order reaction period prior to equilibrium.

**Conclusions:** Estuarine suspended sediment as biofilm masses harboring large numbers of microorganisms can be recalcitrant to disinfectant treatment and present challenges to microbial control operations. Strategies that combine dispersion with disinfection could provide the basis for more effective biofilm treatment. In addition to supporting efforts to meet recent ship ballast microbial control regulations, the model biofilm system and findings of this study could be applied to control other complex water systems.
Delivering Antibiotics Locally to Biofilms: Targeted Drug Delivery vs. Local Synthesis from Non-toxic Prodrugs

Background: Antibiotic treatment of biofilm infections often fail because the dose that can be delivered without adverse side effects is not high enough to eradicate the biofilm. We explore two different approaches to delivering a high local dose of antibiotics at the site of infection: Targeted drug delivery and local drug synthesis from nontherapeutic prodrugs. Targeted drug delivery relies on drug encapsulation and accumulation in the biofilm, followed by burst release of the drug. Local drug synthesis, however, uses immobilized enzymes to convert less toxic prodrugs to the active drug at the site of infection. This approach was not previously available for antibiotics, but our development of a novel synthesis method for generating glucuronide-prodrugs of two fluoroquinolones have opened the door for using prodrug therapy in microbiology.

Methods: For targeted drug delivery, we encapsulated vancomycin and rifampicin in temperature-sensitive 100 nm liposomes decorated with aptamers that bind specifically to Staphylococcus aureus biofilms. Particle accumulation, drug release, and kill efficiency was studied on S. aureus biofilms. Local drug synthesis was achieved by immobilizing the enzyme catalyst (β-glucuronidase) in a polyelectrolyte layer-by-layer coating on titanium surfaces representing an implant. Drug release was achieved by supplying prodrugs of moxifloxacin in solution, and the effect on bacterial growth, survival, and biofilm formation was studied on S. aureus. Results: Drug-loaded aptamer-targeted liposomes accumulated in S. aureus biofilms and resulted in eradication of biofilms in vitro, while non-targeted liposomes were less effective. Although this result is promising, one could argue that the released drug will diffuse out of the biofilm in vivo, resulting in short exposure. We therefore investigated the effect of continued drug synthesis at the implant surface, using prodrug therapy. The embedded enzyme quickly converted the prodrug to moxifloxacin, which inhibited bacterial growth near the implant surface and prevented biofilm formation. The antimicrobial effect of prodrug therapy on pre-formed biofilms was comparable to the effect of the active drug at the same concentration. Advantages of prodrug therapy can therefore be expected if prodrugs can be administered in higher doses than conventional drugs due to their lower toxicity. Conclusions: Targeted drug delivery and pro-drug therapy are both able to expose biofilms to high local antibiotic concentrations. Drug release is better controlled in prodrug therapy, but it requires the placement of a catalyst near the biofilm unless it is already embedded in the implant. Future developments of long-term stable catalysts may therefore determine which of these strategies that is better suited for treatment in vivo. This work paves the way for advances in the use of locally induced antimicrobial treatment.
**Background:** The increased incidence of *Staphylococcus Aureus* (*S. aureus*) biofilm infections that are resistant to various antimicrobial drugs necessitates the development of novel treatment strategies. It has been shown that hyperthermia treatment to increase the temperature up to 45°C could enhance the antibiotic susceptibility of *S. aureus*. The question is how heat can be applied topically without risking thermal burns in the host tissue. We previously validated a magnetic nanoparticle thermotherapy platform that can target and substantially reduce the viability of bacterial pathogen using both *in vitro* and *in vivo* mouse model of *S. aureus* infection. The principle of this method is to induce a localized increase in temperature in bacteria by targeted activation of magnetic nanoparticles (MNPs) with externally applied energy source of high frequency alternating magnetic field (AMF). In this study, we have examined whether MNP/AMF hyperthermia treatment can be synergistic with conventional antibiotic therapy on *S. aureus* biofilm. **Methods:** Varying concentrations (1-3 mg/mL) of MNPs (100 nm, super paramagnetic nanoparticles, Micromod Inc.) were added to the wells of biofilm formed by *S. aureus* (2x10^8 CFU, ATCC 6538) and incubated for 2 hour. Then, the samples were treated with an AMF for 6 min duration at a field strength of 30 kA/m and at a frequency of 2.1 MHz. Following the application of MNP/AMF, biofilms were treated with antibiotics (ciprofloxacin and vancomycin) for 24 hours, at MIC determined from the planktonic phase of *S. aureus*. **Results:** The MIC values of ciprofloxacin and vancomycin for planktonic phase of *S. aureus* (2x10^8 CFU) were measured to be 16 μg/mL. However, biofilm phase of *S. aureus* exhibited ~100-fold higher resistance to both antibiotics than those seen in planktonic culture. The application of AMF to the *S. aureus* biofilm resulted in increase of ambient temperature in the solution via MNP concentration dependent manner (from 23°C to 39°C for 1 mg/mL, to 44°C for 2 mg/mL, and to 48°C for 3 mg/mL MNPs over 6 min exposure of AMF), which was associated with a concomitant reduction in bacterial CFU number (by 1 log reduction for 2 mg/mL and 2 log reduction for 3 mg/mL MNPs). The pretreatment of MNP/AMF hyperthermia to the biofilm phase of *S. aureus* has significantly augmented the antibiotic susceptibility of *S. aureus* biofilm, up to 5 log reduction in CFU number for ciprofloxacin and vancomycin at 16 μg/mL, while antibiotics alone did not alter CFU numbers. This was associated with increased uptake of antibiotics to the bacterial cells and generation of reactive oxygen species. **Conclusion:** Our study validates that non-invasive MNP/AMF hyperthermia can be successfully applied to eradicate biofilm infections. Importantly, the combined use of MNP/AMF hyperthermia with antibiotic treatment can synergistically improve treatment efficacy compared to either therapeutic approach alone.
Session Title: **THURSDAY Poster Session 4**
Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 022
Abstract Topic: Antibiofilm Strategies
Abstract Title: Silver Carboxylate Complex-Eluting Skin Surgical Prep Eliminates Methicillin-resistant *Staphylococcus aureus* and *Propionibacterium acnes* Colonization
Abstract Author: D. R. Garcia, T. Li, D. Roque, C. T. Born;
Abstract Block: Brown University, Providence, RI.

**Background:** Post-operative *Propionibacterium acnes* (P. acnes) and Methicillin-resistant *Staphylococcus aureus* (MRSA) surgical site infections (SSIs) continuously prove to be hard to prevent and treat. These infections can be attributed to inadequate skin prep techniques that fail to eradicate bacteria at the site of injury. The current methods of sterilizing a patient’s body include the application of an antiseptic compound that can include Chloraprep, Duraprep, or Betadine. However, it has been shown that P. acnes and MRSA may be resistant to preparations using these compounds as strains of Chlorhexidine-resistant pathogens have been emerging in hospital environments. This presents a problem as incisions made through skin (ie: scalpel or sutures) can inoculate the incision site with bacteria; allowing biofilm formation and infection. This study validated the efficacy of a silver carboxylate antimicrobial skin prep penetrance into the dermis and the pilosebaceous glands of porcine skin and as an effective antimicrobial agent against P. acnes and MRSA.

**Methods:** Kirby Bauer assay was performed with 10mm Whatman filter paper as a vehicle. MRSA was plated at a concentration of 1E9 CFU/mL. P. acnes was plated at a concentration of 1E7 CFU/mL. Zones of inhibition for MRSA were visualized at the 24, 48, and 72-hour time points. P. acnes visualization was offset by an additional 24 hours. With IACUC approval, the axillary regions of 12 Yorkshire and Yucatan pigs were treated on 3 sites with Chlorhexidine alone, Silver carboxylate alone, and silver carboxylate coating followed by Chlorhexidine. The pig skin was excised, fixed in 10% neutral buffered saline, and stained with Sirius fast-red/fast-green. Penetrance of silver hybrid skin prep and Chlorhexidine was measured via ImageJ. **Results:** The Silver Carboxylate Skin Prep is effective at completely inhibiting MRSA and P. acnes growth up to 72 and 96 hours respectively. Higher concentrations of the skin prep showed the ability to eliminate all bacteria in 24 hours. Additionally, Chlorhexidine was found to have no penetration into the pilosebaceous glands of the pig skin whereas the silver carboxylate skin prep was shown to penetrate the pilosebaceous glands and deposit antimicrobial silver. **Conclusion:** The Silver Carboxylate Skin Prep may be more effective in a clinical setting against P. acnes and MRSA than currently utilized surgical site preps due to its high bactericidal activity and ability to penetrate deeper into the skin.
Next Science It is widely documented that biofilm is present in the oral cavity as dental plaque. Not only is dental plaque difficult to remove using physical means such as brushing and flossing, but microorganisms that promote diseases such as gingivitis can be found on soft tissue surfaces in the mouth. As physical removal techniques are commonly not used on soft tissues, it is suggested that oral rinse solutions be used to control the microorganisms present thereby delaying the formation of dental plaque and development of gingivitis. Next Science has developed a mouthwash using antibiofilm technologies that aim at removing this dental plaque and reducing prevalence of gingivitis. In-vitro tests were conducted on biofilms produced in a drip-flow reactor from spit collected samples. The Next Science product was tested alongside Listerine® and Perioguard® with the Next Science formulation showing equivalent efficacy to Listerine against Candida albicans with a 5-log reduction in biofilm. The Next Science product showed superior efficacy to Listerine® against both subgingival (2.8 log reduction) and supragingival (1.5 log reduction) bacterial biofilms. The Next Science mouthwash and Listerine® both outperformed Perioguard® against all microorganism types. Clinical studies were completed with 200 patients demonstrating active gingivitis. Plaque scores, gingivitis indices and the composition of oral microbial flora were determined following 6 and 12 weeks of product use. A significant reduction in plaque (p<0.05) was demonstrated though the reduction in plaque did not show an improvement in the gingival index (p>0.35). The DNA analysis for composition of microbial flora is ongoing. These data suggest that the formation of plaque may play a role in development of gingivitis though that role may be minor.
Numerous bacteria communicates using small, diffusible signalling molecules to adapt to environmental challenges. A fascinating bacterial communication system is quorum sensing (QS), a system used to regulate gene expression, and thereby coordinate actions in a cell density-dependent manner. Bacteria constantly produce signaling molecules, whose concentration increase proportionally with cell density: when a specific cell density is reached, termed “quorum”, a certain concentration of the signaling molecule is reached and will result in a population-wide changes in behavior. These changes, in bacterial pathogens, relate to the expression of virulence factors and the formation of biofilms. Interfering with bacterial communication is an appealing strategy in our fight against multi-antibiotics resistant strains and bacterial biofilms. We have identified enzymes, called lactonases that can efficiently degrade bacterial signalling molecules, and interfere with bacterial communication. These enzymes, isolated from hyperthermophilic organisms, are extremely stable and resistant to harsh chemical processes. We have engineered these enzymes to further increase their stability and efficiency against specific signalling molecules, and demonstrated that they effectively inhibit biofilm formation and virulence factors production. We will show their effects on complex microbial communities and highlight that the availability of highly stable enzymatic quenchers opens up new fields of study, including biofilm and biofouling studies in complex community contexts, and in vivo studies in animal and plant models.
Abstract Topic: Antibiofilm Strategies
Abstract Title: Atmospheric Pressure Plasma Decontamination of Clin. Candida parapsilosis Biofilm Grown on Silicone

Author Block: A. Doria¹, F. Figueira¹, J. Lima¹, H. Maciel¹, S. Khouri¹, R. Pessoa³; ¹Universidade do Vale do Paraíba, Sao Jose dos Campos, BRAZIL, ²Universidade de Taubaté, Taubaté, BRAZIL, ³Instituto Tecnológico de Aeronáutica, Sao Jose dos Campos, BRAZIL.

Background: Biofilm formation in central venous catheter is one of the main causes of bloodstream infection of hospitalized patients. Currently, this represents an important global public health problem, leading to death of 25 to 38% of patients, where the yeasts of the genus Candida spp are the most frequently isolated. Such infections exhibit an increased tolerance for antifungal agents, biocides and immunological variations, making it difficult to treat with conventional therapeutic agents rendering remediation even impossible. In biomedical area, atmospheric plasmas have shown great potential in several applications, however, one of the main researches focuses on microbial inactivation, both in the form of biofilms and planktonic cells of bacteria or fungi. It has been shown that plasma efficacy is due to reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Methods: This study aims to evaluate the antimicrobial action of a plasma jet generated by a surfatron source using different gas compositions on silicone samples contaminated with clinical Candida parapsilosis biofilm. The strain was obtained in a previous study duly approved by the Ethics Committee (CEP/ UNITAU No. 542/11). The samples were treated with plasma of 4 SLM of Ar + 6 SLM of air at 100W of power (group 1), 4 SLM of Ar + 9 SLM of Ar plus water vapor at 50W (group 2) and 4 SLM of Ar + 9 SLM of Ar plus water vapor at 150W (group 3). Treatments were performed in the post-discharge region (30 mm) during 10 min. The characterization of the plasmas/substrate surface was performed by optical emission spectroscopy (OES) and IR camera, while for the treated samples was used colony forming units (CFU) counting and scanning electron microscopy (SEM).

Results: Temperature monitoring showed that no substantial substrate heating occurred during plasma treatment (maximum of 36.2 °C). Results show that all investigated plasma compositions were able to significantly reduce the contamination of the samples. The obtained reductions were 88.14%, 93.81% and 90.21% for groups A, B and C, respectively. The analysis of variance (ANOVA) and Tukey's test were used to compare the values obtained in the groups treated with their respective control groups, all were statistically significant. SEM micrographs evidenced morphological alterations of the cells for all treated groups, i.e. the cells are lysed or have completely lost the structure, forming a "mass".

Conclusions: In this work we used the surfatron technique for the generation of highly reactive plasma jets that allowed an efficient inactivation of clinical C. parapsilosis biofilms on silicon.
Sensitizing Pseudomonas aeruginosa to Antibiotics by Shape Recovery Triggered Biofilm Dispersion

S. Lee, H. Gu, J. B. Kilberg, D. Ren;
Syracuse University, Syracuse, NY.

Background: Due to high-level tolerance to antimicrobial agents, microbial biofilms are a leading cause of chronic infections in humans and persistent biofouling in industries. Due to the protection of extracellular matrix, mature biofilm remains challenging to eradicate. Thus, the need to develop better biofilm control strategies is urgent. Recently, we engineered surfaces with dynamic changes in topography using a tert-butyl acrylate (tBA) based shape memory polymer (SMP) and demonstrated up to 99.9% removal of established Pseudomonas aeruginosa PAO1 biofilm upon triggered shape recovery.

Methods: P. aeruginosa was cultured for 48 h to form biofilm on tBA based SMP that was stretched by 50% using a manual stretcher. Shape recovery was triggered at 40°C for 10 min. The biofilm cells detached by shape recovery or sonication (control using unstretched substrate) were compared for antibiotic susceptibility, the level of intracellular adenosine triphosphate (ATP), and rrnB gene expression. RNA-seq and quantitative PCR (qPCR) were used to study the effects of shape recovery triggered dispersion at the genome-wide scale.

Results: The results show that shape recovery triggered dispersion caused physiological changes in P. aeruginosa and the detached cells were sensitized to bactericidal antibiotics. Shape recovery in the presence of 50 µg/mL tobramycin reduced biofilm cell count by more than 3 logs (2,479 fold) compared to the untreated control. The observed effects were attributed to the disruption of biofilm structure and increase in cellular activities as evidenced by an 11.8-fold increase in intracellular level of adenosine triphosphate (ATP), and 4.1-fold increase in expression of the rrnB gene in detached cells. The results suggest that, unlike other dispersion methods based on strong physical forces, shape recovery triggered biofilm dispersion does not kill bacterial cells but render them to enter a more active physiological stage (but not with fully resumed growth) and opens the door for antibiotic attack.

Conclusions: The results of antibiotic susceptibility, intracellular ATP level, rrnB expression level, and the genomic-wide gene expression analyses suggest that the shape recovery triggered dispersion rendered bacterial cells to leave the physiological stage of biofilm growth and entered a more active and drug susceptible stage. These results indicate that the biofilm cells may actively sense physical changes in the substratum and more effective control strategies can be developed to eradicate biofilm cells with combined physical (dynamic surface) and chemical (antibiotics) factors.
Background:Persisters are multidrug tolerant cells that present within antibiotic sensitive population. Large populations of persisters are common in biofilms, which lead to stress conditions and can result in relapses of bacterial infections. In contrast to antibiotic resistant bacteria, the tolerance arises from transient phenotypic variants rather than genetic mutations. Cells which express persister gene stochastically can switch to persister states. Although previous study identified many interesting genes, molecular mechanisms of bacterial persistence remain unclear because of their redundancy. In order to identify novel persister genes, we developed a marker for E.coli persisters and performed transcriptome analysis of isolated persisters.

Objective: The objective is to reveal molecular mechanisms of persister formation via lactate dehydrogenase (ldhA) and aldehyde dehydrogenase (aldB) which were identified from our transcriptome data. Methods: Transcriptome data suggested expression of anaerobic respiration and fermentation genes. We constructed overexpression and knockdown strains derived from E.coli MG1655. Persisters were measured by determining the number of CFUs after exposure to ofloxacin. CRISPR interference was used for gene knockdown. To visualize ldhA expression, YFP was cloned into pSC101 vector that contain ldhA promoter. The YFP fluorescence of reporter strain was analyzed in microfluidic devise. Conclusions: ldhA overexpression increased persister population 1000 times, and knockdown decrease the population 10 times. Stochastic expression of ldhA use central metabolite pyruvate and NADH. Time-lapse microscopy images of ldhA reporter strain showed the most of cells did not express ldhA, but few (~1%) cells highly expressed ldhA. These cells stop dividing and tolerated lethal concentration of ampicillin. Interestingly, although ldhA was expressed transiently, the cells showed dormant phenotype over 1 hour. These results suggest that stochastic expression of ldhA trigger persister formation.
The 2-aminoimidazole (2-AI) class of compounds can inhibit the formation of bacterial biofilms, disperse existing biofilms, and even resensitize multidrug resistant bacteria to antibiotics. These compounds are active against both Gram-positive and Gram-negative bacteria, making them a powerful weapon against biofilms and antibiotic resistance. We have found that 2-AI compounds interact with bacterial response regulators, the transcriptional regulatory component of canonical two-component systems. Two-component systems allow the cell to detect and respond to changes in its environment, and often trigger quorum sensing, virulence factors, and initiation of biofilm formation. As such, response regulators have been highly sought after as therapeutic targets, but have yet to be successfully exploited. Here, we explore the interaction between response regulators and 2-AI compounds. BfmR from *Acinetobacter baumannii* and QseB from *Francisella tularensis/novicida* are well known for regulating biofilm formation. We demonstrate that BfmR and QseB are cellular targets of 2-AI compounds, and that 2-AI compounds are potent inhibitors of biofilms in *A. baumannii* and *F. tularensis/novicida*. We propose a model for how 2-AI compounds inhibit response regulator activity, based on a combination of structural biology and molecular modeling. Understanding the mechanism of action between 2-AI compounds and response regulators will lead to the development of more potent, specific, and broad-range compounds.
Abstract Body:

Background: In clinical settings, antibiotics are widely applied and problems caused by MRSA biofilm formation have been frequently reported. However, antibiotic pressure on biofilm formation of MRSA, as well as the mechanism, remains unclear. Materials/methods: Twelve clinical MRSA isolates were subjected to MIC determination on 12 commonly used antibiotics, including ampicillin, penicillin G, metropenem, streptomycin, kanamycin, gentamycin, erythromycin, ciprofloxacin, tetracycline, oxytetracycline, trimethoprim and vancomycin. Viability and biomass were determined during biofilm formation at 8, 16, 24 and 48 h under 10 concentrations (from 4 to 1/128 MIC with 1/2 dilution) of the 12 antibiotics, by MTT assay and crystal violet assay. The biofilm samples of 2 MRSA isolates under specific concentrations of antibiotics were further analyzed by RNA sequencing (in triplicate). Expression of genes related to two-component system (TCS) and biofilm formation were selected to be quantified by Q-PCR. Results: Dramatical increase was obtained under 1/4 MIC of ampicillin and streptomycin for biomass, and 1/4 MIC of ampicillin, ciprofloxacin and kanamycin for viability. Five hour biofilm samples of 2 MRSA isolates under these specific sublethal concentration of antibiotics were subjected to transcriptomics study. Based on bioinformatics analyses, 24 up-regulated and 27 down-regulated genes were identified in all sublethal concentration of antibiotics treated samples. Differentially expressed genes were enriched to TCS which plays important role in the response of microbes to adverse environment. Combined with Q-PCR results, tagA, lytR, arlR, hssR, clfB, and altA genes were hypothesized to be critical during the biofilm formation under sublethal concentration of antibiotics environment. Conclusions: Dramatical increase in biofilm formation for biomass or viability was obtained under sublethal concentration of antibiotics environment. Transcriptomics study revealed tagA, lytR, arlR, hssR, clfB, and altA genes may play important roles.
Biofilm Antimicrobial Tolerance

SpoT is Involved in Biofilm Formation of *Helicobacter pylori* with Multidrug Resistance by Upregulating Efflux Pump *Hp1174* (gluP)

**Author** Y. Sun, X. Ge, Y. Cai;

**Block:** Shandong University, Jinan, CHINA.

The drug resistance of *Helicobacter pylori* (*H. pylori*) is gradually becoming a serious problem. Biofilm formation is an important factor that leads to multidrug resistance in bacteria. The ability of *H. pylori* to form biofilms on the gastric mucosa has been known. However, there are few studies on the regulation mechanisms of *H. pylori* biofilm formation and multidrug resistance.

Guanosine 3'-diphosphate 5'-triphosphate and guanosine 3',5'-bispyrophosphate [(p)ppGpp] are global regulatory factors and are synthesized in *H. pylori* by the bifunctional enzyme SpoT. It has been reported that (p)ppGpp is involved in the biofilm formation and multidrug resistance of various bacteria. However, whether SpoT is involved in *H. pylori* biofilm formation and multi-drug resistance remains unknown. Based on the successful construction of spoT mutant strain (ΔspoT) and complemented-strain (spoT*), the difference in biofilm formation between ΔspoT strain and wild-type strain was compared by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The difference of MIC (minimum inhibitory concentration) between ΔspoT strain and wild-type strain was compared. We found that SpoT also plays an important role in *H. pylori* biofilm formation and multidrug resistance. Therefore, it is necessary to carry out some further studies regarding its regulatory mechanism.

Considering that efflux pumps are of great importance in the biofilm formation and multidrug resistance of bacteria, we tried to find if efflux pumps controlled by SpoT participate in these activities. Then, we found that *Hp1174* (glucose/galactose transporter, gluP), an efflux pump of the MFS (Major Facilitator Super) family, is highly expressed in biofilm-forming and multidrug resistance (MDR) *H. pylori* and is upregulated by SpoT. Through further research, we determined that gluP involved in *H. pylori* biofilm formation and multidrug resistance. Furthermore, the average expression level of gluP in clinical MDR strain was considerably higher than that in clinical drug-sensitive strain. Taken together, our results revealed a novel molecular mechanism of *H. pylori* tolerance to multidrug.
Session Title: THURSDAY Poster Session 4

Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 032

Abstract Topic: Biofilm Antimicrobial Tolerance

Abstract Title: Candida albicans Susceptibility to Scorpion-venom Derived Antimicrobial Peptides

Author Block: S. S. Snyder, J. Gleaton, D. Kirui, J. Talackine, N. J. Millenbaugh; Naval Medical Research Unit-San Antonio, San Antonio, TX.

