Abstract: Developing a Novel Bioactive Root Canal Sealer with Antibiofilm and Remineralization Properties

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 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 6876/2001 ISO specifications. 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.
**Title:** Influence of Gold and Platinum Nanoparticles on *Pseudomonas aeruginosa* Biofilms

**Author:** A. Cejkova, M. Koukalova, V. Musilova, J. Masak; ICT Prague, Prague 6, CZECH REPUBLIC.

**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 15424. 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-Postive 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.
**Abstract:**

**Background:** Biofilm, a complex microbial community, is associated with high rates of nosocomial infections. Quorum sensing (QS) plays an important role in biofilm formation, and also development of antibiotic-resistance among pathogenic microbial populations. Single active compound-based antibiotic development approach has failed to withstand the rapid acquisition of resistance by infectious microbes. Plant products with strong traditional medicine base are likely to emerge as useful alternatives to conventional antibiotics, given their immense structural diversity. In the present study, the anti-biofilm activities of 5 different plant products [including plant extracts (Panchvalkal formulation (PF), Punica granatum peel extract (PGPE), Phyllanthus emblica seed extract (PESE), castor oil (CO), and ricinoleic acid (RA)] were evaluated against Pseudomonas aeruginosa and Staphylococcus aureus in their planktonic as well as biofilm forms. **Methods:** Effect of plant products on bacterial growth and QS-regulated pigment production was assessed through broth dilution assay. Biofilm quantification was done by crystal violet assay, and biofilm viability was checked by MTT assay. Effect of plant products on susceptibility of the test pathogens to antibiotics and serum, and haemolytic potential was also checked. **Results:** All the test products in vitro were found to affect quorum sensing (QS)-regulated pigment production in both test bacteria. PESE (50 μg/mL) was found to inhibit biofilm formation of P. aeruginosa (31.42%) and S. aureus (44.61%). PGPE (50 μg/mL), PESE (50 μg/mL) and CO (2%v/v) were able to eradicate pre-formed biofilm of P. aeruginosa in range of ~15-30%. Production of polymeric substances in P. aeruginosa was found to increase in presence of PF. Among all the tested products, only PGPE (10-50 μg/mL ) could reduce biofilm viability (by ~12-21%) of P. aeruginosa. Pre-formed S. aureus biofilm was eradicated by PESE up to 20%, and its viability was reduced by CO by ~63%. All the test products were able to reduce bacterial virulence towards C. elegans. Gene expression study of PF-treated P. aeruginosa culture showed that the gene coding for biofilm dispersion autoinducer (PA1021) was upregulated, whereas genes essential for biofilm development including flagellar assembly, twitching motility, pili biogenesis, and lipopolysaccharide biosynthesis were down-regulated. **Conclusions:** This study is a good demonstration of the therapeutic value of the ‘polyherbalism’ concept in managing bacterial infections. **Keywords:** Biofilm, Quorum sensing, Plant products, Pseudomonas aeruginosa, Staphylococcus aureus, Gene expression, Caenorhabditis elegans
Abstract:

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
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 sensitize 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 forming, 972 isolates were moderate biofilm forming and the 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, ceftazidime+ 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.
Bacterial adhesion to the surface is an initial prerequisite to bacterial fouling and biofilm formation. Therefore, understanding how surface characteristics influence adhesion processes is crucial to identify surfaces with greater ability to support bacterial adhesion, and also to develop novel surfaces and coatings with bacterial antifouling characteristics. Our study is concerned with investigating the effect of substrate hydrophobicity and zeta potential on the dynamics and kinetics of the initial stages of bacterial adhesion and biofilm formation. For this purpose, bacterial pathogens *Staphylococcus aureus* and *Escherichia coli* O157:H7 were inoculated on ultra-smooth substrates with varying hydrophobicity and zeta potential. Self-assembled monolayers are selected as model surfaces in this study because it is possible to eliminate the effect of roughness and texture on bacterial adhesion and make surfaces with similar surface coverages but varying surface energies and chemistries. The main goal of this research is to understand the initial stages of bacterial adhesion onto surfaces varying hydrophobicity and surface charge of the abiotic surfaces. The dewetting of extracellular polymeric substances (EPS) produced by *E. coli* was observed on hydrophobic substrates, but not for *S. aureus*, which is attributed to the more amphiphilic character of staphylococcal EPS. The interplay between the timescale of EPS dewetting and the inverse of the adhesion rate constant modulated the distribution of *E. coli* within microcolonies and the resultant microcolonial morphology on hydrophobic substrates. Bacterial adhesion was greatest on hydrophilic substrates with positive surface charge, followed by hydrophobic substrates with increasing hydrophobicity, and the smallest on hydrophilic substrates with negative surface charge. The time constant of adhesion was about two to four times greater for *E. coli* O157:H7 compared to *S. aureus*, indicating a lower number attached per unit time for *E. coli* O157:H7 but a comparable mass attached per unit time due to the difference in size of these species. Lysis (rupture) of cellular membrane of