Abstract Body:

Background: Bacterial and fungal biofilms in wounds significantly increase morbidity and mortality, and the increasing prevalence of drug resistant strains has prompted the need for new antimicrobial compounds. Kn2-7 is a scorpion venom-derived peptide with broad-spectrum activity against multiple bacterial strains at <10 µg/mL in vitro. In this study, anti-fungal activity, protease stability, and mammalian cell toxicity of Kn2-7 and its D-isoform (dKn2-7) are described. Methods: Planktonic fungal susceptibility: Seven Candida albicans strains were exposed to Kn2-7 and dKn2-7 at concentrations up to 100 µg/mL. Optical density measurements at 600 nm and the colony forming unit (CFU) assay were used to determine the minimum inhibitory and fungicidal concentrations (MIC and MFC, respectively). Fungal biofilm susceptibility: Five C. albicans strains formed biofilms, which were exposed to the peptides at concentrations up to 1000 µg/mL for 24 hours. The XTT assay was then used to determine metabolic activity within the biofilms. Protease stability: Peptides were exposed to purified trypsin in phosphate buffered saline for 4 and 24 hours. The concentration of peptide in solution was determined using LC-MS. Fungal time kill assay: Planktonic C. albicans was exposed to dKn2-7 up to 50 µg/mL and the CFU assay was used to determine fungal viability at various time points up to 24 hours. Hemolysis assay: Human erythrocytes were exposed to peptides up to 400 µg/mL and percent hemolysis was measured using supernatant absorbance at 490 nm. Results: In six of the C. albicans strains, Kn2-7 and dKn2-7 exhibited MICs of 12.5-100 µg/mL and 6.25-25 µg/mL, respectively, and MFCs of 25-100 µg/mL and 12.5-50 µg/mL, respectively; one strain exhibited MICs of 100 µg/mL and MFCs of >100 µg/mL for both peptides. Biofilm IC₅₀ values were between 62.5-125 µg/mL in all five strains for dKn2-7 and 500-1000 µg/mL in four strains for Kn2-7. In the presence of purified trypsin, dKn2-7 exhibited significantly higher stability than Kn2-7. dKn2-7 was thus selected for further study due to its greater antifungal efficacy and stability. dKn2-7 at ≥25 µg/mL caused complete C. albicans cell death in the 24 hour time kill assay, whereas 12.5 µg/mL of dKn2-7 initially decreased cell viability, but could not maintain low cell counts over time. Hemolysis assays indicated no significant hemolysis at concentrations below 100 µg/mL and <50% hemolysis at 400 µg/mL for both peptides. Conclusions: The D-form of the Kn2-7 peptide is a potent, stable, broad-spectrum antimicrobial compound capable of killing planktonic C. albicans and significantly affecting biofilm viability at <125 µg/mL. Cytotoxicity testing of dKn2-7 against mammalian cells is currently ongoing.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 033  
**Abstract Topic:** Biofilm Antimicrobial Tolerance  
**Abstract Title:** Nutrient Conditions Impact Effects of Silver Nanoparticles and Aztreonam on *Pseudomonas aeruginosa* PAO1 Biofilms  
**Author Block:** D. Lee, M. Kornya, H. Lee, M. Habash; University of Guelph, Guelph, ON, CANADA.

**Background:** The specific environment in which microbial biofilms develop, are challenged with antimicrobials, and recover following removal of that challenge is a critical factor affecting their antimicrobial tolerance. The objective of this study was to evaluate the impact of minimal and clinically relevant nutritional conditions on the interaction of aztreonam and silver NPs against *P. aeruginosa* PAO1 biofilms. **Methods:** MBEC assays were used for *P. aeruginosa* PAO1 biofilm formation, antimicrobial challenge, and biofilm recovery following antimicrobial challenge. Aztreonam (Azt) and citrate-capped, 10 nm-diameter silver NPs (Ag-NPs) were the antimicrobial agents selected for testing. The nutrient environments tested were modified Whiteley’s medium (MWM), a nutrient-rich synthetic sputum medium, and defined minimal media (DMM). Biofilm biomass was evaluated using crystal violet staining and biofilm viability was evaluated using an ATP assay and planktonic growth following biofilm recovery. **Results:** Combinations of Ag-NP and Azt against *P. aeruginosa* PAO1 biofilms developed, challenged and recovered in MWM or DMM were tested. For testing in MWM, biofilms were not inhibited even at the highest combined concentration tested (4096 µg/ml Azt and 5 µg/ml Ag-NP). For testing in DMM, biofilms were inhibited at combined concentrations as low as 2 µg/ml Azt and 0.625 µg/ml Ag-NP. The synergistic interaction between Azt and Ag-NPs against *P. aeruginosa* biofilms in DMM was not observed with MWM. Biofilms formed in MWM or DMM produced similar amounts of biomass (OD595nm = 0.2-0.4 after 20h). Biofilms formed, challenged with Azt and recovered in MWM had about 400% more biomass than the untreated control following recovery from antibiotic challenge, up to 64 µg/ml Azt. Further, biofilm recovery following challenge with Azt concentrations ≥128 µg/ml resulted in decreasing amounts of biomass to a minimum of about 50% less than the untreated control. Biofilms formed in DMM or MWM, and challenged and recovered in DMM had biomass amounts about equivalent to the untreated control, following recovery from antibiotic challenge up to 128 µg/ml Azt. Above 128 µg/ml Azt, biofilm recovery resulted in increasing biomass to a maximum of 670% more than the untreated control following recovery from challenge with increasing concentrations of Azt. The only condition resulting in biofilm inhibition were biofilms formed in DMM, but challenged and recovered in MWM, with challenge at 4096 µg/ml Azt. **Conclusions:** The use of MWM and/or DMM during *P. aeruginosa* biofilm formation, antibiotic challenge and recovery resulted in distinctive biofilm recoveries in response to challenge by increasing aztreonam concentrations.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 034

**Abstract Topic:** Biofilm Antimicrobial Tolerance

**Abstract Title:** Role of Extracellular Vesicles from *Staphylococcus epidermidis* on Bacterial Growth and Biofilm Formation Under Antimicrobial Selective Pressure Conditions

**Author Block:** M. Zaborowska, C. Taulé Flores, F. Vazirisani, P. Thomsen, M. Trobos; Department of Biomaterials, Sahlgrenska Academy, University of Gothenburg, Gothenburg, SWEDEN.

**Abstract Body:**

**Background:** Implanted medical devices provide surfaces for bacterial attachment and biofilm formation, which may lead to implant-associated infection. It is a feared complication commonly caused by staphylococci, often requiring implant removal and long-term use of antimicrobial agents. In addition, it is known that bacteria secrete extracellular vesicles (EVs), however fundamental understanding on their secretion and function in staphylococci is lacking. The aims of this study were to study (i) the effect of sub-inhibitory concentrations of antibiotics on the formation and secretion of EVs, and (ii) if EVs play a role in antimicrobial tolerance and biofilm formation.

**Methods:** The secretion of EVs under sub-inhibitory concentrations of gentamicin (GEN; 0, 0.03 and 0.06 µg/mL) was investigated in a clinical *Staphylococcus epidermidis* strain (CCUG 64523; MIC<sub>GEN</sub>=0.094 µg/mL) isolated from a patient with implant-related osteomyelitis. The secreted EVs (5, 60 µg/mL) were then added to the same strain to investigate their potential protective effect under selective pressure (0, 0.03, 0.06 and 0.12 µg/mL GEN). Bacterial growth was measured with optical density over 18 h, thereafter viability of planktonic bacteria was measured with LIVE/DEAD staining. Adhered bacterial cells to polystyrene were evaluated using the microtiter plate assay. In a similar experiment, EVs derived from a strong-biofilm producing GEN resistant *S. epidermidis* strain (ATCC 35984) were cultured with the non-biofilm producing GEN susceptible clinical strain (CCUG 64523) under the selective pressure of GEN (0, 0.03, 0.12, 0.5 µg/mL). **Results:** The mean size of EVs was the same for the different culture conditions. In the presence of GEN, the number of EVs per CFU was higher and contained significantly more proteins than control (0 µg/mL GEN). In the presence and absence of GEN, addition of 60 µg/mL EVs significantly decreased the growth of *S. epidermidis* compared to un-stimulated control (no EVs). Stimulation with 5 µg/mL EVs reduced adhesion (27-82%) compared to unstimulated control in the absence and presence of GEN (0, 0.03 µg/mL), whereas 60 µg/mL EVs reduced adhesion in the presence of GEN. Addition of EVs derived from a biofilm producing strain to the clinical strain significantly increased the total growth compared to unstimulated control, by increasing the growth rate and decreasing the generation time by 5 min. The EVs significantly decreased bacterial adherence by more than 90% compared to control (no EVs). **Conclusions:** Sub-inhibitory concentrations of GEN altered the production and content of EVs, which in turn affected cell growth and adherence to polystyrene, both under normal and pressure conditions. These findings could have potential clinical implications, with consequences for staphylococcal survival and biofilm formation on implants, in the presence of sub-inhibitory therapeutic doses.
Biofilm Antimicrobial Tolerance

Examining the Correlation between Antibiotic Susceptibility and Biofilm Formation of Group B Streptococcus Isolates of Indian Origin

S. Verma¹, P. Yadav¹, A. Johri²; ¹Central University of Haryana, Mahendragarh, INDIA, ²Jawaharlal Nehru University, Delhi, INDIA.

Streptococcus agalactiae (Group B streptococcus), is a common commensal organism that colonizes the gastrointestinal and genitourinary tract and causes severe infection in neonates, adults, and immune-compromised patients. Many microorganisms such as s. aureus and p. aeruginosa, have ability to form biofilm which is important in the infection establishment and generate antibiotic resistance by decreasing the antibiotic penetration rate and mediating bacterial gene expression. The mechanism of biofilm formation in S. agalactiae and its association with antibiotic resistance have not been investigated yet. This study purposed to examining the biofilm formation among S. agalactiae isolates from diverse sources and the antibiotic susceptibilities of S. agalactiae strains. As GBS can switch from planktonic stage to biofilm formation which is an important for the establishment of infection as well as providing resistance to antibiotics. In the present work, we determined, in vitro resistance of GBS isolates to different antibiotics for examining any changes in antibiotic resistance pattern with its correlation to biofilm formation. These outcomes may throw light on the knowledge of mechanism by which biofilm formation in S. agalactiae is contributing to antibiotic resistance. If this would be the possible outcomes, the involved virulence factors could establish new therapeutic and precautionary goals against this important human pathogen.
**Background:** *Pseudomonas aeruginosa* is an important biofilm forming, opportunistic, bacterial pathogen that causes approximately 51,000 nosocomial infections per year including periprosthetic joint infections (PJI). Orthopedic surgeons routinely place antibiotic loaded CaSO₄ beads into PJI surgical sites to allow for local antibiotic therapy. Variant like antibiotic tolerant or resistant colony generation has previously been shown as a response to tobramycin loaded CaSO₄ beads which could lead to recurrent or persistent infections. The aim of this study was to further characterize the variant colonies produced in response to a tobramycin loaded CaSO₄ bead.

**Methods:** In this study, variant colonies were generated by placing a tobramycin loaded CaSO₄ bead into an agar plate containing a pre-grown lawn biofilm of *P. aeruginosa*. After generation, colonies were isolated and cultured, before being plated for minimum inhibitory concentration (MIC) testing. Growth curves were also generated for the isolates to look for the presence of a growth defect which could explain their survival. Finally, generation of variant colonies was repeated with a biofilm lawn that had been exposed to an efflux pump inhibitor (CCCP) to examine efflux pumps as a possible mechanism for the variants’ antibiotic tolerance. In addition to variant colony characterization, the antibiotic concentration throughout the agar over time was also characterized. Tobramycin loaded CaSO₄ beads were placed in sterile agar plates and punches were taken from the agar at varying radii and time points. The punches were melted and used in a Kirby Baeur test to approximate the tobramycin concentration.

**Results:** Three phenotypes were identified from the variant colonies which survived the tobramycin bone CaSO₄ therapy - classically resistant colonies, and two novel variants, viable but non culturable colonies (VBNCs), and a tolerant phenotype which we call “phoenix” colonies. Phoenix colonies are variants which grow up within the zone of inhibition of the tobramycin bead while there are still high levels of antibiotic present, but upon subculturing, return to wild-type susceptibility. They show no growth defects indicating they are not persistor cells, are morphologically similar to wild-type colonies aside from a pigmentation change from green to white, and are not efflux pump driven. While variant colonies were produced in response to the tobramycin beads, there was also a consistent zone of killing around the bead from which nothing could be cultured. **Conclusions:** These findings suggest that resistant colonies, VBNCs, and phoenix colonies may be generated in biofilms in response to antibiotic therapies, but by achieving high enough local antibiotic concentrations, we may be able to kill classically resistant mutants, phoenix colonies, VBNCs, and persister cells thus reducing the incidence of recurrent or persistent infection.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 037

**Abstract Topic:** Biofilm Mechanics

**Abstract Title:** Mechanical Characterization of Biofilms Under Oxidative Stress Induced by Photocatalytic Reactions

**Author Block:** E. A. López-Guerra, H. Shen, S. D. Solares, D. Shuai; The George Washington University, Washington, D.C., DC.

**Abstract Body:**

**Background:** Photocatalytic reactions hold promise in biofilms control for healthcare, food, and environmental applications. Graphitic carbon nitride (g-C₃N₄) has proved to be an effective photocatalyst, because of its visible-light-response, low cost in fabrication, stability, and biocompatibility. We select g-C₃N₄ for biofilm control, and aim to understand how the mechanical properties of biofilms change when subject to photoreactions. Elucidating the mechanism is the key to the rational design of effective photocatalytic materials for antimicrobial applications. The atomic force microscope (AFM) is a powerful characterization tool that provides simultaneous information about sample surface topography and probe-sample interacting forces with high spatial resolution and sensitivity. This unique capability has helped us to highlight the mechanical response of biofilms to photocatalysis in our study.

**Methods:** *Staphylococcus epidermidis* biofilms were grown over g-C₃N₄ coupons in a multi-well cell culture plate at 37 °C for 72 h in the dark. For assessing the change of biofilm morphology, viability, and mechanical properties, the biofilms were next exposed to visible white LED light for 6 h. A control sample of bacterial cells with minimal extracellular polymeric substances (EPS) was prepared by washing repeatedly the suspended cells with phosphate buffered saline (PBS) solution. We used two basic modes of AFM operation to get knowledge of surface morphology and tip-sample force interactions, namely, tapping mode and static force spectroscopy (SFS). From SFS measurements we derived the elastic moduli of the biofilms by using the Derjaguin-Muller-Toporov (DMT) theory. **Results:** Topographical exploration with tapping mode AFM evidenced substantial morphological changes occurring in the biofilms when exposed to light on g-C₃N₄ coupons. The biofilms lost the integrated structure after photoreactions, and only single cells or small clusters of cells were able to be imaged. Results from SFS data revealed that the biofilms exposed to light experience an increase in their (Young’s) elastic modulus, which we ascribe to a reduction in the amount of EPS. We have confirmed this hypothesis with the aid of a control sample of bacterial cells with minimal EPS (see methods section). **Conclusions:** g-C₃N₄ has shown to be an efficient photocatalyst for biofilm eradication by degrading EPS, which is critical for maintaining a cohesive structure of the biofilms. This exploratory study sheds important light into the mechanical changes experienced by the biofilms upon the imposition of oxidative processes. As a future step, we plan to consider the viscoelastic nature of the biofilms in the mechanical characterization using more sophisticated mechanical models, and identify key mechanical properties that control biofilm eradication.
**Abstract**

**Background:** *Pseudomonas aeruginosa* evolves during chronic pulmonary infections of Cystic Fibrosis (CF) patients, forming adapted variants that are selected for. Mucoid and rugose small-colony variants (RSCVs) are isolated from CF sputum samples. The emergence of these variants is associated with increased treatment difficulties and a worsening patient outcome. RSCV and mucoid variants overproduce different exopolysaccharides in the biofilm extracellular polymeric substance (EPS). Mucoid variants overproduce alginate, whereas RSCVs overproduce Psl and Pel. Changes to the mechanical properties of their biofilms, due to the overproduction of the EPS exopolysaccharides and associated advantages in an infection are not well understood.

**Methods:** Here, we analyze *P. aeruginosa* RSCV (PAO1ΔwspF) and mucoid (PAO1ΔmucA) biofilms compared to their isogenic wildtype parent (PAO1). Colony biofilms were grown on sterile filter discs on *Pseudomonas* isolation agar, with the biofilm transferred to a new plate every 24h. The mechanical properties of the colony-biofilms were measured at 2-d, 4-d and 6-d using mechanical indentation and flat spinning disk rheometry on a TA instruments Discovery Hybrid HR2 rheometer.

**Results:** We identified that the mechanical properties of wildtype biofilms undergo extensive temporal changes. Initially, wildtype biofilms are relatively stiff and transition to become more fluid-like on 4-d before returning to a stiff phenotype on 6-d. The mechanical properties of RSCV and mucoid biofilms showed a gradual progression to more elastic-solid behavior. Theoretical mucociliary and cough clearance indices have been determined for sputum which correlate sputum viscoelasticity to predicted clearance by either mechanism from the lung. The viscoelasticity of bacterial biofilms has not been considered regarding their mechanical clearance from the lung during infection. The mechanical properties of *P. aeruginosa* biofilms determined here predict that mucoid biofilms are more resistant to both mechanisms of clearance, whereas wildtype and RSCV biofilms would show reduced clearance by cough at later timepoints when biofilms are highly elastic.

**Conclusion:** Our results suggest that adaptation to evolve phenotypes with different mechanical properties may allow *P. aeruginosa* biofilms to insure against mucociliary and cough clearance from the lung. This may be a mechanism for facilitating persistence within an infection. We propose that the mechanical properties of a biofilm be included in the virulence factors that these communities possess.
Abstract Title: Extracellular RNA Contributes to Robust Biofilm Organization

Abstract Body:

**Background:** Biofilms are complex surface-bound microbial communities. Microbes within biofilms are embedded in a self-produced extracellular matrix (ECM) composed of proteins, polysaccharides, and/or DNA that protects them from various stressors. Thus, biofilm-forming bacteria often cause various human chronic infectious diseases. Knowledge of how these communities develop is important for their eradication; however, the mechanistic basis for biofilm formation remains poorly understood at the molecular level. Identifying ECM components contributing to biofilm structural integrity can provide insight into the process of biofilm development that can lead to the development of strategies for their inhibition. In this study, we explored the presence of extracellular RNA (eRNA) in bacterial biofilms and analyzed its roles in biofilm development. **Methods:** Several clinically isolated strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* were used. ECM components were extracted from the biofilms with 1.5 M NaCl and were subsequently analyzed by electrophoresis. Localization of eRNA in the biofilms was analyzed by confocal laser scanning microscopy (CLSM). To identify eRNA in the ECM, RNA-sequencing (RNA-seq) was performed. Physiological role of eRNA in the biofilm ECM was clarified by investigating the effects of RNase A on biofilm formation and dispersal. Molecular interaction between eRNA and polysaccharides was examined by surface plasmon resonance (SPR) analysis. **Results:** Nucleic acids were detected in the ECM of *S. aureus* and *S. epidermidis*. The nucleic acids were degraded by RNase A but neither by dispersin B, proteinase K, nor DNase I, indicating the presence of eRNA in the ECM. The molecular size of the eRNA was estimated 20 to 100 nucleotides by denaturing polyacrylamide gel electrophoresis. RNase A inhibited biofilm formation and dispersed pre-formed biofilms, representing the importance of eRNA in the structural integrity of the biofilms. The results of time-course experiments suggested the requirement for eRNA at various stages of biofilm formation. RNA-seq revealed that eRNA originated from bacteria were tRNA and rRNA. CLSM visualized colocalization of extracellular polysaccharides with eRNA in the biofilm. SPR analysis showed direct interaction between synthesized RNA with an abundant sequence in the ECM and purified polysaccharides. **Conclusions:** Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology and the treatment of biofilm-associated problems.
Abstract
Biofilms consist of a consortium of cells affixed to a surface, embedded in a matrix of proteins, polysaccharides and DNA. This lifestyle differs vastly from that of free floating, planktonic counterparts, as spatial and temporal architecture can directly alter the behavior, properties and composition of a biofilm. Biofilms present unique challenges to medical treatment and industrial biofouling remediation. Such obstacles have only worsened as antimicrobial resistance increases. Phage therapy recently resurfaced as a candidate to supplement traditional antibiotics and biofilm removal, yet we are only beginning to understand the ramifications of phage exposure on microbial ecosystems and community assembly. *E. coli* biofilms resist bacteriophage infection by secretion of a polymeric protein, curli, forming a diffusion barrier on the surface. However, the implications of phage tolerance in this system extend beyond survival or death of biofilm-dwelling cells. Community composition often changes as a result of biofilm formation and can promote or obstruct multi-species coexistence. Coexistence or co-occurrence of multiple species, strains or subpopulations is often evaluated by “invasibility” or the ability of an organism to increase in abundance when it is less numerous. Given that phages are trapped by the curli mesh of mature *E. coli* biofilm matrix, we speculated that this may influence the ability of other cells to subsequently colonize the biofilm-liquid interface. Under control conditions in the absence of phage, invading cells do not penetrate into the interior of mature biofilms, but they can become established along a biofilm’s outer periphery. However, when resident biofilms are pretreated with phage, a protective barrier is created by the phages themselves: resident cells remain uninfected due to their embedding within the curli mesh, whereas potential invaders land on the phage coated matrix, become infected and die. On the other hand, if the invasion occurs prior to phage application and ample time is given for acquisition or production of curli, the invaders are likewise protected, and rates of invasion are comparable to control conditions. Large scale phage protection via matrix production and biofilm formation thus has the potential to drastically alter community assembly and coexistence of bacteria within the same family of phage susceptibility.
Curli Amyloids Confer Rigidity to Biofilms Reducing Density Dependent Bead Movement and Prolonging Bead Interactions During 20-Minute Time Courses

Background: Our long-term interest is to model how planktonic cells can introduce plasmids into a biofilm community. Plasmid transfer begins with interaction of planktonic cells with the biofilm. The composition of the matrix may influence interaction of planktonic cells with the biofilm. The goal of the current study was to develop a technique to compare the interaction of 1 micrometer negatively charged glyoxylate beads with bacterial biofilms with and without bacterial amyloids over a 20-minute time period.

Methods: Bead movement was tracked in a 20 micrometer thick biofilm over 20 minutes using laser-scanning confocal microscopy. Software was developed to track the bead movement over time, calculate the velocity of movement, follow the tracks, determine bounding boxes to measure total area covered by a bead and determine the density around each bead. This information was used to plot bead velocities, determine average velocities, variance and weighted velocities (density-dependence) as well as determine the stability of the interactions over twenty minutes and the amount of area covered by each bead. Bead movement was compared between Salmonella enterica serotype Typhimurium, an isogenic amyloid curli mutant and Enterococcus faecalis biofilms.

Results: Beads in low density regions of Salmonella biofilms appeared to have reduced movement as compared to low density regions of E. faecalis biofilms. Consistent with the visual observations, curli containing Salmonella biofilms had average bead velocities ranging from 0.2-1.1 nm/sec, E. faecalis biofilms from 21-64 nm/sec and Salmonella curli mutants from 15-91 nm/sec. The weighted velocity of the beads (density dependence) was 0.001 to 0.06 in Salmonella, 0.04-0.13 in Salmonella curli mutants and 0.1-0.65 for E. faecalis biofilms. In general, curli containing biofilms tended to have more beads with stable interactions (trajectories that could be followed for all 20 minutes) than biofilms lacking curli. However, in biofilms lacking curli, beads with longer trajectories had a greater range of movement (larger bounding box). Ongoing analysis is being done to compare overall stability of interactions and range of movement of the beads. Preliminary data suggests that E. coli and isogenic curli mutant biofilms behave much the same as the respective Salmonella biofilms. The technique is currently being used to characterize Staphylococcus aureus and Streptococcus mutans biofilms under amyloid-inducing and non-inducing conditions. Conclusions: The presence of curli conferred rigidity to S. Typhimurium biofilm. This rigidity was visually evident in low density areas; it reduced average biofilm movement speeds, increased bead contact time and decreased density dependence of the bead movement when compared to E. faecalis biofilms lacking amyloids and isogenic curli mutant Salmonella biofilms.
For our research, we define electrochemically active biofilms (EAB) as biofilms that exchange electrons with electrodes. The biofilms grown on electrodes allow us to quantify electron transfer rates and identify electron transfer processes. The limitations in electron transfer processes need to be determined to maximize utilization of these biofilms, especially in scaling up. We have demonstrated that the current density decreased with the increased electrode size. We hypothesize that the loss of current density is attributed to substrate transport limitations and microscale heterogeneity in biofilms. The heterogeneity of local conditions on biofilm electrodes at low substrate environments are expected to decrease the current density of large electrodes. Microelectrodes can be used to measure depth profiles in biofilms and determine electron donors/acceptor limitations as well as measure the redox and pH gradients. Here we present how chemical and electrochemical gradients in biofilms are influenced by electron donor/acceptor availability. Acetate and formate microbiosensors along with pH and redox microelectrodes were used to record depth profiles and stationary measurements in electrochemically active biofilms to understand the role of electron donor/acceptor limitations and electrochemical gradients in scaling up. Therefore, we developed new redox and pH microelectrodes with built-in reference electrodes. We found that 1) the biofilms can grow thicker in the absence of electron acceptor or electron donor because the bottom part of the biofilms is conductive, 2) the redox potential gradients depends on the electrode potentials, and 3) pH decreases towards the bottom of the biofilm. When we tested the effect of electron donor and acceptor concentrations on current density of anodes and cathodes at different sizes, we found that scaling up is controlled by the mass transfer. Finally, we found almost identical microbial communities on electrodes at different size demonstrating that the microbial community structure was not the main limitation controlling scale up.
Abstract

Background: The Microtitter assay is one of the most widely used methods to assess biofilm formation. Though, high throughput this assay is notoriously irreproducible in its outcome from experiment to experiment, and even from well to well. Since the assay constitute one of the pillars of biofilm research, we decided to examine the wells of a microtitter tray directly during growth, treatment and the steps involved in Crystal Violet (CV) measurements. Methods: We used an inverted Zeiss LSM 880 confocal laser scanning microscope (CLSM) to visualize and quantify biomass directly in the wells of the microtitter tray. We applied both GFP-tagged Pseudomonas aeruginosa, PAO1, and Live/dead stains to assess structure and compression of biomass build up. With the use of CLSM we were able to image the development of whole well biofilms in real-time. All observations were compared with CFU counts and CV assays. Results: We were intrigued to see a development of a distinctly structured architecture of the biomass in the wells over time. We were able to capture 3D images of all biomass throughout the entirety of the microtitter wells. These structures were highly variable from well to well, which may introduce variable outcomes. Even though there was an accumulation of biomass from 24 towards 72 hours, the number of variable cells in this biomass declined significant over the same period. We saw large variations in biomass, depending of the chosen method of removing the supernatant prior CV staining (etc. pipetting or throwing the fluid out, washed or unwashed wells). Remaining biomass was highly inconsistent, even with the same method performed by the same person. It was clear that CV measurements did not reflect living biomass, biomass found in the wells before staining or provided any consistent picture of the state of biomass grown in the wells. Conclusions: This study was conducted in an effort to expand our knowledge of a seemingly trivial and widely used method of assessing biofilm formation and treatment. We found that most steps involved in CV assay causes high deviation due to divergence in the structure of biomass between the individual wells and how these steps effect the biomass. Additionally we found that over time, the biomass of a microtitter well would develop into complex structures by increasing biomass while the viable fraction of the biomass decreases. The highly structured microenvironment of biomass in these microtitter wells needs to be taken into account when designing and analyzing experiments using this model.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 046

**Abstract Topic:** Biofilm: From Nature to Models

**Abstract Title:** An Artificial Sputum *in vitro* model for Evaluation of Antibiotics for Treatment of Cystic Fibrosis

**Author:** E. Bevels, U. Schwab, N. Bionda; iFyber, Ithaca, NY.

**Background:** *Pseudomonas aeruginosa* is the major pathogen responsible for lung infections and respiratory failure in cystic fibrosis (CF) patients. The multidrug-resistant nature of these infections is facilitated by the viscous sputum of the CF patient, which contains high concentrations of mucin, DNA, and amino acids. *P. aeruginosa* exists in CF sputum as a suspended mucoid biofilm with a thick extracellular matrix. The evaluation of *P. aeruginosa* antibiotic susceptibility using sputum from CF patients is impractical due to several factors, including patient-to-patient variability, history of antibiotic treatment, and changes imposed by sterilization. Artificial Sputum Medium (ASM) has emerged as an *in vitro* model that can produce suspended mucoid biofilms like those found in CF sputum. This study uses ASM to develop mature, suspended *P. aeruginosa* biofilms for antibiotic efficacy testing. **Method:** ASM was inoculated with *P. aeruginosa* BAA-47 at a final concentration of approximately $5 \times 10^5$ CFU/mL. Inoculated ASM was aliquoted into a 24-well plate and incubated for 6 days, with the daily addition of fresh media to counter evaporation and provide additional nutrients. The 6-day old biofilms were treated with gentamicin (10, 30, 50, and 100 µg/mL; n=4) or tobramycin (10, 20, 40, 80 µg/mL; n=4) for 24 hours. Untreated controls (n=4) were included for each antibiotic. After the designated treatment period, antibiotics were neutralized, and biofilms were disrupted via sonication. The resulting suspension was spot-plated or spread-plated to enumerate bacterial survivors, expressed as colony-forming units (CFU)/mL. **Results:** Visible aggregates of *P. aeruginosa* were formed in ASM that were found to be antibiotic resistant. Both gentamicin and tobramycin showed dose-response in terms of efficacy but failed to eradicate *P. aeruginosa* biofilms at the highest tested concentrations. Biofilms treated with 100 µg/mL gentamicin (25x the MIC value) survived with over $10^4$ CFU/mL, approximately a 5-log reduction from the untreated control at $10^9$ CFU/mL. Biofilms treated with 80 µg/mL tobramycin (80x the MIC value) also survived with over $10^4$ CFU/mL. **Conclusion:** The ASM model produces suspended, antibiotic-resistant biofilms resembling those found in the CF lung. This new model is clinically relevant due to its sensitivity and low detection limit, proving new opportunities to evaluate antibiotics against chronic CF infections.
Session Title: THURSDAY Poster Session 4

Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm

Poster Board Number: 047

Abstract Topic: Biofilm: From Nature to Models

Abstract Title: Diffusion Retardation by Buffering of Tobramycin in Alginate Biofilms

Author Block:
- B. Cao¹, L. Christophersen², M. Kolpen², P. O. Jensen³, K. Sneppen⁴, N. Hoiby², C. Moser¹, T. Sams⁵;
  ¹Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, DENMARK;
  ²Department of Clinical Microbiology, Rigshospitalet, Costerton Biofilm Center, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, DENMARK;
  ³Department of Clinical Microbiology, Rigshospitalet, DK-2100 Copenhagen, Denmark, Costerton Biofilm Center, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark, Copenhagen, DENMARK;
  ⁴Niels Bohr Institute, University of Copenhagen, Copenhagen, DENMARK;
  ⁵Biomedical Engineering, Technical University of Denmark, Lyngby, DENMARK.

Abstract Body:
The killing of bacteria by antibiotics in biofilms is known to be reduced by 100-1000 times relative to planktonic bacteria. This makes such infections difficult to treat. We suggest that a biofilm should be regarded as an independent compartment with distinct pharmacokinetics. To elucidate this, we have measured the penetration of the tobramycin into seaweed alginate beads which serve as a model of the extracellular polysaccharide matrix in P. aeruginosa biofilm. We find that, rather than a normal first order saturation curve, the concentration of tobramycin in the alginate beads follows a power-law as a function of the external concentration. The power-law appears to be a consequence of binding to a multitude of different binding sites. In a diffusion model these results are shown to produce pronounced retardation of the penetration of tobramycin into the biofilm. This filtering of the free tobramycin concentration inside biofilm beads is expected to aid in augmenting the survival probability of bacteria residing in the biofilm. PLOS ONE, 11, 4, e0153616, 2016.
Abstract Body:

Introduction: Presence of bacteria is essential for hosts’ function. The main form of bacterial survival is a biofilm. Growth of bacterial biofilms is complex process, which involves three generalized phases: adhesion, biofilm maturation and dispersion. Lactobacilli spp. are critical components of gut and reproductive health. Despite the fact, that biofilm growth of LB has been described extensively, the temporal characteristics of biofilm maturation has not been validated in real time mode yet. Here we are describing two methods of label-free quantification of biofilm growth of *L. Plantarum*: analyses of video-tracing and electrical impedance-based technologies. Additionally, we are comparing real time growth of *L. Plantarum* *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*. Material and Methods: Biofilm growth was studied in 1) Biofermenter system (Lous Pasteur, France) with video-recording and subsequent analyses, using IMARIS software (Bitplane, South Windsor, Connecticut, United States) and in 2) real time cell analyzer: xCELLigence (ACEA Bioscience Inc., San Diego, CA), based on impedance measurement. Results: The dynamic of biofilm growth of *L. Plantarum* was similar in both systems with the exception of detachment phases. Four LB species differed in the duration of attachment phases, demonstrating negative cell index, while growth phases were similar. Conclusion: Temporal dynamic of biofilm growth is in agreement with published physiological and pathophysiological data and points out, that real time detection of this phase is an important tool in understanding growth of microbial communities.
Abstract

Oxygenated unsaturated fatty acids, known as oxylipins, are signaling molecules commonly used for cell-to-cell communication in eukaryotes. However, a role for oxylipins in mediating such communication in prokaryotes was not previously known until the recent description of the novel Oxylipin-Dependent Quorum-Sensing System (ODS) of Pseudomonas aeruginosa. We have shown that ODS regulates important phenotypes of P. aeruginosa, such as biofilm formation, virulence and antibiotic resistance, provided that oleic acid, the precursor of the oxylipin autoinducers, is present in the bacterial environment. Quorum sensing is a known mechanism of bacterial cell-to-cell communication, which involves the production and detection of diverse small molecule autoinducers. Our data show that ODS uses oleic acid scavenged from host tissues as a substrate to synthesize the oxylipins autoinducers, which in turn control the expression of a gene subset in a cell density-dependent manner. We identified the LysR-type transcriptional regulator encoded by gene PA2076 in the model strain of P. aeruginosa, PAO1, as the primary receptor of the oxylipin signal. ODS is unique not only because it uses prokaryote oxylipins as autoinducers, but also because these signaling molecules are synthesized extracellularly by P. aeruginosa, as opposed to all other autoinducers described thus far that are synthesized intracellularly, and because the ODS is regulated independently of the hierarchical quorum sensing network of this bacterium.
Background: The extracellular polymeric substances (EPS) matrix of biofilms is a complex mixture of compounds, with varying composition depending on environmental conditions and bacterial species present. The common practice in EPS modelling is to treat it as a pool of organic carbon, neglecting its complex chemistry. This work presents a novel methodology for modelling EPS production, by proposing a general EPS chemical formula and using a microbial thermodynamics framework. Methods: The EPS matrix is assumed to contain only proteins (general formula C_{16}H_{24}O_{8}N_{5}) and polysaccharides (general formula C_{6}H_{10}O_{5}), neglecting humic acids, eDNA or lipids. The EPS chemical formula (i.e. CH_{x}O_{y}N_{z}) is proposed by considering the ratios of protein to sugars experimentally reported in activated sludge treatment systems. The thermodynamic approach represents metabolism as the sum of two simplified reactions: the anabolism, in which biomass is synthesized, and the catabolism, which provides the energy for anabolism. EPS formation is modelled as a product in the anabolic reaction, eq. (1). 

\[
\text{a C-source + b N-source} \rightarrow \text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2} + c \text{HCO}_{3}^{-} + d \text{H}_{2}\text{O} + e \text{H}^{+} + f \text{EPS}
\]

The stoichiometry is obtained by solving the element and charge balance, for a given a stoichiometric coefficient of EPS. The thermodynamic properties of EPS (enthalpy, entropy and free energy of formation) are estimated using empirical relations. The stoichiometric coefficient of the electron donor in the overall growth reaction represents the reciprocal of the biomass yield on substrate. Biofilm development is modelled using a 2D individual based model. For the simulations, heterotrophic bacteria using glucose as electron donor and ammonia as nitrogen source were used. Results: The influence of the protein to polysaccharides ratio of EPS on bacterial growth yield was examined. The computed free Gibbs energy is lower for polysaccharides than for proteins. As a result, higher maximum yields correspond to EPS with lower protein content. The stoichiometric coefficient of EPS in the anabolic reaction is varied between 0 to 1, to study biofilm growth with EPS production. Despite the energetic burden, bacteria able to synthesize high EPS amounts produce higher biofilms, in the same simulation conditions. The lower density of EPS is responsible for better diffusion of the nutrients in the biofilm and the better performance of high EPS producers. Conclusions: Modelling the EPS formation using a thermodynamic framework is a novel approach that allows the quantification of the energy cost of its production on the development of microorganisms. The model can be improved by considering the effect of EPS chemical composition on the matrix’s physical properties (e.g. density, viscosity). References: 1. Gonzalez-Cabaleiro, R., et al. (2015). ISME J 9(12), 2630-2641. 2. Battley, E. H. (1999). Thermochimica Acta 326(1-2), 7-15
Biofilm Architecture Inference Tool (BAIT): An Image Analysis Software to Study the Architecture of Oral Multi-Species Biofilms

T. Luo¹, M. Hayashi¹, M. Zsiska², B. Circello², M. Eisenberg¹, C. Gonzalez-Cabezas¹, B. Foxman¹, C. F. Marrs¹

¹University of Michigan, Ann Arbor, MI, ²Procter & Gamble, Mason, OH.

Background: Standard image analysis programs are required to analyze and compare the effects of various treatments on biofilms grown in vitro. Many in vitro biofilm model systems combine confocal laser scanning microscopy (CLSM) with image analysis tools to study biofilms. However, currently, there is no standard approach to analyze biofilm images grown in vitro. This study introduces and evaluates an in-house developed image analysis software that we call BAIT (Biofilm Architecture Inference Tool). BAIT quantifies the architecture of oral multi-species biofilms enabling quantitative comparisons of biofilms grown under different treatment conditions.

Methods: In vitro biofilms representative of oral biofilms were developed over the course of 22 hours using a 24-well Bioflux™ system. Pooled human saliva served as both the growth medium and initial inoculum. During development, nascent biofilms were either untreated or treated with a formulation 8 and 18 hours into development. Treatment formulations included water (negative control), sodium gluconate (placebo) or stannous fluoride (anti-biofilm agent) at active stannous concentrations of 1,000, 3,439, and 10,000 PPM. Mature biofilms were stained with LIVE/DEAD and imaged with CLSM. Digital 3-dimensional image stacks were then quantified with the BAIT software and evaluated for seven outcomes: biovolume, surface area, number of objects, fluffiness, connectivity, convex hull porosity, and viability.

Results: Treatment with 3,439 and 10,000 PPM stannous formulations visibly and significantly decreased bioburden. Analysis with BAIT demonstrated that biovolume, surface area, number of objects, and biofilm connectivity decreased while fluffiness increased (p<0.01). The lowest concentration of stannous, 1,000 PPM, did not visibly decrease biofilm burden, but BAIT indicated an altered biofilm architecture: the number of objects and fluffiness increased while connectivity decreased (p<0.05), suggesting fragmentation of biofilm.

Conclusions: In conclusion, BAIT was able to measure clearly visible as well as more subtle changes in in vitro biofilm architecture. The software enhances the analysis of 3-dimensional biofilm images and can quickly evaluate the efficacy of candidate antimicrobial and anti-biofilm agents.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 052

**Abstract Topic:** Biofilm: From Nature to Models

**Abstract Title:** *ex vivo* Porcine Dermal Model for the Evaluation of Anti-Biofilm Efficacy of a Nitric Oxide-Releasing Polymer

**Author:** N. Bionda, J. Gras, E. J. Bevels, A. D. Strickland;

**Block:** Ifyber LLC, Ithaca, NY.

**Background:** An *ex vivo* porcine dermal model of mature biofilm has been implemented, in several variations, for the evaluation of the efficacy of wound care therapies. The major advantages of this model system are the use of a natural porcine skin matrix and the relatively high throughput format of the assays in determining the effect of wound care technologies on biofilm prevention and/or the eradication of existing biofilm. Here we describe the development and use of this model system to study the efficacy of a nitric oxide (NO)-releasing polyvinylpyrrolidone (PVP) with respect to anti-biofilm efficacy. Microbial biofilms play an important role in the prolonged inflammation state of wounds, and NO has been recognized for its broad-spectrum efficacy, where even sub-bactericidal concentrations lead to biofilm dispersal. The goal of this study was to devise a series of assays that would serve as a prediction of success in the *in vivo* studies, taking into consideration the relevant wound size and the formulation of the active material.

**Method:** Aliquots of the porcine dermal tissue were prepared either by punching out the tissue using a 7/16” biopsy punch or by preparing square shaped pieces approximately 2x2” in size. In the assay using the small tissue, the artificial wound was created using a Dremel tool, while wounds in the larger tissue were done using a scalpel. The artificially wounded tissue samples were then excessively washed and sterilized using chlorine gas. The biofilm was established by inoculating sterilized tissue with approximately $10^5$ CFUs of the bacteria or yeast and incubated for up to 1-3 days on soft agar in the presence of appropriate antimicrobial agents. The biofilms were then challenged in multiple formats with solutions containing the active material or wound care appropriate formulations thereof.

**Results:** We have developed and implemented several variants of an *ex vivo* porcine dermal model of mature biofilm, including *C. albicans* and mixed species biofilms comprised of *P. aeruginosa* and *S. aureus*. The size of the artificial wound was varied from approximately 2-15 mm in diameter to provide a scenario that more closely resembles the wounds found in the clinic. While aqueous solutions of PVP/NO ranging from 0.5% to 5% showed very potent anti-biofilm efficacy with $\geq 6$ log reduction of the biofilm load, the efficacy of PVP/NO semi-solid formulations was significantly lower, especially when the larger wound model was used. Ongoing efforts are geared towards optimizing the NO release from the formulations, the key factor needed for the efficacy of the material.

**Conclusion:** The *ex vivo* porcine dermal model of mature biofilm is a particularly useful tool for guiding the optimization of formulations for wound care applications prior to *in vivo* studies. Additionally, the selection of appropriate experimental set up (i.e., "wound" size) is critical when considering the clinical use of the technology.
Background: Subgingival plaque plays a primary role in initiation and progression of gingivitis and periodontitis. It is a complex and diverse biofilm formed predominantly by normal oral flora in addition to small amounts of potentially pathogenic microbes. The objective of this study was to optimize growth conditions for developing a biofilm model that mimics the human subgingival plaque. Methods: Subgingival plaque was used as a seed culture and was collected using paper points and placed into pre-reduced transport medium. Biofilms were grown from the subgingival plaque sample on saliva pre-coated pegs of a MBEC assay 96 well plate (formerly called Calgary device) in 5 different culture media; modified SHI (mSHI), mSHI with glucose (GmSHI), Brain Heart Infusion supplemented with vitamin K, hemin and mucin (sBHI), mucin-only medium supplemented with 10 % serum (MS10) or 20% serum (MS20). Biofilms were allowed to grow for a period of 14 days in anaerobic conditions, with a replacement of medium every 3.5 days. The microbiomes were then harvested and DNA was extracted. 16S rRNA gene sequencing (Illumina, V1-V3, 2x300 bp chemistry) was performed on DNA from biofilms as well as from original subgingival plaque sample. Post sequencing data cleaning, taxonomic assignment and diversity analysis were carried out using MOTHUR, BlastN and QIIME, respectively. Results: An average of 234 species were identified in the original subgingival sample, whereas the number of species identified in the in vitro microbiomes were in the range of 64-94. All 5 media showed an excess growth of Firmicutes (Parvimonas, Peptostreptococcus, Solobacterium and Mogibacterium). sBHI harbored Veillonella, Prevotella and Fusobacterium in proportions close to that observed in subgingival sample; mSHI captured the TM7s. Shannon and Simpson alpha diversity indices were lowest for MS10 and MS20 media, and highest for mSHI and sBHI. Principal coordinate analysis (PCoA) based on unweighted unifrac and weighted unifrac distance matrix biofilms grown in mSHI and sBHI were closest to the original sample on the PC1 while those grown in MS10, MS20 and mSHI were closest on the PC2 and PC3. Conclusions: Both alpha and beta diversity analyses indicate that biofilm developed using mSHI and sBHI media were closest to the original plaque sample. However the overgrowth of some species and absence of growth of some others was observed. Further optimization and development of this model can be useful for understanding the complex nature of oral biofilms and can have implications for the screening of microbiome modulators.
**Abstract**

**Background:** Catheter-associated urinary tract infections (CAUTI) are the most common cause of hospital-associated infections in the US and can result in significant morbidity, including bacteremia, and in some cases death. Increasing drug resistance among uropathogens and their ability to form recalcitrant, biofilm-associated diseases have made appropriate management of CAUTI difficult. Problematically, the presence of a urinary catheter increases the risk of infection, especially to atypical uropathogens. One such pathogen that has received little attention is *Staphylococcus aureus*. Recent studies show that urinary catheterization is the strongest predisposing factor for the development of *S. aureus* UTI and the majority of isolates are methicillin-resistant *S. aureus* (MRSA). The frequency with which *S. aureus* CAUTI results in bacteremia and toxic shock and the difficulty in treating these infections is particularly concerning. Thus, this study aimed to gain a better understanding of the mechanisms by which urinary catheters facilitate MRSA CAUTI.

**Methods:** We adapted a mouse model of CAUTI to assess the bacterial load on urinary implants and in the bladders, dissemination to other organs, and the immune responses following urinary catheter implantation and MRSA infection. Additionally, we collected patient urinary catheters to confirm the results from our mouse model accurately recapitulated human disease. **Results:** Our adapted MRSA CAUTI model indicated: i) MRSA required a catheter to establish persistent UTI; ii) the host protein fibrinogen (Fg), which plays a major role in clotting and wound healing, accumulated in the bladder and subsequently coated the catheter; iii) MRSA co-localized with Fg deposited on catheters and the bladder epithelium; and iv) MRSA rapidly disseminated to bacteremia and subsequently colonized the spleen and heart. Furthermore, the clumping factor B (ClfB) adhesin and the urease enzyme significantly contributed to MRSA CAUTI. While, ClfB contributes to CAUTI by interacting with Fg recruited following catheter-induced damage, the role urease plays is less clear. Urease breaks down urea in urine, which induces crystal formation, and results in catheter encrustation. Urease mutants grow comparably to wildtype in human urine in vitro but in the CAUTI mouse model results in a 2 log decrease in bacterial load at 1 dpi. Importantly, analysis of patient catheters indicates that *S. aureus* is present on catheters and long term colonization results in urease-induced crystal formation, which the pathogen attaches to form biofilm. **Conclusions:** These studies indicate the catheterized bladder facilitates MRSA infection by providing additional binding ligands and MRSA employs several mechanisms to establish CAUTI. These studies will provide insights into the development of more effective treatment options to prevent or treat MRSA CAUTI.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 055  
**Abstract Topic:** Biofilms and Infection  
**Abstract Title:** *Salmonella* Biofilm Extracellular Polymeric Substances: A Role for Curli Fimbriae and Vi Antigen in Innate Immune Resistance  
**Author Block:** M. M. Hahn, J. S. Gunn; The Ohio State University, Columbus, OH.

**Background:** Typhoid fever is a major health concern that affects 21 million individuals and causes 200,000 deaths each year. Acute illness is primarily caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S. Typhi*). The only known reservoir for *S. Typhi* is chronically-infected human carriers, who harbor the organism in the gallbladder and are usually asymptomatic but shed and transmit the organism via the fecal-oral route. A hallmark of chronic *S. Typhi* infections is the ability to produce biofilms on cholesterol gallstones that are encased in *Salmonella*-produced extracellular polymeric substances (EPS). Biofilms are thought to permit bacterial evasion of host immunity, but the involvement of specific EPS components in immune tolerance is poorly characterized. In particular, knowledge on subversion of the innate immune response during early stages of infection is lacking. **Methods:** We characterized *S. Typhi* and *S. Typhimurium* for susceptibility to serum complement and to the antimicrobial peptides (AMPs) polymyxin B and melittin. These immune factors attack bacterial surfaces by pore-forming mechanisms. Following exposure to each immune factor in the planktonic or biofilm state, bacterial viability was quantified by CFU analysis. We further investigated the contribution of individual EPS components by deleting genes essential for expression of curli fimbriae, cellulose, O-antigen capsule, colanic acid, or Vi antigen. **Results:** The biofilm lifestyle clearly enhances tolerance to serum complement and to AMPs. EPS mutations do not affect tolerance to serum complement. Loss of curli fimbriae, O-antigen capsule, and colanic acid reduces tolerance to polymyxin B. However, only loss of curli fimbriae reduces tolerance to melittin, suggesting curli fimbriae should be a prioritized target for anti-biofilm agents. *S. Typhi* Vi antigen mutants have hyper-biofilm forming phenotypes. Despite having excess biofilm, these mutants are more susceptible than wildtype to AMPs, indicating a protective function of Vi antigen and that biofilm recalcitrance cannot be attributed to biomass alone. **Conclusions:** By identifying mutants exhibiting less tolerance than wildtype *Salmonella*, we have identified EPS components crucial for the biofilm immunotolerant phenotype. These data improve our understanding of biofilm-mediated recalcitrance to innate immunity and provide new directions for therapeutics that specifically target critical EPS components.
In Silico Identification of the Indispensable Quorum Sensing Proteins of Multidrug Resistant Proteus mirabilis

S. Mujawar¹, C. Lahiri², S. Pawar¹, M. Ashraf⁴;
¹Sunway University, Subang Jaya, MALAYSIA, ²Sunway University, Bandar Sunway, MALAYSIA, ³Georgia State University, Atlanta, GA, ⁴B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, INDIA.

Catheter-associated urinary tract infections (CAUTI) is an alarming hospital based disease with the increase of multidrug resistance (MDR) strains of Proteus mirabilis. Cases of long term hospitalized patients with multiple episodes of antibiotic treatments along with urinary tract obstruction and/or undergoing catheterization have been reported to be associated with CAUTI. The cases are complicated due to the opportunistic approach of the pathogen having robust swimming and swarming capability. The latter giving rise to biofilms and probably inducible through autoinducers make the scenario quite complex. High prevalence of long-term hospital based CAUTI for patients along with moderate percentage of morbidity, cropping from ignorance about drug usage and failure to cure due to MDR, necessitates an immediate intervention strategy effective enough to combat the deadly disease. Several reports and reviews focus on revealing the important genes and proteins, essential to tackle CAUTI caused by P. mirabilis. Despite longitudinal countrywide studies and methodical strategies to circumvent the issues, effective means of unearthing the most indispensable proteins to target for therapeutic uses have been meager. Here, we report a strategic approach for identifying the most indispensable proteins from the genome of P. mirabilis strain HI4320, besides comparing the interactomes comprising the autoinducer-2 (AI-2) biosynthetic pathway along with other proteins involved in biofilm formation and responsible for virulence. Essentially, we have adopted a theoretical network model based approach to construct a set of small protein interaction networks (SPINs) along with the whole genome (GPIN) to computationally identify the crucial proteins involved in the phenomenon of quorum sensing (QS) and biofilm formation and thus, could be therapeutically targeted to fight out the MDR threats to antibiotics of P. mirabilis. Our approach utilizes the functional modularity coupled with k-core analysis and centrality scores of eigenvector as a measure to address the pressing issues.
Session Title: **THURSDAY Poster Session 4**
Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 058
Abstract Topic: Biofilms and Infection
Abstract Title: Re-direction of the Natural Immune Response to Integration Host Factor with a Chimeric Peptide Immunogen Directed against Immunoprotective Domains
Author Block: L. A. Novotny¹, S. D. Goodman², L. O. Bakaletz²;
¹The Research Institute at Nationwide Children's Hospital, Columbus, OH, ²The Research Institute at Nationwide Children's Hospital and The Ohio State University College of Medicine, Columbus, OH.

**Background:** Biofilms contribute to the chronicity and recurrence of many diseases and eradication of these bacterial communities is confounded by a multicomponent extracellular polymeric substance which shields the resident bacteria from antimicrobials and host immune effectors. The bacterial DNABII protein, integration host factor (IHF), is a critical element of the extracellular DNA matrix that supports the biofilm structure. Whereas the adaptive immune response to IHF is against non-protective epitopes within the carboxy-terminal (or ‘tail’) region, antibodies against the DNA-binding ‘tip’ regions induce catastrophic collapse of biofilms formed by all human pathogens tested. To therefore redirect the adaptive immune response away from the non-protective tail region and toward protective tip domains within IHF, we designed a chimeric peptide immunogen comprised of 20-mer portions within the tip regions of IHF α and β subunits. **Methods:** Nontypeable *Haemophilus influenzae* (NTHI) biofilms were established *in vitro* then incubated with anti-tip chimer peptide or anti-whole native IHF protein. Antibodies against a ‘tail chimer peptide’ that represented non-protective domains within IHF were also tested. Further, we used an experimental model of otitis media wherein NTHI biofilms established within the middle ears of chinchillas were treated with IgG-enriched anti-tip chimer peptide, anti-IHF or anti-tail chimer peptide that was delivered directly to this site. **Results:** *In vitro*, NTHI biofilm biomass was significantly reduced within 5 min of exposure to anti-tip chimer peptide or anti-IHF compared to naive serum, whereas biofilms incubated with anti-tail chimer peptide sera were not disrupted. Moreover, at the earliest (5 min) and latest (60 & 120 min) time points tested, biofilms incubated with anti-tip chimer peptide were significantly reduced compared to anti-IHF. *In vivo*, middle ears administered anti-tip chimer peptide or anti-IHF had a significant 4-log fewer NTHI adherent to the mucosa and within biofilms compared to receipt of naive serum or anti-tail chimer peptide. Mucosal biofilms were significantly reduced in animals that received anti-tip chimer peptide or anti-IHF. Importantly, anti-tip chimer peptide antibodies were more effective than anti-native whole IHF to induce mucosal biofilm clearance and eradication of NTHI from the middle ear, which validated the premise for the design of this chimeric immunogen. **Conclusions:** Whereas the natural immune response to IHF is directed toward non-protective domains of this protein, re-direction of the adaptive immune response by immunization with a peptide that represents defined immunoprotective regions was highly effective and avoided augmentation of a pre-existing non-protective response that would have likely resulted following immunization with native IHF. Support: NIH R01 DC011818
HVAC (heating, ventilation, and air conditioning) condensate can be a beneficial source of water that is currently going down the drain. As warm air is cooled by an air conditioning system, humidity in the air forms condensation that is essentially distilled water. Typically, this high-quality water is drained into wastewater systems, but it can alternatively be collected to support water conservation and sustainable water use. In hot, humid weather, an air conditioner may produce three to ten gallons of water per day per 1000 square feet of air-conditioned space. The goal of this study was to assess if HVAC condensate water was safe for various reuse purposes by testing bulk water samples and surface biofilms for opportunistic bacterial pathogens. The bulk condensate water from four air handling units in two buildings in North Carolina was monitored biweekly from May through October, 2017. Additionally, 36 biofilm samples were collected a single time from condensate surfaces (100 cm² swabbed), collection pans (25 cm² swabbed), and drain pipes (variable sized areas swabbed) of each handler using sterile swabs. Microbial analyses included heterotrophic plate counts (HPC), culture and qPCR for nontuberculous mycobacteria (NTM), and qPCR for *Legionella pneumophila* serogroup 1 (Lp SG1). The average HPC counts for the bulk condensate water were 6.05 x 10³ CFU/ml. The average HPC counts for biofilm drain pipe samples were 1.83 x 10⁶ CFU/ml, 4.15 x 10³ CFU/cm² for collection pans, and 6.24 x 10³ CFU/cm² for condensation surfaces. No biofilm samples and 2.5% (1/40) of condensate samples were positive for *Mycobacterium avium* (MA) by qPCR. Six percent (2/36) of biofilm samples and 7.5 % (3/40) of condensate samples were positive for *M. intracellulare/chimaera* (MI/C). Lp SG1 was detected by qPCR in one biofilm sample and one condensate sample, both quantities below the assay’s limit of quantification. Nearly 50 putative mycobacteria were isolated from condensate water samples and two biofilm samples. In this study, the microbiological quality of untreated HVAC condensate was evaluated to determine its safety for reuse purposes. Heterotrophic bacteria are not necessarily pathogenic but concentrations were higher than the <500 CFU/ml recommended for potable water. While species-specific pathogen detection by qPCR (MA, MI/C, Lp SG1) was rare and concentrations were low, clinically relevant species were also isolated by the general *Mycobacterium* culture method. Disinfection could provide sufficient bacterial log reduction to utilize HVAC condensate water safely for many non-potable uses including irrigation, toilet flushing, and cooling tower makeup water. This research will inform States on concentrations of opportunistic bacterial pathogens and non-pathogens in untreated HVAC condensate and aid in recommending fit-for-purpose treatment options for this type of reclaimed water.
Pseudomonas aeruginosa (Pa) is a ubiquitous gram-negative bacterium that thrives in diverse environments. The ability of Pa to exist in these different environments can be linked to its ability to adhere to surfaces and produce a protective matrix. The Pa biofilm matrix is composed of proteins, exopolysaccharides (EPS), and self-produced extracellular DNA (eDNA). In addition to occupying environmental niches, Pa can cause chronic upper respiratory infections in immunocompromised individuals, such as cystic fibrosis (CF) patients. We performed immunohistochemistry staining of sputum and explanted lungs of CF patients and confirmed that the EPS Pel is produced by Pa in CF airways and that it remains localized to Pa aggregates.

Previous work in our lab revealed that the Pa EPS Pel is composed of N-acetyl-glucosamine and N-acetyl-galactosamine and carries a positive charge at pH<6.9. Producing Pel and no other EPS is sufficient to support Pa biofilm structures and Pel was also found to be a major contributor to the antibiotic resistance of structures containing multiple EPS. However, the interactions between Pel and other matrix components or host products remains largely unexplored. Since the upper airway of CF individuals is known to be mildly acidified and contain high concentrations of eDNA from lysed host cells, we hypothesized that Pel could interact with host eDNA in the cell-free system. Additionally, eDNA bound by Pel is protected from nuclease degradation. In biofilms grown under continuous flow, exogenously added eDNA exclusively colocalizes with Pel and does not bind to structures lacking Pel. Furthermore, Pel-containing biofilms displayed an increased tolerance to tobramycin when grown with excess eDNA. Our work demonstrates that Pel produced in the CF airway can interact with host eDNA and that this may influence Pa susceptibility to antibiotic treatment.
The opportunistic pathogen *Pseudomonas aeruginosa* forms chronic biofilm-based infections in the lungs of cystic fibrosis (CF) patients. *P. aeruginosa* biofilms in the CF lung environment are often characterized by the overproduction of the exopolysaccharide alginate, due to acquired mutations in *mucA*. MucA inhibits the sigma factor AlgU, and it is thought that the clinical mutations in *mucA* lead to truncated proteins that are fully degraded, which results in misregulation of AlgU and overproduction of alginate. Paradoxically, our work shows that a portion of *mucA* is required for bacterial viability in a variety of *P. aeruginosa* strains. Here we examine why *mucA* is essential. Our results show that *mucA* was no longer essential in a strain lacking *algU* and that *mucA* alleles that abolished interaction with AlgU were not sufficient for viability. These results suggest that the loss of the MucA-AlgU interaction results in bacterial cell death because MucA is no longer available to regulate AlgU, leading to aberrant expression of AlgU-regulated genes. To determine if alginate is responsible for *mucA* essentiality, we deleted *algD*, a key alginate biosynthetic gene. We also created a strain in which three transcription factors that regulate alginate production (*algB*, *algR*, and *amrZ*) were deleted. In both strains, *mucA* was still essential, suggesting that alginate overproduction is not, at least solely, responsible for the *mucA* viability defect. To help determine the cause of the cell viability defect, we imaged cells depleted of MucA. We observed that MucA-depleted cells were shorter, suggesting that the cells were under some form of stress. Because MucA is involved in the response to envelope stress and removal of the envelope stress response via the deletion of *algU* suppresses *mucA* essentiality, we wondered if removal of other stress response pathways would alleviate the requirement for *mucA*. We, therefore, deleted two transcription factors involved in stress response, *rpoS* and *oxyR*. We found that *mucA* was no longer required in these backgrounds. While more work is still needed to understand why *mucA* is required for bacterial viability in *P. aeruginosa*, our results suggest that in the absence of the ability to respond to certain stresses, *mucA* is no longer required for bacterial viability.
The Relationship between Hyperbiofilm Capacity and Enhanced Pathogenic Phenotypes of Currently Circulating *Bordetella pertussis* strains may Explain the Resurgence of Pertussis

N. Cattelan¹, J. L. Gutierrez-Ferman², E. Garza-Gonzalez², O. M. Yantorno¹, R. Deora³;
¹Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI), CONICET, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, ARGENTINA, ²Servicio de Gastroenterología, Hospital Universitario "Dr. José Eleuterio González", Monterrey, MEXICO, ³Department of Microbial Infection and Immunity, The Ohio State University Wexner Medical Center, Columbus, OH.

**Background:** *Bordetella pertussis* is a gram-negative human pathogen and the primary etiological agent of the disease whooping cough or pertussis. Despite widespread and very high levels of vaccination coverage, pertussis has re-emerged with significant morbidity and mortality in infants, adolescents and adults. We have hypothesized that *B. pertussis* biofilms allow bacteria to escape from immune defenses and vaccine-mediated immunity thereby enhancing their persistence, transmission, and continued circulation. Little is known about how *Bordetella* biofilm growth has adapted with respect to time, region, and changing immunization regimens. To address this, a side-by-side comparison of the biofilm-forming abilities, structures and pathogenic phenotypes of currently circulating strains from the USA, Mexico and Argentina, three countries utilizing different vaccines and vaccination schedules, was conducted.

**Methods:** *B. pertussis* clinical isolates recovered in USA, Mexico and Argentina, from 2001 to 2014, the laboratory reference strains Tohama I and Bp536, and an avirulent mutant strain (Bp369) were used in this study. Biofilm forming capacity was evaluated by the microtiter dish assay, and by confocal microscopy. In addition, autoaggregation index and biofilm-promoting and -inhibitory factors (Filamentous hemagglutinin and Adenylate cyclase) were determined. Adhesion to respiratory epithelial cells A549 and colonization of the mouse respiratory tract were also analysed.

**Results:** Irrespective of the country of origin and compared to the reference laboratory strains, all the recently isolated strains formed hyperbiofilms. However, biofilm structural analyses revealed country-specific differences, with strains from the USA forming more structured biofilms. Bacterial hyperaggregation and reciprocal expression of biofilm-promoting and -inhibitory factors were also observed in current isolates. Strikingly, hyperbiofilm capacity of current isolates was associated with augmented epithelial cell adherence and higher levels of bacterial colonization in the mouse nose and trachea.

**Conclusions:** Taken together, our data provide a link between increased biofilm formation in *B. pertussis* clinical isolates with altered pathogenic phenotypes and a colonization advantage in an animal model, even among those isolated in countries with different vaccination regimens.
A Rapid Visual Quantitative Diagnostic Methodology for the Detection of Bacterial Infections in Orthopaedics

**Background:** Every year, there are up to six million bone fractures in the United States; many of these fractures require surgical fixation, which contribute to the nearly two million fracture fixation devices deployed annually. In addition to fracture fixation devices, there are millions of prosthetic joint replacements inserted yearly. The burden of infection for these surgeries is one of the biggest problems in orthopedics. Infection rates can climb to nearly 2% for closed fractures, 30% for open fractures, and cause nearly 26,000 prosthetic joint infections yearly. These infections can extend patient hospital stays by 9.7 days per patient, cost $20,000 extra in hospital costs per person, and decrease overall quality of life. The current methods used to diagnose these infections are traditional bacterial culturing, PCR, and gram staining. However, these methods have issues with accuracy, efficiency, and cost. This study aims to assess the capabilities of a rapid visualization assay using fluorescently conjugated antibodies and Confocal Laser Scanning Microscopy (CLSM) to detect bacterial presence on surgical explants, tissue, and synovial fluid.

**Methods:** With an IRB approved protocol, samples from 6 faculty surgeons at Rhode Island Hospital were collected and stored in 10% neutral buffered formalin. Synovial fluid samples were fixed onto slides with methanol and stained with a cocktail of serums, while explants and tissue were stained in 15 mL falcon tubes. Fluorescently conjugated anti-LPS (Dylight 594 and AlexaFluor 568) and anti-LTA (FITC 488) antibodies were added to the samples to label gram negative bacteria and gram positive bacteria. A DAPI stain was used to mark the presence of eukaryotic cells. Positive controls of Methicillin Sensitive Staphylococcus aureus and Acinetobacter baumannii and negative controls lacking bacteria are used to determine antibody quality and viability. Images were quantified through Confocal Laser Scanning Microscopy, analyzed with ImageJ (NIH), and compared to the results of the hospital data.

**Results:** In the course of our experiment, we have obtained 43 synovial fluid samples and 32 hardware and tissue samples. Our assay results agree with hospital data and gram staining trials in 100% (43 out of 43) of cases for synovial fluid samples. For the hardware and tissue samples, hospital data found that 31.25% (10 of 32) of samples were infected, but our assay showed that 65.63% (21 of 32) had significant bacterial presence. **Conclusion:** This assay has demonstrated the ability to visualize bacteria on surgical explants, tissue, and synovial fluid with high contrast and specificity in 30 minutes. The assay also allows for the determination of bacterial morphology regardless of sample heterogeneity. In solid samples, the technology is able to pinpoint bacterial presence directly on the samples, allowing for directed debridement and localize treatment.
**Abstract**

**Background:** Managing infections of orthopedic implant sites remains a considerable challenge despite the abundant availability of therapeutic compounds in this antibiotic era. A poignant example is Type IIIB open fractures reduced with internal fixation, displaying infection rates upwards of 50%. These high rates of infection have scarcely changed since the Type IIIB fracture was defined in 1984; persisting because significant environmental biofilm contamination at the time of injury overwhelms clinical approaches. A sheep model for open fracture biofilm contamination was developed by Williams et al. and has previously been used extensively to test experimental anti-biofilm approaches. We used this sheep model of biofilm contamination to test a clinical prophylactic course of antibiotics typical for open fracture cases. **Methods:** The sheep model used *S. aureus* biofilms grown on polyether ether ketone (PEEK) membranes using a modified CDC biofilm reactor; the two PEEK-biofilms were each placed on the medial aspect of the right tibia and secured atop with two corresponding metal fixation plates. One of the biofilm-plate combinations was removed post-necropsy for microbiological analysis; the other remained on the bone and was processed for histological analysis. Each PEEK membrane contained $1.58 \pm 0.42 \times 10^{10}$ CFU at the time of inoculation. There were two study groups (n=2 sheep/group). The first group did not receive any post-procedural antibiotics whereas the second group received a 48 h prophylactic dose of IM antibiotics: gentamycin 10mg/kg every 24 h and cefazolin 25 mg/kg every 8 h. Sheep were sacrificed at 21 days. **Results:** Both groups had an approximate 4 log10 reduction in CFUs in the PEEK-biofilms, with little difference between the group which received prophylactic antibiotics and the positive control group. The PEEK-biofilms from the antibiotic group were slightly more robust at $4.0 \pm 1.2 \times 10^6$ CFU/membrane compared with $0.6 \pm 0.2 \times 10^6$ from the group which received no antibiotics (Figure 1). Histology is currently being performed. **Conclusions:** Clinical prophylactic antibiotics had no measurable effect on the biofilm bacteria used in this model of open fracture infection. The model simulated well the difficulty to treat infections, and may indicate why open fracture infections remain upwards of 50%. Results validated the utility of the model and may benefit future work to develop antibiofilm technologies.
Bartonella henselae (Bh) is a Gram-negative rod that is typically transmitted to humans by a scratch from the common house cat. Infection of humans with Bh can result in a range of clinical disease including lymphadenopathy observed in cat-scratch disease and more serious disease from persistent bacteremia. It is a common cause of blood-culture negative endocarditis as the bacterium is capable of growing as aggregates and forming biofilms on infected native and prosthetic heart valves. Its aggregative growth requires a high molecular weight trimeric autotransporter adhesin (TAA) called Bartonella Adhesin A (BadA), a member of the TAAs found in all Bartonella species and other Gram-negative bacteria. Using Bh Houston-1, Bh Houston-1 ∆badA (a complete badA deletion mutant) and Bh Houston-1 ∆badA/pNS2P;badA (a partial complement of badA consisting of the N-terminal head region, a truncated neck region and the C-terminal membrane anchor), we analyze bacterial adhesion, biofilm formation, and biofilm composition. Immunofluorescence microscopy using anti-BadA primary antibody and fluorophore conjugate secondary antibody demonstrated surface expression of the truncated version of the badA expressed by the partial complement. Real time monitoring using ACEA Biosciences’ xCelligence RTCA system and microscopy experiments both show that Bh Houston-1 quickly adheres and forms biofilm more efficiently than the Bh Houston-1 ∆badA. The partial complement Bh Houston-1 ∆badA/pNS2P;badA displayed an intermediate ability to form biofilms indicating an incomplete restoration of the parental phenotype. In addition, a novel 3D nano-fibrous scaffold biofilm model was stained with the cationic dye Alcian Blue and employed to preserve the EPS structure and show biofilm formed by Bh Houston-1. The deletion of the badA gene significantly decreased adhesion, aggregation and biofilm formation in-vitro that was partially restored with a partial complement of the badA gene. Addition of proteinase K and DNase1 both reduced adhesion and biofilm formation for all three strains suggesting the presence of both protein and extracellular DNA as components of the Bh biofilm EPS. We conclude that badA is required for optimal adhesion, auto agglutination and biofilm formation by Bartonella henselae.
Dispersion is a mechanism by which bacterial cells leave the biofilm in response to various environmental endogenous and exogenous cues including carbon sources and nitric oxide, resulting in a phenotypic switch of dispersing cells returning to the planktonic mode of growth. Compared to biofilms dispersed cells have been demonstrated to have reduced c-di-GMP levels, increased motility, reduced matrix, and altered virulence and susceptibility. While it is now apparent that dispersion occurs as a result of complex spatial differentiation and molecular events in biofilm cells, little is known about the mechanism contributing to dispersion. The aim of the current study was to characterize dispersal mechanisms by elucidating genes necessary to enable dispersion. We therefore determined the transcriptome of planktonic, biofilm, and dispersed cells by using RNA-Seq, with dispersion being induced by nitric oxide or glutamate. Genes identified to be induced upon induction of dispersion or found to be dispersion-specific comprised those involved in adaptation and protection, transcriptional regulators, and matrix degradation. The latter comprised genes encoding secreted degradative enzymes including nucleases (EndA, EddA) and hydrolases (PslG, PelA, PA3429 and PA0480). Given that dispersion coincides with the release of bacteria from biofilms enmeshed in a polymeric matrix composed of polysaccharides and eDNA, we asked whether genes encoding matrix degrading enzymes are required for dispersion. While inactivation of eddA only impaired dispersion in response to glutamate, biofilms by endA::IS were impaired in the dispersion response regardless of the dispersion cues used. Moreover, induction of endA gene expression coincided with dispersion and an overall reduction in eDNA present in biofilms. We furthermore asked if degradation of polysaccharides present in the biofilm matrix were necessary for the dispersion response. While inactivation of PA0480 only reduced dispersion in response to nitric oxide, inactivation of PA3429 impaired the dispersion response to both cues. Moreover, while inactivation of pel and psl coincided with biofilms being impaired in the dispersion response, only the induction of the Pel specific hydrolase pelA but not pslG, the Psl specific hydrolase, resulted in dispersion. Our findings indicate that nucleases and hydrolases are essential to the liberation of bacterial cells from the biofilm. Moreover, our findings suggest bacteria to be actively released through specific regulation of degradative factors resulting in the overall reduction specific components of the biofilm matrix.
Background: Outer membrane vesicles (OMVs) are ubiquitously produced by Gram-negative bacteria and have extensive roles in virulence and pathogenicity. For these reasons, elucidating what factors control OMV biogenesis is critical for developing therapeutic strategies against pathogenic bacteria. Our lab and others have shown that the Pseudomonas quinolone signal (PQS) induces the formation of OMVs in *Pseudomonas aeruginosa* as well as in other species. PQS is part of a hierarchy of quorum sensing pathways, and its production is dynamic and dependent on many genetic and environmental cues. This work identifies a novel effector of PQS induced OMV formation: cyclic dimeric guanosine monophosphate (c-di-GMP). This universal molecule acts as a lifestyle switch for bacteria inducing the shift from planktonic to biofilm growth. Previous studies have shown that c-di-GMP downregulates the virulence factor regulator (Vfr). Separately, Vfr has been shown to upregulate LasR, which is a positive regulator of PqsR. As a result, we hypothesized that PQS induced OMV production is negatively regulated by c-di-GMP.

Methods: PQS induced OMV production was measured under low and high c-di-GMP conditions. These conditions were achieved using c-di-GMP mutants in planktonic conditions and a natural biofilm model. In our genetic approach, ΔwspF (which overproduces c-di-GMP due to its inability to regulate the WspR diguanylate cyclase) and PAO1/pMJT-PA2133 (which overproduces the PA2133 phosphodiesterase and depletes intracellular c-di-GMP levels) were analyzed after 12 hours of planktonic growth. In our natural model, tube reactor biofilms that have different c-di-GMP concentrations at each stage of biofilm formation were analyzed for PQS, OMVs, and c-di-GMP. PQS was extracted with ethyl acetate and quantified by TLC. OMVs were isolated by differential centrifugation and measured by nano-particle tracking analysis (NTA) and lipid analysis. C-di-GMP was extracted with ethanol and quantified by HPLC.

Results: In our planktonic studies we found that under high intracellular c-di-GMP conditions (ΔwspF) PQS production significantly decreased. Consequently, we also observed that OMV production was reduced in ΔwspF. On the contrary, when planktonic cultures were grown under low c-di-GMP conditions (PAO1/pMJT-PA2133) PQS production significantly increased. This increase was accompanied by a significant increase in OMV formation. We were very excited to show that the trends observed in planktonic conditions upheld in a biofilm model. We observed that as biofilms developed through attachment, maturation and dispersion, PQS production was inversely correlated to intracellular c-di-GMP concentration. Conclusion: This work provides new insights into PQS-controlled OMV biogenesis as it connects this mode of OMV production to a major modulator of pathogenicity in *P. aeruginosa*, c-di-GMP.
Evolutionary Innovation in Biofilm Formation in *Pseudomonas aeruginosa*

Abstract Body:
The pathogen *Pseudomonas aeruginosa* (PA) exhibits complex social behaviors. Although the physiological basis of processes like biofilm formation has been studied for several years, it is still unclear how gene content differences shape this process across different environments and selective pressures. We used a 138-strain library of sequenced PA isolates from environmental and human sources and phenotypically characterized these isolates biofilm formation and antibiotic resistance. Using a combination of LASSO (with Bayesian parameter estimation) and phylogenetic signal assignment we were able to estimate genetic predictors for all the traits based on the size of their effect and their phylogenetic correlation. This mapping of genotypes to phenotypes enabled us to explore the evolution of biofilm formation. We found that several transcription factors which shape these processes and cluster together in the PA regulatory network. Based on these findings we used mutual information to build a co-occurrence network of genes. This network indicates that traits evolve in a modular fashion. We also found instances where modules with predicted effects biofilm formation have an inverse effect on antibiotic resistance. Finally, we mapped the genes of PA to lower phylogenetic levels to date the age of genes involved in biofilm formation and characterize the historical times where most genes appear.
Background: Autotransporter (AT) proteins make up the largest group of non-frimbrial adhesins in Gram-negative bacteria. The autotransporter Antigen 43 (Ag43) is a highly abundant AT, which through self-association between neighbouring Escherichia coli cell surfaces was known to promote both cell aggregation and biofilm formation. Ag43 is found in all E. coli pathotypes, including uropathogenic E. coli (UPEC), the main cause of urinary tract infections and enterohaemorrhagic E. coli (EHEC), causative agent of severe foodborne diseases. The chronic forms of these infections involve bacterial biofilms, which promote bacteria persistency in hostile environments and resistance against the effects of conventional antibiotics. Methods: We have investigated and compared two distant Ag43 homologues from UPEC and EHEC in order to define similarities and differences in their molecular mechanisms for forming bacterial aggregates and biofilms. We have accomplished this by utilising a unique combination of X-ray crystallography, biophysical techniques, mutagenesis and cell-based assays. Results: My two new X-ray crystal structures of Ag43 from UPEC and EHEC have shown a surprising degree of structural conservation of their three stranded beta-helix structures and L-shaped bending. Despite this similarity, both adhesins remarkably showed significant differences in their mechanism of self-association, when their interfaces were mapped using site-directed mutagenesis. We further found that these differences in Ag43 self-association caused variations in their dimerization affinities. These differences in structure and mechanism of action translated into differing bacterial aggregation phenotypes. Conclusions: The different modes of dimerisation observed in Ag43 may reflect the adaptations to the different environmental niches that the they colonize. These findings suggest that there is a possibility of further diversity in the self-association of autotransporter. These differences and common themes of self-association could be used to create both narrow and broad spectrum anti-microbials.
**Abstract Topic:** Grapping Hooks Involved in Biofilm Development

**Abstract Title:** The Effect of Bile Application on Biofilm Matrix of *Listeria monocytogenes*

**Abstract Body:**

*Listeria monocytogenes* is a biofilm-forming microorganism capable of survival in a wide range of conditions. Even a high bile salt concentration with antimicrobial activity did not prevent its biofilm growth in the gallbladder. The biofilm formation and the ability to attach to abiotic surfaces or biotic surfaces are facilitated by extracellular polymeric substances, the biofilm matrix. The matrix contributes to the resilience of the microorganism to stress conditions, including bile. Therefore, we aimed to assess the effect of bile on biofilm formation and each matrix component. The biofilm formation of 25 strains was assessed with and without the presence of bile. All experiments were performed at least in three independent replicates and evaluated statistically. The biofilm treated with bile was visualized by confocal laser scanning microscopy. The ability of strains to form a biofilm increased in the presence of bile (p<0.001). The preliminary tests revealed that the total amount of proteins was similar in both treatments. Surprisingly, overall eDNA content was higher after the application of bile in contrast to saccharides that decreased significantly. The information about the proportion of biofilm matrix components could expand the understanding matrix functions in the presence of bile.
Grapping Hooks Involved in Biofilm Development

The Conformation of Fibronectin Determines the Success of Bacterial Attachment

N. Khan1, H. Aslan1, H. Buttner2, H. Rohde2, R. Meyer1;

1Aarhus University, Aarhus, DENMARK, 2University Medical Centre Hamburg-Eppendorf, Hamburg, GERMANY.

Background: Staphylococcus epidermidis is responsible for many implant-associated infections, and its pathology is linked to its ability to form biofilms on the implant. Attachment to the implant occurs through specific interactions with adsorbed host proteins. One important receptor for S. epidermidis is the giant extracellular matrix binding protein (Embp), which binds to fibronectin (Fn). Embp, like many other bacterial adhesins, bind to host proteins that are available in solution in the blood as well as adsorbed to the surface of biomedical implants. So how can interaction with these proteins promote attachment to the surface? We hypothesized that Embp only mediates attachment to immobilized fibronectin, and that the distinction between soluble and immobilized fibronectin lies in the availability of binding domains in Fn. These domains may become exposed only when the protein adsorbs to an implant surface and undergoes conformational changes that lead to fibrillation.

Methods: To investigate these hypotheses, we first used fluorescently labelled Fn and confocal microscopy to show that S. epidermidis interacted with adsorbed but not soluble Fn. Soluble Fn is in a globular conformation, while adsorbed Fn can either remain globular, or change conformation to form fibrils in a similar way as it does in the extracellular matrix of host tissue. To study the bacterial interaction with Fn in these two conformations, we produced surfaces coated with (poly)methyl acrylate (PMA) and (poly)ethyl acrylate (PEA), which adsorb Fn in the two different conformations.

Results: Atomic force microscopy confirmed that Fn adsorbed to PMA remained globular, while Fn adsorbed to PEA fibrillated. We then quantified Embp-mediated bacterial attachment to the two surfaces, using Staphylococcus carnosus expressing a recombinant fragment of Embp. Fibrillar Fn promoted bacterial attachment while globular Fn did not. These differences were also reflected in the adhesion forces.

Conclusions: This result supports our hypothesis that adsorption-induced conformational changes dictate if a host protein promotes or prevents bacterial attachment to an implant surface. Our results underline that the materials properties of implants affect biofilm formation indirectly by making host proteins available in the right or wrong conformation. This knowledge adds a new layer to the considerations made in materials design for novel implant materials that prevent biofilm infections.
Host Microbe Biofilms

Metabolites Produced by Lung Epithelial Cells Potentiate Aminoglycoside Activity Against *Pseudomonas aeruginosa* Biofilms by Increasing Bacterial Intracellular pH

Universiteit Gent, Gent, BELGIUM.

**Background:** During the infection process, bacterial pathogens are exposed to a variety of host factors that may influence their susceptibility to antimicrobial agents. While many compounds that modulate antibiotic activity are known, the influence of the local environment at the host-pathogen interface on bacterial responses to antibiotics is still poorly understood. Gaining insights into these host factors that influence the efficacy of antibiotics may help improve our understanding of why there is such a poor correlation between antibiotic activity *in vitro* and *in vivo*. This is particularly relevant for biofilm-associated infections for which antibiotic therapy chosen based on susceptibility assays frequently does not lead to clinical improvement, as is the case in for example respiratory tract infections by *Pseudomonas aeruginosa* in people with cystic fibrosis. **Study goals:** This study aims to evaluate the influence of lung epithelial cell secretions on antibiotic activity against *P. aeruginosa* biofilm formation. We investigated (i) whether lung epithelial cell secretions modulate the activity of antibiotics, (ii) which host factors are responsible, and (iii) what the underlying mode of action is. **Methods:** We assessed antibiotic activity against *P. aeruginosa* in the presence of conditioned medium of an *in vivo*-like 3-D lung epithelial cell model (3-D CM). Antibiotic activity in the presence of 3-D CM or control medium was tested using a biofilm inhibition assay and time-kill curve. Tobramycin uptake was measured using BODIPY-labelled tobramycin in combination with flow cytometry analysis. The intracellular pH was determined using a 2,7-bis(2-carboxyethyl)-5(6)-carboxyfluorescein-acetoxymethyl (BCECF-AM) assay. A series of chemical inhibitors of metabolic pathways were used to assess which compounds produced by the host were responsible for increased tobramycin activity. **Results:** The 3-D CM potentiated the biofilm inhibitory and bactericidal activity of aminoglycosides, including tobramycin. The effect was observed for the reference strain PAO1 and most of the tested clinical and environment isolates of *P. aeruginosa*. Mechanistic studies indicated that 3-D CM enhanced intracellular pH of *P. aeruginosa* (hereby increasing the ΔpH component of the proton motive force, PMF), resulting in an enhanced tobramycin uptake. Finally, our data suggest that metabolites of the host, generated through pyruvate metabolism, stimulate bacterial metabolism - hereby increasing the PMF and aminoglycoside uptake in *P. aeruginosa* biofilm cells. **Conclusions:** We demonstrate that lung epithelial cells contribute to the innate defence against biofilm-grown *P. aeruginosa* by acting in concert with antibiotics.
Session Title: **THURSDAY Poster Session 4**
Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 074

Abstract Topic: Host Microbe Biofilms

**Abstract Title:** Staphylococcus aureus Modulates Expression of a Novel Antimicrobial Protein Perforin-2 to Establish Chronic Wound Biofilms

**Author Block:** I. Pastar, N. Srbo, A. Sawaya, V. Chen, K. O’Neill, C. Head, A. Ferreira, L. Romero, R. Kirsner, M. Tomic-Canic; University of Miami Miller School of Medicine, Miami, FL.

**Abstract Body:**

*Staphylococcus aureus*, in both methicillin sensitive and methicillin resistant form, is the common pathogen associated with chronic wound infections. Despite the fact that *S. aureus* is the most prevalent microorganism in multiple cutaneous diseases associated with biofilm, the mechanisms by which this pathogen is causing persistent skin infections remain largely unknown. We examined the role of recently-discovered anti-microbial protein, Perforin-2 (P-2) a membrane-attack-complex-perforin domain containing protein important for cutaneous innate immunity, in *S. aureus* wound biofilms. We have previously shown that wound infection with *S. aureus* in P-2 deficient mice results in systemic bacterial dissemination and death. Necrosis and inflammation were absent in P-2 KO despite systemic dissemination and massive replication of *S. aureus* *in vivo*. We further analyzed P-2 expression in human skin wound model and biofilm infected chronic ulcers at a single cell resolution. We describe a novel approach for the measurement of host reponse to biofilm infection by analyses of P-2 mRNA within individual skin cells using an amplified fluorescence *in situ* hybridization (FISH) technique. The unique aspect of this approach (FISH-Flow) is simultaneous detection of P-2 mRNA in combination with immune-phenotyping for cell surface proteins using fluorochrome-conjugated antibodies. We detected P-2 transcript in both hematopoietic (CD45+) and non-hematopoietic (CD45-) cutaneous cell populations, confirming the P-2 expression in both professional and non-professional phagocytes. Next, we investigated P-2 role in restoration of barrier function utilizing human *ex vivo* wound model. We found induction of P-2 during early inflammatory phase of wound healing confirming its role in cutaneous immunity. In contrast to healthy skin and non-infected wounds, FISH-Flow analyses from human infected chronic diabetic ulcers revealed P-2 suppression in CD45+ cells and basal keratinocytes, suggesting their inability to eliminate *S. aureus* biofilms. The P-2 mediated response to *S. aureus* biofilms was measured in human *ex vivo* wound infection model. Human wound infection with *S. aureus* biofilm resulted in suppression of P-2 expression in a cell specific manner, revealing a novel mechanism by which *S. aureus* escapes cutaneous immunity to cause persistent biofilm wound infections. Our data suggest that P-2 may have dual property during wound healing, acting as an innate immunity effector preventing biofilm formation and also wound healing stimulator, while its suppression by *S. aureus* contributes to chronicity of wound infections. Thus, P-2 may be a new target for prevention of staphylococcal skin infections and associated complications.
Background: We show that the eukaryotic HMGB1 protein which is known to function as a pro-inflammatory effector, is also the primary guardian of the innate immune system to control bacterial biofilms by virtue of its potent anti-biofilm activity. HMGB1 is a ubiquitously expressed and highly conserved eukaryotic nuclear protein that functions as a structural component in a gamut of nucleoprotein interactions in the intracellular milieu by virtue of its ability to bind to and bend DNA. HMGB1 can be secreted and/or released into the extracellular milieu by activated immune cells, epithelial cells and fibroblasts and is associated with neutrophil extracellular traps to combat microbial infection. We have previously demonstrated that the bacterial DNABII proteins ubiquitously expressed by prokaryotes bind to and bend DNA in the minor groove and are integral to the structure of biofilms formed by multiple human pathogens. HMGB1 shares no sequence or structural homology to DNABII proteins, yet has been shown to functionally complement DNABII proteins in vitro. Moreover, whereas both the DNABII proteins and HMGB1 bind DNA in the minor groove, HMGB1 binds on the convex surface of DNA while the DNABII proteins bind on the concave side to implement a bend.

Methods: To evaluate its effect on bacterial biofilms in vitro, we added HMGB1 (or its anti-inflammatory variant; 200 nM) to pre-formed biofilms at 24h. At 40h, the biofilms were stained with LIVE/DEAD® and analyzed using confocal laser scanning microscopy. To assess the ability of HMGB1 to eradicate (0.2 nmole HMGB1 added to an established biofilm) or prevent (HMGB1 added simultaneously with the bacteria) biofilm development in vivo, we employed a chinchilla model of otitis media and a mouse model of lung infection.

Results: We demonstrated that recombinant HMGB1 disrupts preformed biofilms in vitro (formed by multiple bacterial species including the ESKAPE pathogens) and in vivo (nontypeable Haemophilus influenzae biofilms in a chinchilla model of otitis media, and Burkholderia cenocepacia biofilms in murine airways). Despite the latter successes, HMGB1 induced a substantial inflammatory response akin to its native function, hence we engineered a mutant HMGB1 variant (mHMGB1) to inactivate its proinflammatory properties. The engineered variant mHMGB1 retained the anti-biofilm function against multiple human pathogens but exhibited an attenuated inflammatory response in both animal models.

Conclusions: Thus, we hypothesize that while HMGB1 has a native extracellular role in both biofilm control and inflammatory response, the latter function can be sufficiently attenuated, which demonstrates the potential to deliver mHMGB1 and thereby provide a significant therapeutic benefit against disease caused by multiple human bacterial pathogens without induction of hyper-inflammatory sequelae.
**Abstract Topic:** Host Microbe Biofilms

**Abstract Title:** A Suppressor Mutation of Eep-Mediated Lysozyme Resistance Leads to Permanent Alterations of the *Enterococcus faecalis* Cell Surface

**Author:** C. N. Rouchon, A. Weinstein, K. L. Frank;

**Block:** Uniformed Services University of the Health Sciences, Bethesda, MD.

*Enterococcus faecalis* is an opportunistic pathogen that is resistant to lysozyme, an important antimicrobial of the host innate immune system. Previous studies demonstrated that the *E. faecalis* Eep membrane metalloprotease, a biofilm infection-associated virulence factor, confers lysozyme resistance through a signal transduction cascade that involves activation of the alternative sigma factor SigV via cleavage of the anti-sigma factor RsiV. Our lab has isolated suppressor mutants of Δ*eep* (Δ*eep* lysR) that regain the ability to thrive in the presence of lysozyme. The goal of this study is to elucidate the molecular mechanisms that confer the Δ*eep* lysR phenotype. Using a time-kill assay, in which *E. faecalis* planktonic cultures were grown in broth containing 2.5 mg/ml lysozyme, we found that the number of viable Δ*eep* cells decreased by >2 log₁₀ CFU/ml relative to the inoculum at 6 hours post-exposure. However, after 24 hours of exposure to lysozyme, the number of Δ*eep* cells recovered to a level similar to that of WT, for which growth was unaffected. Colonies of Δ*eep* from 24-hour cultures remained resistant to killing by lysozyme after passage in non-selective, rich medium. These experiments suggested that the observed re-growth of Δ*eep* in the 24-hour lysozyme cultures was due to selection of genetically stable, lysozyme-resistant Δ*eep* suppressor mutants (Δ*eep* lysR). Whole genome sequencing analysis of Δ*eep* lysR isolates revealed that a gene required for techoic acid biosynthesis was mutated in these strains. Using a microtiter well biofilm assay, we found that the biomass of 24-hour Δ*eep* lysR biofilms was reduced relative to that of the lysozyme-sensitive Δ*eep* parent strain. Additionally, through confocal microscopic imaging, we observed that the cell envelope of Δ*eep* lysR biofilms stained with an Alexa Fluor 594-wheat germ agglutinin conjugate fluoresced more intensely than that of similarly-labeled WT and Δ*eep* cells. Further examination of the Δ*eep* lysR cells by transmission electron microscopy demonstrated that the cell surface of these isolates lacked the electron dense structures observed on the surface of WT and Δ*eep* cells. Furthermore, analysis of purified cell wall carbohydrates indicated that Δ*eep* lysR exhibits altered polysaccharide content relative to WT and Δ*eep* cells. The combined findings of this study demonstrate that in the absence of functional Eep protein, Δ*eep* lysR suppressor mutants utilize an alternate mechanism of lysozyme resistance that is associated with stable alterations to the cell surface. Our on-going studies are focused on examining the fitness of Δ*eep* lysR cells following exposure to cell wall-targeting molecules and characterizing the lectin-binding abilities of these isolates.
Altered Crosstalk among Related Histidine Kinases Promoted Biofilm Production and Mediated the Rapid Evolution of Non-
Native \textit{Vibrio fischeri} to a Novel Symbiosis

C. A. Whistler, R. L. Foxall, V. S. Cooper; University of New Hampshire, Durham, NH.

Symbiotic strains of \textit{Vibrio fischeri} must coordinate the expression of multiple traits, including production of a biofilm matrix, in
order to enter into a mutualism with the squid, \textit{Euprymna scolopes}. We utilized an experimental evolution approach to examine
whether non-native and symbiosis impaired strains could adaptively evolve under squid host selection and subsequently identified
the paths of adaptability in evolved lineages (Pankey, Foxall et al.; eLife 2017;6:e24414 doi: 10.7554/eLife.24414). This led to the
discovery of convergent gain-in-function mutations in the gene encoding the BinK biofilm repressor underlay adaptation. The
breadth of phenotypes associated with adaptive \textit{binK} alleles implied a potential pliability of sensory transduction partnerships
could underlie improved symbiotic fitness. We used comparative genomics to identify potential signal transduction partnerships,
which revealed a paralogous, ancestral sensor kinase to BinK we call RemK. BinK and RemK differed in sensory perception
architecture and had divergent REC output domains that could promote phosphotransfer with different downstream signaling
partners but had a conserved core domain sequence that could promote cross-talk. \textit{In vivo} protein interaction assays indicated that
BinK and RemK were capable of forming a heterodimer and genetic analysis indicated that BinK repressed biofilm and squid
colonization by heterodimerization with RemK. BinK and RemK co-repressed cellulose and symbiotic polysaccharide (Syp)
production and loss of either paralog increased expression of \textit{sypA}. However, loss of \textit{remK} additionally enhanced the expression of
all \textit{syp} promoters activated by the key regulator SypG. We predict that the adaptive mutations impaired BinK homodimer activity
and also impaired heterodimer repressor activity thereby extending the BinK-mutant regulon to include impaired RemK repression
of biofilm and that these mutations were favored over mutations that eliminated BinK through the greater impact on biofilm matrix
production that included both Syp and cellulose.
Regulation of Biofilm Development

Oxygen-Driven Adhesion and Biofilm Formation of the Foodborne Pathogen Campylobacter: Role of the Virulence Factor CadF and the Transcriptional Factor CosR

Author Block:
R. Briandet¹, T. Neu², A. Stintzi³, E. Dé⁴, O. Tresse⁵;
¹INRA, Jouy-en-Josas, FRANCE, ²UFZ Helmholtz Centre for Environmental Research, Magdeburg, GERMANY, ³University of Ottawa, Ottawa, ON, CANADA, ⁴University of Rouen, Rouen, FRANCE, ⁵INRA, Nantes, FRANCE.

Background: Campylobacter jejuni has been reported as the leading cause of bacterial foodborne infections worldwide, with significant increase over the past years. Despite the fastidious growth requirements, C. jejuni is able to survive in the environment without permanent loss of viability and virulence. The mechanisms responsible for its survival remain unknown, survival strategies might be associated to adhesion to inert surfaces and biofilm formation. The objectives of this study was to assess the effect of dioxygen (O₂) on adhesion and biofilm formation of C. jejuni. In order to understand the underlying mechanisms of dioxygen on adhesion and biofilm formation, the role of the virulence factor Campylobacter adhesion to fibronectin CadF and the essential transcriptional factor Campylobacter oxidative stress regulator CosR identified to pilot the sub-system of reactive oxygen species (ROS) detoxification in C. jejuni were inspected.

Methods: BioFilm Ring Test was used to assess the adhesion to inert surfaces and Confocal Laser Scanning Microscopy (CLSM) was applied to analyse biofilm formation and spatial structuration. Effect of oxygen-enriched conditions (OEC) on membrane proteome was approached using proteomics analyses. CadF-knock out mutant and CosR-overexpressing transformant were used to investigate their role in adhesion to inert surface and biofilm maturation, respectively.

Results: A high intra and inter-species variability among strains of Campylobacter was observed for adhesion capability. Among the adherent strains, biofilms could be formed within 17 h of incubation. Biofilm architecture could differ from finger-like structure with voids and channels to compact multilayer-like structure. Dioxygen and oxidative stress conditions favoured adhesion to inert surface and biofilm formation. Pre-conditioned cells to oxygen-enriched conditions (OEC) were less favourable to biofilm development than cells incubated in OEC during biofilm formation. CadF was over-expressed under OEC and demonstrated to be involved in the adhesion process to inert surface. The orphan two-component regulator CosR was found to contribute to the entrance to the maturation phase of biofilm in an O₂-independent manner.

Conclusions: C. jejuni is able to survive by forming biofilms. Cellular mechanisms involved in adhesion and biofilm development are enhanced by O₂. Entrance in maturation phase seems to be crucial for biofilm development of C. jejuni. While the microaerophilic Campylobacter is sensitive to oxidative stress, oxygen-enriched conditions enhanced adhesion to inert surface and biofilm formation. Biofilm might play an important role in O₂ distribution and availability to maintain Campylobacter viable in the environment. This adaptation capability might contribute to disseminate pathogenic species of Campylobacter in the environment and food plants.
**Abstract Body:**

*Clostridium perfringens* is a Gram-positive spore-forming anaerobic pathogen that causes gas gangrene and food poisoning. Biofilm formation and self-produced extracellular polymeric substances (EPS) confer increased tolerance, and thus biofilms are widely recognized as causes of infectious diseases and environmental contaminants. We found that *C. perfringens* forms biofilms with different structures in response to temperatures. At 37°C, cells adhere densely to the surface, forming thin biofilms. In contrast, at 25°C cells produce a threadlike extracellular EPS that facilitates the formation of a thick, elastic, pellicle-like biofilm. Temperature is an environmental cue that alters between the outside and inside of the host, which suggests that the morphological changes of biofilms are involved in the pathogenesis of *C. perfringens*. Here, we identified the gene responsible for production of the threadlike extracellular EPS and pellicle-like biofilm formation. We constructed fluorescent protein-fusion reporters to detect the EPS gene expression in *C. perfringens*. Fluorescent reporter analysis indicates that there are two types of cells within the overall population. In one type, the EPS gene promoter is activated. In the other type, the EPS gene promoter is repressed, attaching to the surface. The sub-population size of EPS-expressing cells gradually increase as the temperature decreases. Interestingly, we observed that cells expressing EPS overlie the EPS-repressed cells, which are located near the surface by confocal laser scanning microscopy. The deletion of the gene involved in type IV pili, which are involved in the attachment in *C. perfringens*, activates the EPS gene expression in the whole population through the positive feedback regulation via a two-component system. Furthermore, we also found that a quorum sensing system is also involved in fine-tuning of the EPS gene expression. Heterogeneity within the population determines cell fate; cells may either attach to the surface or produce the threadlike EPS. This heterogeneity is controlled by temperatures and quorum sensing, which greatly influences biofilm morphology. *C. perfringens* may modulate EPS expression to induce morphological changes in the biofilm structure as a strategy for adapting to the inter-host and external environments.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 080

**Abstract Topic:** Regulation of Biofilm Development

**Abstract Title:** Role of Temperature Dependent Regulation on *Pseudomonas aeruginosa* Biofilm Formation

**Author Block:**

- K. Bisht¹, A. G. Brown¹, J. L. Moore², R. M. Caprioli², E. P. Skaar³, C. A. Wakeman¹;
- ¹Department of Biological Sciences, Texas Tech University, Lubbock, TX, ²Department of Chemistry, Vanderbilt University, Nashville, TN, ³Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN.

**Abstract Body:**

Biofilms are surface-associated groups of microorganisms that adhere to surfaces and interact with each other using an extracellular polymeric substance matrix. Microorganisms have developed complex mechanisms to sense and react to their constantly changing environment under these conditions. One key regulatory cue for them is temperature. Studies have shown that different factors, such as temperature, can cause behavioral and morphological changes in the microbial communities. *Pseudomonas aeruginosa* is a common nosocomial gram-negative bacterium, that can cause various serious diseases in infected humans. The severity of the infections is compounded by *P. aeruginosa*’s ability to form robust biofilms in all the various niches it occupies. Biofilm-associated infections are particularly recalcitrant to clearance by both antimicrobial therapy and immune function. We hypothesize that the fluctuations in temperature in the different niches that *P. aeruginosa* occupies drive the formation of biofilms specifically adapted to survival within that niche. Using MALDI IMS, we have demonstrated that biofilms grown under these different temperature conditions exhibit dramatically different protein expression profiles, which supports the contention that these biofilms are uniquely adapted to different niches. The objective of this project is to elucidate the genes involved in the temperature regulation of biofilm formation of *P. aeruginosa*. For this purpose, a biofilm screen was run on a commercially-available transposon mutant library containing over 5,000 unique mutants of *P. aeruginosa* at four different temperatures (room temperature, 30°C, 37°C, and 40°C) to identify genes required for temperature-dependent biofilm formation. This temperature range was chosen to simulate conditions relevant to both medical and industrial settings. These strains were replicated out into 96-well plates and incubated for two days at the specified temperatures. Finally, total biomass and biofilm growth were monitored using specific absorbance readings combined with staining analysis. The biofilm assay becomes more variable as temperature increases, but we were still able to identify potential primary screen hits that exhibit reproducible biofilm phenotypes. We identified mutants with temperature dependent as well as few with temperature-independent phenotypes. The potential hits were then categorized into groups based on their function and carried forward towards the secondary screen.

Effectively this project will reveal the genetic mechanisms utilized by *P. aeruginosa* to establish biofilm growth at temperatures relevant to medical, industrial, and natural environments and will provide a wealth of information regarding the adaptive potential of *Pseudomonas aeruginosa* towards the colonization of various niches including the human.
Oxygen-Regulated Subpopulations Direct the Formation of Architecturally Complex Biofilms Capable of Withstanding Exogenous Insults

C. J. Beebout¹, A. R. Eberly¹, J. L. Moore¹, S. H. Werby², S. A. Reasoner¹, J. R. Brannon¹, S. De³, M. J. Fitzgerald¹, M. M. Huggins¹, D. B. Clayton³, L. A. Cegelski², M. Hadjifrangiskou¹;
¹Vanderbilt University, Nashville, TN, ²Stanford University, Stanford, CA, ³Vanderbilt University Medical Center, Nashville, TN.

Rather than existing as a phenotypically uniform population, bacteria within biofilms differentiate into spatially segregated and metabolically distinct subpopulations. As a result of this metabolic specialization, there is a division of labor within biofilms that is essential for the formation of structurally complex communities capable of withstanding exogenous stressors. The presence of chemical gradients within biofilms is a major driver of metabolic differentiation. Previous studies detected a steep oxygen gradient within biofilms that results from consumption of oxygen by respiring bacteria near the surface of the biofilm. In turn, this oxygen gradient regulates bacterial redox-state and influences expression of key adhesive molecules and matrix components. We hypothesized that the presence of such gradient results in distinct subpopulations that express different respiratory components and are organized along the oxygen gradient. Consistent with this hypothesis, we demonstrate that cytochrome bd, a high affinity quinol oxidase required for aerobic respiration under hypoxic conditions, is the most abundantly expressed respiratory complex in uropathogenic Escherichia coli biofilms. Strikingly, loss of cytochrome bd causes marked disruption biofilm architecture and leads to global dysregulation of protein expression within the biofilm. In addition, the loss of cytochrome bd alters the composition of the biofilm extracellular matrix, such that the mutant biofilms permit increased penetration by aqueous solutions and, as a result, are more susceptible to antibiotic therapy. These data indicate that oxygen gradients spatially regulate expression of respiratory components within biofilms and that the cytochrome bd expressing subpopulation is central to formation of structurally intact biofilms capable of withstanding antibiotics and other exogenous stresses.
Isolation of Biofilm Producing Microorganisms from Packaged Water Sold in the Open Market Within the Three Senatorial District of Lagos Metropolis

W. W. Effiok, L. O. Egwari, L. O. Itaba

1University of Lagos, Lagos Nigeria, Lagos, NIGERIA, 2Covenant University, Canaanland, OTA, Ogun, NIGERIA, 3Scientific Laboratory Services LTD, Lagos, NIGERIA.

Background: Bacteria growing in a biofilm are linked with protracted and recurring human infections; and have been shown to be highly resistant to antimicrobial agents. Although biofilm has become a popular research topic in many areas in recent years; it is still opaque in the continent of Africa as very little or no data is available in this area in most African countries. Objective: This study was conducted to detect biofilm forming Microorganisms in packaged water sold in retail store in Lagos, Nigeria. Method: Samples (130 bottled and 170 sachet) water was purchased using the simple random technique and isolation of microorganism was carried out using the filtration method recommended by WHO; while biofilm formation was detected using the tube method. Results: A pH range of 3.5 was recorded for the 300 samples at 25°C. 82% of the 130 bottled water brands conformed to WHO maximum contamination level while 29% of the 170 sachet water did not. A total of 112 bacteria were isolated of which approximately 70% were capable of producing biofilms of varying degree. Among the strong biofilms producing isolates are E. coli, Klebsiella pneumoniae, P. aeruginosa, Salmonella typhi and Enterococcus faecalis while S. aureus S. epidermidis were moderate biofilm formers whereas species of Bacillus and Pseudomonas were non/weak biofilm producers. Conclusion: The results of the present study exposes the lack of attention paid to biofilms formation within storage tanks and pipes of water distribution system; by this small and medium scale packaged water companies; sprouting up every now and then within the metropolis. We therefore call for strict and routine monitoring of the fast growing packaged water industry within the metropolis with a view that ensures adequate periodic back washing activity and anti-biofilms equipment are used within the industry, in order to safeguard public health.
Prophages are regions in bacterial genomes that have originated from lysogenic viral infections. Recent research showed that in a pool of 2110 bacterial genomes, almost half of the genomes contain such regions. Among Pseudomonas aeruginosa strains, the prevalence of prophages is even higher; at least one prophage region was found in every strain analysed.

To examine the contribution of prophage genes, a comprehensive screening was done on five P. aeruginosa strains (laboratory and clinical strains) using several bioinformatic tools, which revealed a total of ~750 prophage-derived genes. Two of these genes, Prevent host death protein (phdP) and Plasmid stabilization system protein (pssP) were found in two of the examined strains (PAO1 and C3719), and are estimated to constitute a Toxin-Antitoxin (TA) system. TA systems are genetic elements that consist of two components - a toxin and antitoxin that counteract the activity of the toxic protein. There are a variety of functions that have been assigned to TA systems, ranging from persistence to DNA stabilization to protection against mobile genetic elements. PssP is assumed to be a member of the ParE toxin family, which is toxic towards DNA gyrase, but is neutralized by the antitoxin. We provide experimental evidence demonstrating that PssP acts as a toxin most likely through interaction with GyrA. Another pair of genes, Accessory Cholera Enterotoxin (Ace) and Zona Occludens Toxin (Zot), were found in three of the examined strains (PAO1, C3719 and 2191), and are localized in one operon along with coat protein A of bacteriophage Pf1. Ace and Zot, together with Cholera Toxin, are known to comprise the Vibrio cholerae's "virulence cassette", which originated from bacteriophage CTXΦ. A previous study suggested that Zot might not have virulent functionality in P. aeruginosa and has an additional role which was speculated to be related to phage assembly. We characterized the role of Ace and Zot on a range of P. aeruginosa phenotypes including biofilm formation and motility.
Regulation of Biofilm Development

Title: Linking form and function of *Mycobacterium tuberculosis* Biofilms

Author: J. P. Richards¹, A. K. Ojha²;

¹Wadsworth Center, NYSDOH/University of Pittsburgh, Albany, NY, ²Wadsworth Center, NYSDOH, Albany, NY.

Abstract: Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is among the first clonally purified bacterial species described to form multicellular communities, appearing like parallel bundles of cords. Our work over the last decade have led us to define these communities as surface-associated, matrix-encapsulated, and antibiotic tolerant three-dimensional architecture, providing consistency with the broader framework of microbial biofilms. However, factors regulating the architecture development of Mtb biofilms and their relationship, if any, to the emergence of antibiotic tolerance remained unclear. Using a high throughput TnSeq-based approach, we screened a mutant library of Mtb to rank fitness of individual mutants in biofilms, relative to planktonic culture. Mutants thus identified were further analyzed for their sensitivity to rifampicin (RIF) in planktonic cultures.

Majority of mutants with deficiency in biofilm formation were also hypersensitive to RIF, suggesting that growth of Mtb in biofilms enriches drug tolerant clones at the expense of their drug-sensitive counterparts. A proof-of-concept at molecular levels was offered by mutants of genes involved in phosphorous homeostasis. While mutations in the phosphorous sensing pathway cause hypersensitivity to RIF through a constitutive activation of RegX3-SenX3 two-component system, these mutations also abrogate biofilm-specific induction of a gene cluster involved in remodeling of the cell envelope during architecture development. In summary, our findings provide a direct linkage between architecture development and emergence of drug tolerant cells in Mtb biofilms.
Regulation of Biofilm Development

Transcriptomic Analysis Revealed Unexpected Role Played by Flagella in *Helicobacter pylori* Biofilm

**Author:** S. Hathroubi, J. Zerebinski, K. Ottemann;
**Affiliation:** UC Santa Cruz, Santa Cruz, CA.

Around the half of the world's population is infected by *Helicobacter pylori* which is a major cause of duodenal and gastric ulcers as well as gastric cancer. *H. pylori* has an impressive ability to persist chronically in the human stomach and to have increased tolerance towards conventional antibiotic treatments. Similar characteristics are associated with biofilm formation in other bacteria. However, *H. pylori* biofilm process is poorly understood. To gain insight into this mode of growth, we carried out comparative transcriptomic analysis between *H. pylori* biofilm and planktonic cells, using the mouse colonizing strain SS1. Optimal biofilm formation was obtained with low serum and three-day growth. RNA-seq analysis found that 8.18% of genes were differentially expressed between biofilm and planktonic transcriptomes. Biofilm-downregulated genes included those involved in metabolism and translation, suggesting cells in that setting have low metabolic activity. Biofilm-upregulated genes included those whose products were predicted to be at the cell envelope, involved in regulating different stress response, and surprisingly, genes related to formation of the flagellar apparatus. Using scanning electron microscopy, flagella appeared to be a in high abundance and an integral component of the biofilm matrix, supported by the observation that an aflagellated mutant displayed a less robust biofilm with no apparent filaments. We observed flagella in the biofilm matrix of additional *H. pylori* strains, supporting that this is a common occurrence. Flagella are know to play a role in initial biofilm formation, but are often considered to be down-regulated after this stage. In this study, we demonstrated that *H. pylori* have co-opted these structures for non-motility roles, including biofilm and thus support recent studies in *Escherichia coli* that suggested an architectural role of flagella in biofilms.
Controlling Chronic *Pseudomonas aeruginosa* Infections by Strategically Interfering with the Sensory Function of SagS

J. Dingemans¹, R. E. Al-Feghali¹, G. W. Lau², K. Sauer¹;

1Binghamton University, Vestal, NY, 2Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL.

The hybrid sensor SagS plays a central role in the formation of *Pseudomonas aeruginosa* biofilms, by enabling the switch from the planktonic to the biofilm mode of growth, and by facilitating the transition of biofilm cells to a highly tolerant state. In agreement with the transition to the sessile lifestyle having been associated with global shifts in virulence, SagS has been furthermore shown to contribute to the virulence of *P. aeruginosa*. However, the contribution of SagS to *P. aeruginosa* pathogenicity is not well understood. The goal of this study was to determine whether SagS contributes to virulence in a manner dependent on biofilm formation, biofilm drug tolerance, or both. To address this question, we made use of the finding that SagS contributes to biofilm formation and drug tolerance via two distinct sets of amino acid residues located within the HmsP sensory domain of SagS. Specifically, residue D105 was found to only contribute to drug tolerance, while residue L154 affected the ability of *P. aeruginosa* to transition to the surface-associated lifestyle. ΔsagS mutant strains harboring the empty CTX integration vector or expressing intact sagS or sagS variants (harboring alanine substitutions in residues D105 and L154 affecting drug tolerance and biofilm formation, respectively), under the control of the native promoter, were used. Virulence phenotypes were assessed using romaine lettuce, murine lung and agarose bead-based chronic bronchopulmonary mouse infection models. The transcript abundance of select genes was determined using qRT-PCR. Virulence was first assessed using a murine model of chronic pneumonia. Three days post infection, the bacterial burden in mice infected with ΔsagS was significantly reduced relative to the complemented strain. Attenuation of sagS was further supported by ΔsagS only being 17% as competitive as the parental strain PAO1 using competitive index infection assays. Moreover, alanine substitution of residue D105 had no effect on the bacterial burden while expression of sagS-L154A coincided with attenuation. Given that SagS also contributes to the drug tolerance of biofilms in a manner dependent on residue D105, we repeated the virulence study in combination with antibiotic treatment. Tobramycin treatment greatly affected the bacterial burden in mice infected with ΔsagS and ΔsagS expressing sagS-D105 than strains expressing sagS or sagS-L154A. Interestingly, no difference in the disease progression or bacterial burden was noted post 24h of infection of romaine lettuce or the murine lung. Moreover, with the exception of biofilm marker genes, only minor differences in the expression of virulence genes were detected, regardless of the mode of growth tested. Our findings suggest that interference with the biofilm or tolerance regulatory circuits of the sensory domain of SagS affects *P. aeruginosa* pathogenicity in chronic, but not acute, infections.
**Background:** The intricate process of biofilm formation in *S. aureus* involves distinct stages during which a complex mixture of matrix molecules including polysaccharides, proteins, and extracellular DNA (eDNA) is produced and modified throughout the developmental cycle. Early in biofilm development the accumulated cells have been shown to detach from the surface of the microfluidic chamber in an event termed “exodus”. This event is mediated by the production of a secreted staphylococcal nuclease, which degrades the eDNA within the matrix, causing the release of cells, and allowing for the formation of metabolically heterogeneous microcolonies in subsequent stages of development. Recent studies have shown that the nuclease gene (*nuc*) is regulated by the SaePQRS multi-component regulatory system, which is also responsible for regulating the transcription of several additional virulence factors.

**Methods:** To characterize virulence gene expression patterns within the dynamic biofilm context, fluorescent gene reporter plasmids were introduced into strains of interest. These strains were then grown in a Bioflux 1000 microfluidic system which supplies the bacterial populations with a constant flow of fresh media while simultaneously performing time-lapse microscopy to image these developing biofilms in five-minute intervals. To assess the impact of various regulatory systems influence on gene expression within this context, mutant strains in which these systems have been rendered non-functional were transduced with the same plasmids and compared to their wildtype counterparts.

**Results:** Our results indicate the *nuc* gene is expressed in only a subpopulation of biofilm-associated cells via an unknown mechanism involving the SaePQRS system. Interestingly, these studies also demonstrate that SaePQRS coordinates temporal and stochastic expression of other virulence genes during biofilm development. Additional experiments have focused on exploring the effects of SaePQRS as well as the Agr quorum sensing system on expression of virulence factors during biofilm development and have shown that the two regulatory systems have distinct effects on spatial and temporal expression. **Conclusions:** The results of this study demonstrate that stochastic gene expression in *S. aureus* is not limited to the *nuc* gene. Rather, multiple virulence genes were shown to be expressed in this specialized subpopulation of cells. Importantly, these studies also demonstrated that virulence genes regulated by the SaePQRS regulatory system are stochastically expressed, while Agr-regulated genes are generally expressed more homogeneously within growing micro-colonies. Overall our results indicate that the Sae system may function as a bistable switch similar to regulators controlling competence gene expression in *Bacillus subtilis* and persister cell formation in *Escherichia coli*. 
Regulation of Biofilm Development

Unraveling O104:H4 Escherichia coli Chemical Language during Biofilm Formation

T. R. Ribeiro¹, W. P. Elias², C. G. Moreira¹;
¹São Paulo State University, Araraquara, BRAZIL, ²Butantan Institute, São Paulo, BRAZIL.

Enteroaggregative Escherichia coli O104:H4 Stx+ has caused a major diarrheagenic hemolytic-uremic syndrome (HUS) outbreak in many countries in Europe in 2011. Specifically, the C227-11 O104:H4 strain produces Shiga toxin (Stx2a subtype), also found in enterohemorrhagic E. coli O157:H7 serotype, a deadly combination for humans. The two-component system QseBC is closely related to expression of virulence genes in Enterobacteriaceae. The chemical signaling here occurs via Autoinducer-3/Epinephrine/Norepinephrine employing the QseC sensor kinase, as previously described by our group (Curtis et al., 2014). The VisP periplasmic protein is a pleiotropic function player during chemical signal and virulence of different pathogenic E. coli strains (Moreira et al., 2013). A novel compound described by our group, the LED209, blocks the QseBC signaling pathway by directly affecting virulence in Gram-negative pathogens (Rasko., 2008). Therefore, this study aimed to investigate the chemical signaling virulence in EAEC O104:H4 C227-11 (Stx2a+) and EAEC O104:H4 BA3826 (Stx-) in vivo. We have orally challenged C57BL/6 mice with distinct O104:H4 Stx+ and Stx-. This treatment of O104:H4 employing the LED209 compound have shown distinct colonization during a total period of 14 days pi and the expression levels of qseC and visP genes during in vivo challenge.

The qseC overexpression was observed in throughout in vivo experiment, both in the presence and absence of Shiga toxin. It was 3.8-fold higher by C227-11 at day 5 and 9.5-fold for the BA3826 strain. Noteworthy, the qseC expression reduction at day 14 in the absence of Shiga toxin, thus the BA3826 strain, with approximately 1-fold decrease. The visP expression was highly upregulated in the presence of LED209, upon its potential to block the sensor kinase QseC, C227-11 strain between day 3-14 pi has peaked at 30-fold increase. Moreover, the BA3826 strain has shown higher levels of visP expression, specifically at day 5 pi, but much lower in comparison to the Shiga toxin O104:H4 strain. To date, the VisP important role in enteric bacterial stress response seems accentuated upon LED209 in vivo treatment. The QseC sensor kinase may trigger alternative mechanisms of intestinal colonization, bacterial survival and overexpression of important chemical signaling players. Herein, we highlighted the importance of QseC sensor kinase in the O104:H4 (Shiga +) outbreak E. coli strain during colonization in vivo and in the presence of a promising anti-virulence drug to intestinal pathogens.
**Abstract**

**Topic:** Regulation of Biofilm Development

**Title:** Biofilm Dispersion Activities of Ginger (*Zingiber officinale*) Extracts against some Isolates of *E. coli* 0157:H7 from Retailed Dispensed Powdered Milk in Ibadan, Nigeria

**Author:** M. I. Adedeji, V. O. Adetunji; University of Ibadan, Ibadan, NIGERIA.

**Background:** Biofilm formation occurs in natural and man-made environment, on wide variety of surfaces including medical devices, water system piping, aquatic environments, etc. Its formation in food processing environments and surfaces can lead to pre or post production contamination of foods which is of public health significance. It is of great concern because of the contamination of foods which may lead to food poisoning and resistance to antimicrobials, disinfectants and other cleaning agents. *Escherichia coli* (*E. coli*) O157:H7 is an organism which is capable of forming biofilm and causes food borne disease outbreaks. It is one of the major milk borne pathogens and is capable of causing diseases. Ginger is well known for its medicinal and preservative values but its use for inhibition and dispersion of *E. coli* O157:H7 biofilm has not been sufficiently explored. This study was therefore designed to evaluate the biofilm inhibition and dispersion activities of *Zingiber officinale* crude extract and fractions on biofilms of *E. coli* O157:H7 isolated from dispensed powdered milk in Ibadan, South-West Nigeria. **Methods:** Ginger root samples were obtained, identified (UIH-22558), extracted, fractionated using four solvents (hexane, chloroform, ethyl acetate and ethanol) and concentrated. Three *E. coli* O157:H7 isolates were obtained from dispensed powdered milk and a reference strain (*E. coli* ATCC 35218) was obtained from microbiology laboratory. Biofilm quantification was done using the crystal violet binding assay. The effect of the plant extracts on biofilm formation and biofilm dispersal were tested at 37°C for 48 h and 24 h respectively. The experiment was done in triplicates. **Results:** All the isolates developed biofilm (0.08±0.07). Biofilm development was suppressed with ethanolic (0.01±0.00) and crude extracts (0.05±0.01), while highest biofilm dispersion was exhibited with the ethanolic (0.04±0.05) and crude extract fractions (0.05±0.06). The chloroform fractions had little or no effect on biofilm formation and dispersal at 50mg/ml conc. **Conclusion:** Ginger (*Zingiber officinale*) showed potential use for dispersion of already formed biofilm by *E. coli* O157:H7 strains, hence, can be used in food processing plants, surfaces and industries to combat biofilm forming organisms, disperse their biofilms and enhance food safety.
Surface-bound bacteria in matrix-coated aggregates, called biofilms, are up to 1,000 times more resistant to antibiotics than planktonic cells (Rasmussen 2006). Rather than attempting to create new antibiotic treatments, disrupting biofilms would allow for a more effective way to use already existing ones. Pseudomonas aeruginosa is a model organism in biofilm research due to its proficiency forming biofilms, its mechanisms for antibiotic resistance, and its medical impact in chronic infections. The formation and survival of a biofilm is paramount on having a strong initial cell-surface attachment by way of exo-polysaccharides (Palmer 2017). We hypothesize that interrupting bacterial attachment to surfaces will reduce disease burden. RNA sequence results of cells attached within the first hour showed that 437 genes were regulated upon surface contact in the initial attachment phase of biofilm formation. Transposon mutants of these genes were screened for attachment to polyvinyl chloride. Of the 437 mutants of surface-regulated genes, 36% exhibit enhanced attachment and 15% have an attachment defect compared to the parental strain, PAO1. Through prioritization by magnitude of attachment alteration, 8 mutants were selected for clean deletions of highest priority genes. Future work will test the biofilm formation of clean deletion mutants. Identification of essential genes for biofilm formation will facilitate the development of treatments specifically preventing biofilms.
Session Title: **THURSDAY Poster Session 4**

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 091

**Abstract Topic:** Regulation of Biofilm Development

**Abstract Title:** Antiformation of *Streptococcus mutans* Biofilm by *Streptococcus salivarius* BD3900

**Author Block:**

Z. Wu\(^1\), Y. Yu\(^2\), C. You\(^1\), Z. Liu\(^1\);

\(^1\)State Key Laboratory of Dairy Biotechnology, Brightdairy & Food Co. Ltd, Shanghai, CHINA; \(^2\)College of Food Science and Technology, Shanghai Ocean University, Shanghai, CHINA.

Biofilm formed by *Streptococcus mutans* is recognized to play important roles in the development of dental caries. *Streptococcus salivarius* BD3900, isolated from the oral cavity of a healthy volunteer, displayed the potential to be an oral probiotics. The bacterial strain BD3900 displays strong hydrophobicity (95.8%) as well as high surface charges (≥88.6%), which endure its high capability to adhere in the oral cavity. Furthermore, the bacterial strain BD3900 exhibited high auto-aggregation (15%-20%) and co-aggregation (13.1%-64.3%) with *S. mutans*. When co-cultured with *S. mutans* in vitro, the biofilm formation by *S. mutans* could be reduced by 67.1%. To explore the substances and/or mechanism involved in the inhibition of *S. mutans* biofilm formation by *S. salivarius* BD3900, the cell free supernatant of the cultivated M17 broth supplemented with 0.5%(w/v) sucrose by BD3900 was precipitated by ammonium sulphate at 60% saturation. The active components responsible for the inhibition of *S. mutans* biofilm in the precipitated substance were with molecular mass exceeding 10kD. When heated at 70°C for 5min, The precipitated substances lost almost all of the inhibitory activity on the formation of *S. mutans* biofilm, whilst the existence of Fe\(^{3+}\),Zn\(^{2+}\),Ca\(^{2+}\) could enhance the antibiofilm activity. When the precipitated substances were co-incubated with *S. mutans* at the presence of sucrose, the biofilm formed by the latter was significantly decreased, and also the amount of insoluble exopolysaccharides synthesized by *S. mutans*. Therefore, it is postulated that some enzymes secreted by *S. salivarius* BD3900 might interfere the synthesis of insoluble exopolysaccharides by *S. mutans* and thus inhibited the biofilm formation by the latter.

**Keywords:** *Streptococcus salivarius* BD3900; *Streptococcus mutans*; biofilm; dental plaque.
**Regulation of Biofilm Development**

**A Global Analysis of Gene Expression within *Staphylococcus aureus* Biofilms Across Multiple Clonal Lineages**

**Author:** B. R. Tomlinson, J. L. Adams, L. N. Shaw;

**Block:** University of South Florida, Tampa, FL.

*Staphylococcus aureus* is a highly virulent, opportunistic pathogen and a leading cause of both nosocomial and community acquired infections. Although a variety of methods exist for clonally typing widely different isolates of this organism, the CDC derived USA lineages is perhaps most commonly used in North America. When comparing these clonal lineages, it is clear that infection characteristics vary greatly as demonstrated by distinct disease progression and altered expression profiles of virulence determinants. A key characteristic of *S. aureus* infections, and one that also varies phenotypically between clones, is that of biofilm formation, which aids in bacterial persistence through increased adherence and immune evasion. Using newly developed real-time tracking technology known as xCELLigence, we measured biofilm initiation, progression, and dispersal across the 5 major *S. aureus* CDC-types (USA100-USA500). In so doing we observed patterned adherence phases that markedly differed between the various strains. To gain insight into this, we used RNA-seq based transcriptomic profiling of these isolates, compared to planktonically growing counterparts (and to each other), at a variety of different time points. As a consequence, we uncovered distinct and specific global expression profiles for each clonal lineage that reveals complex and unique regulation across the different *S. aureus* lineages. This data is currently being used to compile a global and comprehensive map of regulatory circuits during *S. aureus* biofilm growth that has the potential to inform not only on the basic mechanism of this aggregate lifestyle but may also provide new insight into future therapeutic targets.
Regulation of Holdfast Production in *Caulobacter crescentus* by Flagellum Assembly and c-di-GMP

A. Justice, C. Berne, Y. V. Brun; Indiana University, Bloomington, IN.

Bacteria spend most of their lives attached to surfaces as multicellular communities called biofilms. In a biofilm, bacteria can share nutrients, synchronize behaviors, and stay protected from the environment and xenobiotic stresses. *Caulobacter crescentus* is a freshwater bacterium that produces an adhesive holdfast to permanently attach to surfaces and form biofilms. *C. crescentus* asymmetrically divides and produces two different cell types, a motile swarmer cell and a sessile stalked cell, at each division. The swarmer cell harbors a polar flagellum and several pili at the same pole. It differentiates into a replication-competent stalked cell by shedding its flagellum, retracting its pili, and synthesizing a holdfast and a stalk at the same pole of the cell. Thus, holdfast synthesis is developmentally regulated by cell cycle cues. Furthermore, holdfast synthesis can also be stimulated by surface sensing, in which swarmer cells rapidly synthesize holdfasts in direct response to contact with a surface. The goal of this study is to better understand the regulation of the transition between motile and sessile lifestyles and holdfast synthesis in *C. crescentus*. The intracellular signaling molecule c-di-GMP is involved in the switch between motile and sessile lifestyles in *C. crescentus*, signaling proper flagellum functions and holdfast synthesis. c-di-GMP plays a role in both the developmentally regulated and the surface contact stimulated pathways that lead to holdfast production. In this study, we are investigating the connection between c-di-GMP signaling, flagellum and holdfast production. We determined that, while more than 60% of a WT cells population harbor a holdfast in a mixed culture, this number drops to less than 10% in a ΔpleDΔflgEΔmotB triple mutant that produces lower amounts of c-di-GMP (pleD deletion) and lacks its flagellum (flgE deletion) and flagellar motor (motB deletion). This number is similar to the number of holdfasts produced by a mutant unable to produce c-di-GMP (rcdG0). To gain a better understanding of the interplay between c-di-GMP, the holdfast, and flagellum, we constructed a mutant transposon library in the ΔpleDΔflgEΔmotB triple mutant and screened for strains where the low holdfast production phenotype was suppressed. We screened more than 24,000 mutants and isolated eight strains with increased holdfast production and biofilm formation compared to the parent strain. Once we have characterized the mutations, we will determine how the identified genes are involved in the regulation of flagellum and holdfast production by c-di-GMP. This study will provide a better understanding of the pathway that starts with c-di-GMP production and leads to the switch between motile and sessile lifestyles. We expect to identify novel players that play a role in this complex regulatory cascade.
Cyclic-di-AMP Governs Release of Extracellular DNA during Biofilm Formation in *Staphylococcus aureus*

Author: A. K. Syed, R. Losick; Harvard University, Cambridge, MA.

The bacterial pathogen *Staphylococcus aureus* is a leading cause of antibiotic-resistant nosocomial infections and is often found growing as a biofilm in catheters and chronic wounds. Cells in the biofilm are held together by an extracellular matrix that consists of recycled cytoplasmic proteins that moonlight as components of the matrix and extracellular DNA (eDNA), which forms an electrostatic net. Taking an unbiased genetic approach, we previously identified the gene (*gdpP*) for cyclic-di-AMP phosphodiesterase as being required for eDNA release. Using a riboswitch biosensor, we now find that cyclic-di-AMP levels drop prior to biofilm formation and that this drop is prevented in a *gdpP* mutant. Conversely, artificially depleting cyclic-di-AMP levels by overexpressing *gdpP* is sufficient to promote eDNA release under growth conditions that normally do not support biofilm formation. Together, these results support a model in which a drop in c-di-AMP is both necessary and sufficient for eDNA release during biofilm formation *S. aureus*. We have also identified genes upstream and downstream of cyclic-di-AMP in the pathway governing eDNA release.
Social and Asocial Interactions in Biofilms

Cargo Transport Shapes the Spatial Organization of a Microbial Community

A. Shrivastava¹, V. Patel¹, Y. Tang¹, S. C. Yost², F. E. Dewhirst², H. C. Berg¹;
¹Harvard University, CAMBRIDGE, MA, ²The Forsyth Institute, CAMBRIDGE, MA.

The human microbiome is an assemblage of diverse bacteria that interact with one another to form communities. Bacteria in a given community are arranged in a three-dimensional matrix with many degrees of freedom. Snapshots of the community display well-defined structures, but the steps required for the construction of these structures are not understood. Here, we show that this construction is carried out, in part, by gliding bacteria that carry non-motile bacteria on their surface as public cargo. Gliding is defined as the motion of cells over a solid or semi-solid surface without the necessity of growth or the aid of pili or flagella.

Genomic analysis suggests that gliding bacteria are present in human microbial communities. We focus on Capnocytophaga gingivalis which is present in abundance in the human oral microbiome. By adhering to a mobile cell-surface adhesin, SprB, cells of non-motile bacterial species attach to the surface of C. gingivalis and are propelled as cargo. The cargo cell moves along the length of a C. gingivalis cell, looping from one pole to the other. Multi-color fluorescent spectral imaging of cells of different live but non-motile bacterial species reveals their long-range transport in a swarming polymicrobial community. Some non-motile bacterial species use this public transport more efficiently than others. Tracking of fluorescently-labeled single cells and of gas bubbles carried by fluid flow shows that the swarms are layered, with cells in the upper layers moving more rapidly than those in the lower layers. Thus, cells also glide on top of one another, arranging themselves in three-dimensional space.
Understanding the impact of spaceflight on microbial ecosystem dynamics and biofilm formation is critical for spacecraft design, operations, and astronaut health. The ISS is an isolated closed environment that has been inhabited by more than 200 international crew members and their accompanying microbes since 2000. This habitat relies on sustainable life support systems (including those that provide potable water) to support crew health and can be compromised by microbial contamination and overgrowth. The latter can lead to materials degradation, biofouling, operational systems failure, and infections, all of which have been reported on the ISS. The purpose of this study is to understand 1) how microbial communities and their population dynamics have developed and been sustained in the ISS potable water system under low nutrient conditions, and 2) how these complex microbial ecosystems may adapt and evolve in the spaceflight environment. The ultimate goal of this project is to provide a better understanding of the effects of spaceflight on microbial ecosystems in ISS regenerative potable water, their effective control, and implications for spaceflight systems integrity and crew health risks, and to advance our understanding of the microbiome of built environments on Earth.

We received 16 ISS potable water microbial isolates from the NASA Johnson Space Center and profiled the growth kinetics, drug sensitivities, and halotolerance of each of the individual strains. Over 20 antimicrobial compounds were tested at a range of concentrations and assayed for microbial colony forming units (CFUs). Based on our assays, the tested strains are classified as MDR (multidrug resistant) or XDR (extensive drug resistant). No single antibiotic tested in this study completely killed the ISS water microbiota, and a combination of antibiotic cocktails are necessary for complete elimination. Interestingly, our results revealed an association between antibiotic-tolerance and changes in distinct phenotypes. We also investigated key metabolic characteristics, biofilm formation, changes in colony morphology of polymicrobial communities, and multispecies interactions showing synergistic or competitive behaviors. Unexpectedly, we discovered subpopulations in selected Burkholderia species that differed in colony morphology, biofilm formation, sensitivity to antibiotics, and hemolytic potential. This is the first thorough evaluation of microbial characteristics of microbial isolates from the ISS potable water system. This study is funded by a postdoctoral fellowship award from the Alfred P. Sloan Foundation.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 097

**Abstract Topic:** Social and Asocial Interactions in Biofilms

**Abstract Title:** Let Live and Let Die - On the Edge between Competition and Mutualism in Oral Multi-Species-Biofilms

**Author Block:** S. Redanz, U. Redanz, Z. Zou, J. Merritt, J. Kreth; Department of Restorative Dentistry, Oregon Health and Science University, Portland, OR.

**Introduction:** Caries and periodontitis are recognized as polymicrobial diseases caused by an altered abundance of species present in the oral microbiome. This shift is driven by synergistic and antagonistic interactions among the oral species. Streptococci are known as early colonizers of the dental surface. Pyruvate oxidase (SpxB) positive species form H$_2$O$_2$ and thus inhibit the growth of competing bacteria like *Streptococcus mutans* (Sm), a major contributor of initial enamel caries. **Objectives:** The deletion of *spxB* and external addition of H$_2$O$_2$ uncovered the presence of a so far unknown phenotype of *Streptococcus sanguinis* SK36 (SK36) and *Streptococcus gordonii* DL1 (DL1). Both, SK36 and DL1 were able to detoxify H$_2$O$_2$ from the surrounding and thus protect nearby bacteria from its inhibitory effect. Therefore, we aim to investigate cause and ecological meaning of such contradictory phenotype. **Methods:** We investigated *ccpA, spxB* and *ccpA/spxB* double mutants as well as further deletion mutants of SK36 and DL1. H$_2$O$_2$ release was quantified by a chromogenic assay. Growth inhibition assays as well as H$_2$O$_2$-inhibition and H$_2$O$_2$-protection assays (disc diffusion) were performed by co-culturing SK36 or DL1 derivatives with Sm or other streptococcal / staphylococcal isolates. Further, the H$_2$O$_2$-detoxifying capacity of crude extract was determined by the survival rate of an epithelial cell line (OKF4) challenged by H$_2$O$_2$. Directed and transposon based random mutants were constructed and tested to identify candidate genes involved in regulation, release or synthesis of the protective agent. **Results:** Due to the lack of H$_2$O$_2$ production *spxB* mutants of SK36 and DL1 fail to inhibit other bacteria. Remarkably, the external addition of H$_2$O$_2$ revealed a protective effect against H$_2$O$_2$ mediated growth inhibition. Investigation of different growth conditions uncovered this protective substance to be oxygen, glucose and biofilm dependent regulated. Analyses of crude extract revealed the protective substance to be water soluble and heat resistant. The protective effect against H$_2$O$_2$ mediated killing was verified for several bacterial species as well as for eukaryotic cells. Directed and random mutagenesis identified 29 genes involved in regulation / release or synthesis of the protective agent (*ccpA, dps, nox* etc). **Conclusion:** Our findings uncovered the presence of a so far unknown phenotype of SK36 and DL1. The release of components protecting against H$_2$O$_2$-mediated killing provide a new aspect in bacterial mutualism in oral mixed-species-biofilms. Furthermore, considering the importance of H$_2$O$_2$ as an antimicrobial as well as regulatory molecule of the innate immune response, the identified effect might also play a central role in the host-microbe interaction or even have a direct modulatory influence in the immune defense in the oral cavity.
Social and Asocial Interactions in Biofilms

Characterization of Multi-species Biofilms of Significant Bacterial Pathogens

V. Fuchsova, O. Chlumsky, K. Zdeňkova, K. Demnerova; University of Chemistry and Technology Prague, Prague 6, CZECH REPUBLIC.

During their existence bacteria evolve in order to adapt to the surrounding environment. Permanent microbial modification causes an emergence of formation called biofilm. Its composition and structure provide advantages manifested by changes in the phenotype and physiology of microbial population, high stability and resistance. In our environment the prevalent forms of life are multi-species biofilms, which are more complex, virulent, stable and resistant in comparison to mono-species biofilms. These multi-species communities can represent very beneficial part of human microflora. Bacterial biofilms can contribute for example to processes of bioremediation in the industry. However, some pathogenic microorganisms, which cause human diseases (such as Escherichia coli, Staphylococcus aureus and Listeria monocytogenes), also have the ability to form biofilm. The stability of biofilm, the inability of the detection by classical cultivation techniques and the resistance to the antimicrobials have become a major problem in many branches. The main objective of this study was to characterize multi-species biofilms of the E. coli, S. aureus and L. monocytogenes. Biofilms of these bacteria were detected in food and medical industry. In this work the classical microbiologic methods, the molecular-biologic method and the confocal laser scanning microscopy (CLSM), were used to study multi-species biofilms of above mentioned bacterial pathogens. We evaluated the ability of bacterial strains to form a mono- and dual-species biofilms and afterwards we measured biomass of 24 hours biofilms. Biofilms were cultivated under the static condition of 37 °C in tryptone soya broth (TSB). The range of absorbance values was detected by using the crystal violet staining. Most of tested samples had the ability to form a biofilm. In comparison the biomass amount of mono- and dual-species biofilms four species combinations increased the biofilm biomass. The maximum level of biomass was observed in food isolate S. aureus strain 1241 in combination with food isolate L. monocytogenes strain 149. For the monitoring of spatial distribution of multi-species biofilms by CLSM the transformations of plasmids containing genes sequences coding iLOV and mCherry fluorescence proteins were also successfully executed. According to confocal microscopy results, it seems that used bacteria form dual-species biofilms with microcolonies arranged in layers. This study has been supported by GACR project 17-15936S and from specific university research (MSMT No 21-SVV/2018).
Social and Asocial Interactions in Biofilms

Development of Tools for the Analysis of Dual-Species Biofilms between Health- and Disease-Associated Streptococci of the Oral Microbiome

J. Kaspar, K. Lee, R. C. Shields, R. A. Burne; University of Florida, Gainesville, FL.

BACKGROUND: The human oral cavity hosts a diverse microbiome where synergistic and antagonistic interactions between bacterial species govern the balance between health and disease. Health-associated commensal streptococci can antagonize the growth of pathogens through the production of H$_2$O$_2$ and other factors that promote their persistence. In the case of dental caries, they suppress the growth of acidogenic and acid tolerant organisms that create localized acidic microenvironments while enmeshed in sucrose-derived exopolysaccharide (EPS) matrices. We report here the development of tools that will aid in the study of how interbacterial interactions between competing Streptococcus species affects the spatial organization and behaviors of species within microenvironments when these microbes are co-cultured in biofilm communities.

METHODS: To visualize individual species within biofilms, a series of fluorescent reporters were developed using constitutive promoters (P$_{veg}$, P$_{23}$) and common fluorescent genes (gfp, dsRed) on the Streptococcus shuttle vector pDL278. Following transformation of these plasmids into health-associated Streptococcus gordonii DL1, Streptococcus sanguinis SK150 and Streptococcus A12, and into the caries-associated pathogen Streptococcus mutans UA159, competitive fitness of the health-associated streptococci against S. mutans was monitored and compared in 24 h planktonic and biofilms cultures with or without sucrose. 24-h biofilms were imaged by confocal laser scanning microscopy and 3D-images of the resulting biofilms reconstructed.

RESULTS: Health-associated streptococci were present in comparable numbers to S. mutans in planktonic growth conditions, but became less competitive when grown in biofilms. The growth inhibition was further exacerbated by the presence of sucrose. Microscopy showed that the architecture and organization of bacterial species was markedly different with glucose versus sucrose, with S. mutans forming microcolonies that excluded the health-associated strains in the presence of sucrose with enhanced microcolonies only in the presence of other streptococcal species rather than when S. mutans strains were cultured together. Addition of Alexa Fluor 647-labeled dextran conjugate showed that EPS matrix formed in the presence of sucrose associated preferably with S. mutans but not with commensal strains. CONCLUSIONS: Our data confirms and builds upon earlier work showing that S. mutans-produced EPS-matrix modulates the architecture and spatial heterogeneities of oral biofilm communities through the formation of S. mutans-rich microcolonies. Establishment of these tools will allow for closer examination of how health-associated streptococci that could be used in probiotic applications interact with disease-associated oral microbiome members, such as S. mutans.
**Abstract Topic:** Social and Asocial Interactions in Biofilms

**Abstract Title:** Cross-Species Induction of Outer Membrane Vesicle Biogenesis: The Problem with Bacterial Peer Pressure

**Author:** A. M. Horspool, J. W. Schertzer; Binghamton University, Binghamton, NY.

**Background:** Co-infection studies show increased virulence, and multispecies infections are correlated with worse patient outcomes. A common hypothesis is that pathogen synergy results from competition and communication between multiple species at an infection site. Outer Membrane Vesicles (OMVs) are mediators of competition and communication among many species and are increasingly associated with multiple modes of virulence. OMV biogenesis in *P. aeruginosa* is induced by secretion and intercalation into the outer membrane of a self-produced small molecule: the Pseudomonas Quinolone Signal (PQS). The biophysical underpinnings of this model are easily generalizable, raising the possibility that the mechanism is widespread. We developed a tightly controlled experimental system to test the interaction of bacterially-produced factors with target cells. Results from these studies inform on the generality of small molecule-induced OMV biogenesis as well as highlight the role of cross-species induction of OMV biogenesis in pathogen synergy.

**Methods:** A panel of recipient bacteria were exposed to pre-solubilized PQS at low concentration for short duration and resultant OMV production was analyzed by both nanoparticle tracking and lipid analyses. *P. aeruginosa* was exposed to supernatants from donor species and OMV production was likewise analyzed. Pairs of species were cultured together and OMV production was compared to monocultures of either species. Co-culture OMVs were harvested and tested for THP1 cytotoxicity by trypan blue staining and compared to that of monoculture OMVs. **Results:** We show that multiple species respond to PQS by increasing OMV formation, that PQS accumulates in all induced vesicles, and that other bacteria secrete OMV-promoting factors. Analysis of induced vesicles indicates that recipient-mediated mechanisms exist to control vesicle size and that relatedness to the producer organism can dictate susceptibility to OMV-inducing compounds. This work provides evidence that small molecule induced OMV biogenesis is a widely conserved process and that cross-talk between systems may influence OMV production in neighboring bacteria. We extended this work to show that several pairs of bacterial species produce more OMVs per cell when co-cultured than when alone, and that co-culture OMVs are more cytotoxic against THP1 monocytes than monoculture OMVs. **Conclusions:** Our work demonstrates reciprocal cross-species induction of OMV biogenesis mediated by secreted factors. The physiological consequences of this phenomenon were explored, and we showed that such interactions between co-cultured species resulted in more OMV production and greater cytotoxicity toward host cells. Thus, “peer pressure” to produce greater numbers of more potent OMVs in bacterial communities likely contributes to the pathogen synergy observed in many clinical infections.
**Abstract Topic:** Synthesis and Assembly and Function of the Biofilm Matrix

**Abstract Title:** Regulation of Bacterial Amyloid Biogenesis by Multitasking Molecular Chaperone DnaK

**Authors:** S. Sugimoto1, K. Arita-Morioka2, A. Terao1, K. Yamanaka3, T. Ogura3, Y. Tanaka2, Y. Kinjo1, Y. Mizunoe1; 1Jikei University School of Medicine, Tokyo, JAPAN, 2Fukuoka Dental College, Fukuoka, JAPAN, 3Kumamoto University, Kumamoto, JAPAN.

**Background:** Curli are functional amyloid fibers that assemble on the extracellular surface of enteric bacteria such as *Escherichia coli* during biofilm development and colonization. Unlike pathogenic amyloids that result from protein misfolding, curli are generated via a secretory nucleation-precipitation mechanism, also called the type VIII secretion system. In *E. coli*, seven proteins encoded by two operons, *csgBAC* and *csgDEFG*, regulate curli expression, export, and assembly. The major curli subunit CsgA has three domains: an N-terminal signal peptide, the recognition sequence for the curli-specific translocation channel CsgG, and five imperfect amyloidogenic repeats. Following translocation across the cytoplasmic membrane through the Sec translocon, the signal peptide is cleaved, yielding a ~13-kDa mature CsgA subunit that is exported across the outer membrane by CsgG. Exported soluble CsgA is nucleated by CsgB, the minor curli subunit, to induce amyloid assembly. The *csgDEFG* operon encodes CsgD, a master transcriptional regulator of curli biogenesis that acts as a positive regulator of the *csgBAC* operon, the periplasmic accessory protein CsgE, the extracellular accessory protein CsgF, and CsgG. Recently, we found that molecular chaperone DnaK is involved in curli biogenesis and that Myricetin (IC50 = 46.2 μM), a DnaK-inhibiting polyphenol, and Epigallocatechin gallate (IC50 = 5.9 μM), a derivative of Myricetin, prevent curli-dependent biofilm formation by *E. coli* K-12 (Arita-Morioka et al. *Antimicrob. Agents Chemother.* 2015, *Sci. Rep.* 2018). However, molecular mechanisms how DnaK regulates curli biogenesis was unclear. **Methods and Results:** Transcription and immunoblotting analyses using *E. coli* BW25113 and its isogenic ∆dnaK strains showed that DnaK positively regulates the expression of CsgA and CsgB via quantity and quality control of CsgD and RpoS, a stationary phase-specific alternative sigma factor promoting expression of the *csgDEFG* operon. Cytological analysis with fluorescent protein reporters revealed that DnaK also keeps CsgA and CsgB in a translocation-competent state by binding to their signal peptides prone to aggregation, facilitating their translocation in the cell. Molecular interaction analyses by surface plasmon resonance and peptide-scanning indicated that DnaK strongly binds to the N-terminal 8-amino acid peptides of CsgA and CsgB. In vitro translation/folding analysis demonstrated that DnaK suppresses the aggregation of CsgA, but other chaperones GroEL and SecB, both of which facilitate the protein export in *E. coli*, do not. **Conclusion:** Our findings indicate that DnaK controls the homeostasis of curli biogenesis at multiple stages to organize the biofilm matrix (Sugimoto et al. *Commun. Biol.* 2018). Collectively, these results may lead to the development of drugs to treat chronic biofilm-associated infections.
Alcohols are natural major end products of some microbial fermentations. The acetone/butanol/ethanol (ABE) or the isopropanol/butanol/ethanol (IBE) fermentation using solventogenic *Clostridium* strains have a long industrial history but an efficient fermentation system is still required. Recent publications highlight that immobilized cells are the preferred option for obligate anaerobe such as the solventogenic strains belonging to this genus. This operating mode can indeed increase cell density and productivity by maintaining high cell concentrations in the bioreactor (Dolejš et al., 2013; Jiang et al., 2009). However, relatively few data are available regarding the physiological state of the *Clostridium* cells present in such biofilms. Our study aimed at validating a flow cytometric (FC) approach to analyze cellular viability in biofilms of the isopropanol producing strain *Clostridium beijerinckii* DSM6423. Two couples of fluorescent probes, Propidium Iodide [PI] associated with carboxyfluorescein diacetate [cFDA] or Bis-(1,3-dibutylbarbituric acid)trimethine oxonol [Dibac], were validated to screen suspended cells of *Clostridium beijerinckii* in batch or continuous fermentations. As FC analysis require suspended cells, a pretreatment of the biofilm was required. Physical pretreatments were tried but a comparative analysis of cell/event counting, respectively by plating and cytometry, revealed a strong difference. This may be due to the presence of EPS in the cell suspension which can interfere with the FC analysis. Moreover, a significant quantity of extracellular DNA (eDNA) was detected in the biofilm matrix, disturbing the propidium iodide cell staining. Another approach, using sequential enzymatic treatments, were allowed us to limit the background noise linked to the presence of EPS but more particularly eDNA. Interestingly, FC analysis revealed that only a fraction of the cells was viable in *Clostridium beijerinckii* DSM6423 biofilm, suggesting that only a part of the biofilm is permanently active. This new protocol provides a rapid and efficient method to measure cell viability and monitor immobilized cell fermentation. Dolejš I., M. Rebroš, M. Rosenberg. 2013 Immobilisation of Clostridium spp. for production of solvents and organic acids. *Chem. Pap.*, 68: 1-14. L. Jiang, J. Wang, S. Liang, X. Wang, P. Cen, Z. Xu. 2009 Butyric acid fermentation in a fibrous bed bioreactor with immobilized Clostridium tyrobutyricum from cane molasses. *Bioresour. Technol.*, 100: 3403-3409.
**Title:** Insights on *Klebsiella* Biofilm: A complex world

**Authors:** S. D. Desai, K. Sanghrajka, D. Gajjar; The Maharaja Sayajirao University of Baroda, Vadodara, INDIA.

**Abstract Body:**

**Background:** *Klebsiella pneumoniae* (*Kp*) from the *Enterobacteriaceae* family, is a frequent cause of hospital-acquired infections (HAI). It has emerged as an “urgent threat” to public health due to antibiotic resistance. Biofilm formation by *Kp* is a major concern in clinical settings as *Klebsiella* forms biofilms on urinary catheters, ventilators etc. *Kp* is a major etiological agent found in HAI, especially in CAUTIs (70%). To prevent and eradicate biofilms, the dynamics of biofilm formation on different materials needs to be understood. The aim of the present study was to evaluate the biofilm-formation capacity of clinical isolates of *Klebsiella* associated with UTI. Biofilm components were quantified to evaluate the contribution of each component in formation of biofilm matrix. The EPS (extracellular polymeric substances) plays an important role in the resistance and strength of the biofilm.

**Methods:** Pathogenic isolates of *Klebsiella* spp. (*n*=30) from patients suffering from UTI were collected from Gujarat, India and identified using 16s rRNA gene sequencing. Biofilm was studied by crystal violet assay on 96 well-plate and isolates were categorized into 3 categories: weak, moderate and strong biofilm formers using statistical analysis. Biofilm formation capacity of isolates was studied on latex and silicone catheters. Biofilms formed on catheters were further characterized by quantification of each component of biofilms including EPS, extracellular DNA, living and dead cells. Scanning electron microscopy and fluorescent microscopy were also performed to study the difference in the composition of biofilm matrix on different materials and to compare the matrix of weak and strong biofilm. Extraction and quantification of EPS produced by each isolate were done. Results: 30 isolates were identified as *Klebsiella* spp. by 16s rRNA gene sequencing. Among these, 41%, 44% and 15% isolates were found to be strong, moderate and weak biofilm formers respectively. Biofilm formation was found to be more on latex-based catheters than silicone-based catheters. 50% decrease was seen in biofilms formed on silicone. More number of live cells were found to be present in weak biofilms, while in case of stronger biofilms, a large number of dead cells were present. In case of EPS quantification, a wide range of EPS production was observed among the isolates (0 µg EPS/mg protein to 210.79 µg EPS/mg protein) and the isolate with highest EPS (M-27) has strong biofilm and lowest EPS (M-20) has very weak biofilm. Conclusion: A high level of variation in EPS production and biofilm formation among the *Klebsiella* isolates were observed. Interestingly, dead cells embedded in the biofilm matrix seem to play a major role in the formation of strong biofilms by *Klebsiella*. Further work to study the role of cell death in strong biofilm formation is warranted.
**Session Title:** THURSDAY Poster Session 4  
**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm  
**Poster Board Number:** 104  
**Abstract Topic:** Synthesis and Assembly and Function of the Biofilm Matrix  
**Abstract Title:** Biofilm Architecture Confers Individual and Collective Protection against Viral Infection  
**Author Block:** L. Vidakovic¹, P. Singh¹, R. Hartmann¹, C. Nadell², K. Drescher¹;  
¹Max Planck Institute for Terrestrial Microbiology, Marburg, GERMANY, ²Department of Biological Sciences, Dartmouth College, Hanover, NH.  
**Abstract Body:**  
**Background:** In their natural environments, biofilms regularly encounter the presence of viral pathogens, termed bacteriophages which use bacteria as their host for self-replication. Our aim was to understand the interaction of phages and biofilms, as well as their coexistence. **Methods:** In order to study phage-biofilm interactions we developed a method to visualize phage spread inside living *E. coli* biofilms. By insertion of *sfgfp* into the T7 phage genome, the conversion of susceptible to infected cells can be visualized spatiotemporally at the single-cell level. Using a combination of bacterial genetics, molecular biology, and fluorescent reporters, we were able to understand key elements of phage-biofilm interactions. **Results:** We discovered that biofilm susceptibility to phage infection is dependent on the stage of biofilm development and the production of biofilm matrix. *E. coli* biofilms that were grown for 48 h or less were rapidly eradicated due to phage infection. By contrast, biofilms grown for 60 h and more experienced no biomass reduction in the presence of phages. The removal of curli fibers, a major component of the *E. coli* matrix, generated biofilms that were susceptible to phage infection, regardless of the age of the biofilms. Visualization of curli fibers during biofilm growth further demonstrated a dynamic change in matrix composition. The development of phage tolerance in biofilms coincided with the production of curli fibers. We further discovered that curli fibers protect bacterial communities via two mechanisms: (1) Curli prevent phages from diffusion inside biofilms, and (2) curli fibers protect individual cells from phage infection. **Conclusion:** Our results demonstrate that a single component of the biofilm matrix can provide individual as well as collective protection against viral infection.
Session Title: THURSDAY Poster Session 4

Session Date/Time: Thursday, October 11, 2018, 4:15 pm - 5:45 pm
Poster Board Number: 105

Abstract Topic: Synthesis and Assembly and Function of the Biofilm Matrix

Abstract Title: Self-assembly of Bacterial Peptides in a Staphylococcus aureus Biofilm: a Molecular Dynamics Study

Author: Y. Wang, C. Liu, P. Elvati, N. Kotov, J. VanEpps, A. Violi;
Block: University of Michigan, Ann Arbor, MI.

Background: Staphylococcus aureus are among the most frequent causes of infections on indwelling medical devices, which characteristically involve biofilms. Phenol soluble modulins (PSMs) have been identified as one of the key regulators for the S. aureus biofilm lifecycle. As monomers, PSMs promote biofilm dissociation, but they can self-assemble to form extracellular amyloid fibrils that fortify the biofilm matrix to better resist disassembly by enzymes and mechanical stress. Despite the recent advances made in understanding the roles of PSMs and their aggregation in biofilm control, many crucial aspects are still not clear. Here, we discuss recent progress on the molecular mechanisms of PSMs' self-assembly to amyloid fibers in S. aureus biofilm, using molecular dynamics (MD) simulations. Method: PSMα1 and PSMα3 were studied respectively using classical all-atom MD simulation in explicit solvent. These PSMs were chosen due to their ability to form amyloid-like fibrils. We studied dimers configurations to understand the early stages of oligomer formation, then single, double, and triple sheets protofibril structures consisting of 10, 20 and 30 dimers to analyze the structural changes emerging from the fibril growth. The stability of different dimers' configurations was investigated using well-tempered Metadynamics. Protofibrils were extensively equilibrated and then simulated to capture slow structural rearrangements. MD simulations were performed using the NAMD, employing the CHARMM general force field; well-tempered metadynamics were performed using PLUMED plugin. Results: PSMα1 and PSMα3 dimerization is driven by the intermolecular interactions between hydrophobic groups, but the monomers retain a large degree of flexibility in the terminal amino acids. For the transition to single sheet protofibril structure, hydrogen bonds (and potentially salt bridges) among dimers play an important role. While general characteristics of the protofibril agree with crystallographic data, it was found to form a helical structure in water with 0.15M NaCl. This arrangement is barely detectable in the double sheet protofibril and it disappears in the triple sheet structure. As the number of sheets in the protofibril increase, individual peptides become less organized in the central part of the fibril. These two factors suggest that the number of sheets composing the fibril is generally limited to 3-4, which is equivalent to a width of 12-16 nm, in agreement with the average diameter of PSM fiber observed in experiments. Conclusion: We have investigated critical aspect of the molecular mechanism of amyloid fiber formation via self-assembly of common bacterial peptides, PSMα1 and PSMα3 from S. aureus biofilm. Dependencies on the structure and size of the peptides were discovered suggesting potential avenues to target PSMs for the development of anti-staphylococcal biofilm agents.
Salmonella enterica serotype Typhimurium is a Gram negative, motile bacterium that causes infection via the fecal oral route. These bacteria, as well as other Enterobacteriaceae, produce amyloid proteins called curli as a major proteinaceous component of their biofilm. Amyloids, like curli, can be produced by bacteria as well as humans. Accumulation of amyloids in humans can be found in complex human diseases such as Alzheimer’s Disease. It is thought that the amyloids seen in humans are the result of misfolded aggregates, however bacterial amyloids have been found to be functional. Amyloid curli complexes with extracellular DNA within the biofilm. When purified from the matrix, these complexes are recognized by a variety of receptors. Previous studies have shown that the amyloid portion of these curli complexes is recognized by the Toll Like Receptor 2 (TLR2)/TLR1/CD14 complex as well as the NLRP3 inflammasome, leading to the production of proinflammatory cytokines such as interleukin-1 beta (IL-1β). Additionally, the eDNA within the complex has been shown to induce type I Interferon (IFN) production and autoimmunity through activation of TLR9. Recently, we determined variable amounts of DNA within the curli prepared in the lab. We have been testing two protocols and were able to manipulate the amount of DNA in the curli complexes. We found treatment with DNase and RNase in cultures is able to disrupt aggregation of the curli/eDNA complexes making for a weaker biofilm. Using fluorescent microscopy we are able to see the aggregates in our high DNA preps are larger and more robust than those of the low DNA preps. We hypothesize that this difference in aggregate size may affect the downstream recognition and signaling of the complexes. To test this, we will treat bone marrow derived macrophages that are wild-type, TLR2−/−, TLR9-mutant, or TLR2−/−9mutant with low and high curli to see if there are differences in response between the different complexes. We expect that the low DNA curli will not activate TLR9 mutant macrophages as well as the high DNA curli due to lesser DNA incorporation. Additionally, we will look at internalization by the macrophages to see if the aggregate size difference affects TLR2 signaling and entry into the cell. Downstream effects on type I IFN response and in vivo autoantibody production in mice will be investigated to understand how DNA incorporation to produce mature biofilm structure affects the functionality of the complexes.
Pseudomonas aeruginosa biofilms have been linked to a number of chronic infections. The biofilm matrix is composed of exopolysaccharides (EPS), eDNA and proteins, where research historically concentrated in studying EPS. Non-mucoid P. aeruginosa produce two EPS, Pel and Psl, and recent studies determined their chemical structure. Conversely, the role of proteins in P. aeruginosa remains largely understudied. Considering that EPS is the main component of the matrix, proteins that interact with sugars are potentially important for aggregate formation. Currently, CdrA is the only structural protein shown to bind Psl and contribute to the aggregate stability. Additionally, P. aeruginosa is known to produce two small soluble sugar-binding proteins, designated as LecA and LecB. Interestingly, mutation of LecA and LecB has been linked to abnormal biofilm formation suggesting that they play a biofilm-specific role. However, the underlying mechanism for this biofilm phenotype is unclear.

Previous sugar-binding studies of LecA and LecB revealed that they have binding affinities to monosaccharides present in Pel and Psl, respectively. The aim of our study was to determine if LecB could bind to Psl and if these interactions would contribute to biofilm development. First, we assessed the binding of LecB to saccharides via ELISA-based assays and co-immunoprecipitation, and we demonstrated that LecB binds to Psl. These results led us to assess if LecB could interact with Psl in situ using biofilms grown under flow. When fully grown aggregates were stained with LecB-FITC, we observed by microscopy that LecB and Psl were localized in the same areas, suggesting that LecB interacts with Psl in situ. Next, we investigated the redundancy of LecB and the adhesin CdrA. When both LecB and CdrA were mutated, Psl was not retained in the matrix and a thin layer of cells was formed. When the double mutant was partially complemented with LecB, aggregate architecture appeared similar to wild-type PAO1. Furthermore, when the double mutant was partially complemented with CdrA, aggregate formation was impaired with the appearance of a thick layer of cells that contained Psl. These results support the hypothesis that LecB interacts with Psl and this interaction is crucial for Psl placement in the matrix and consequently biofilm development.
Eradicating Bacterial Biofilms by Wireless Electrostimulation

H. Wang, D. Ren; Syracuse University, Syracuse, NY.

Background: Bacteria such as Pseudomonas aeruginosa and Staphylococcus aureus can form biofilms on implanted medical implants and cause serious infections that are incurable by conventional antibiotics. Low-level electric currents have been shown to have potent antimicrobial activities and synergy with antibiotics in bacterial killing. However, electrochemical treatment in vivo requires skin-piercing to introduce the wires for delivering electric current from an outside power source. This can cause discomfort and complications including secondary infections, which hinder the clinical applications of this technology. To address this challenge, we developed a wireless system for more effective control of biofilms.

Methods: Both in vitro and ex vivo studies were conducted to evaluate the killing activities against P. aeruginosa and S. aureus biofilm cells using electric currents generated by wireless electromagnetic induction. We also designed and constructed a prototype device, and validated its anti-biofilm performance. The CRL5803 lung epithelial cells were treated under the same condition to evaluate the cytotoxicity to human cells.

Results: After treatment with 6 µA/cm² of wirelessly delivered DC in vitro for 6 h, the viability of biofilm cells was reduced by 87% and 91% for P. aeruginosa and S. aureus, respectively. Clear synergy between low-level DC and antibiotics in biofilm killing was also observed in concurrent treatment. For example, the viability of P. aeruginosa biofilm cells was reduced by 2.5 logs after treatment with 6 µA/cm² wirelessly delivered DC and 4.5 µg/mL tobramycin. In comparison, treatment with DC or tobramycin alone only showed 0.9 and 0.6 log of killing, respectively. The viability of S. aureus biofilm was reduced by 2.2 logs after concurrent treatment with 6 µA/cm² DC and 10 µg/mL chlorhexidine, while treatment with DC or chlorhexidine alone only showed 1.1 log and 0.6 log of killing, respectively. When treated with 50 µA/cm² of wirelessly delivered DC in an ex vivo model with pork skin, the viability of biofilm cells on the surface of prototype device was reduced by approximately 4 logs and 2.6 logs for P. aeruginosa and S. aureus, respectively. Existence of pork skin tissue (1 - 5 mm thick) did not show significant effects on wireless DC delivery; and these conditions were found safe to tested human cells.

Conclusions: With the capability to kill bacteria without using a directly connected power source, this platform technology has potential applications in developing new systems and devices to effectively control biofilm infections such as those associated with pacemakers, cochlear implants, gastric stimulators and other implanted medical devices.
Antibiofilm Strategies

Clinical Efficacy of a Biofilm Disrupting Surgical Lavage in Reducing Bacterial Plate Counts in Total Knee Arthroplasty Revision Surgery in Known Cases of Prosthetic Joint Infection

M. Myntti1, C. Hunter2;

1Next Science, LLC, Jacksonville, FL; 2Zimmer Biomet, Dover, OH.

Background: Total joint arthroplasty (TJA) is one of the most common surgical procedures performed in the US and worldwide. In 2010, an estimated 332,000 total hip arthroplasty (THA) procedures and over 600,000 total knee arthroplasty (TKA) were performed in the United States. Periprosthetic Joint Infection (PJI) affects 1-2% of total joint arthroplasty patients and remains one of the most serious complications of TJA. While rare, the condition incurs substantial morbidity and costs, and a significant portion of sufferers will bear consequences for the remainder of their lives. By 2020, the predicted cost for infected revision procedures may reach as high as $1.6 billion. Bactisure Wound Lavage solution has been developed to improve removal of planktonic and biofilm bacteria from the articular joint space. Bactisure is a mixture of surfactants, chelating agents and salts to disrupt and dissolve contaminants, providing a cleanser to a wound (articular space) and potentially reducing the risk of future infection.

Methods: A 40-patient clinical trial was designed for patients undergoing the first stage of a 2-stage revision for PJI. To date, 36 patients have completed the surgical portion of the trial. Infection was confirmed following TKA with the PJI occurring within 30 days of the primary procedure or 1 year with primary implant retention. Bactisure lavage was performed at the end of the procedure, prior to closure, and then followed by saline lavage. 3 mL fluid cultures were obtained from deep in the surgical wound both before the Bactisure lavage and after the saline lavage. White blood cell (WBC) counts were obtained from the fluid as a surrogate marker for bioburden. Plate counting was performed to determine the bacterial colonization of the surgical site and DNA analysis was used to identify the bacteria. A 90-day follow-up period for infection is ongoing to determine the effectiveness in preventing recurrence of PJI.

Results: There was a substantial (>99%) reduction in the WBC counts, (2.3 log reduction, 4.2 +/- 0.5 log to 1.9 +/- 0.7 log, p-value <0.01). There was a dramatic decrease in the number of colony forming units in the surgical site. For those patients with a positive culture in the pre-lavage test, 74% had no countable bacteria in the post-lavage test (p-value < 0.01, 19/36 culture positive in the pre-lavage population, 14 of those 19 were culture negative after lavage). For patients with positive cultures, the average plate count decreased by 99.6% (2.4 log), with the log CFU of bacteria being reduced from 2.9 +/- 1.6 log to 0.5 +/- 1.2 log (p-value < 0.01). For all patients, including those with 0 count pre-lavage, the average plate count decreased by 96% (1.6 log), with the log CFU of bacteria being reduced from 1.9 +/- 1.9 log to 0.3 +/- 1.0 log (p-value < 0.01).

Conclusions: The use of Bactisure prior to closure significantly reduces the bioburden and bacterial count within the surgical site.
**Session Title:** THURSDAY Poster Session 4

**Session Date/Time:** Thursday, October 11, 2018, 4:15 pm - 5:45 pm

**Poster Board Number:** 110

**Abstract Topic:** Antibiofilm Strategies

**Abstract Title:** Thiol-Benzo-Triazolo-Quinazolinone Inhibits Alg44 Binding to c-di-GMP and Reduces Alginate Production by *Pseudomonas aeruginosa*

**Author Block:** S. KIM, E. Zhou, A. B. Seminara, C. L. Hall, Y. Wang, V. T. Lee; University of Maryland, College Park, MD.

*Pseudomonas aeruginosa* is an opportunistic pathogen that affects a large proportion of cystic fibrosis (CF) patients. CF patients have dehydrated mucus within the airways that leads to the inability of the mucociliary escalator to expel inhaled microbes. Once inhaled, *P. aeruginosa* can persist in the lungs of the CF patients for the remainder of their lives. During this chronic infection, a phenomenon called mucoid conversion can occur in which *P. aeruginosa* can mutate and inactivate their *mucA* gene. As a consequence, transcription of the *alg* operon is highly expressed, leading to the copious secretion of the alginate exopolysaccharide, which is associated with decreased lung function and increased CF patient morbidity and mortality. Alginate biosynthesis by *P. aeruginosa* is post-translationally regulated by bis(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP), which binds to the receptor protein Alg44 to activate alginate production. The identification of small molecules that disrupt the binding of c-di-GMP to Alg44 could inhibit the ability of *P. aeruginosa* to produce alginate. In this work, a class of thiol-benzo-triazolo-quinazolinone compounds that inhibited Alg44 binding to c-di-GMP in vitro was identified after screening chemical libraries consisting of ~50 000 chemical compounds. Thiol-benzo-triazolo-quinazolinones were shown to specifically inhibit Alg44-c-di-GMP interactions by forming a disulfide bond with the cysteine residue in the PilZ domain of Alg44. The more potent thiol-benzo-triazolo-quinazolinone had the ability to reduce *P. aeruginosa* alginate secretion by up to 30%. These compounds serve as leads in the development of novel inhibitors of alginate production by *P. aeruginosa* after mucoid conversion.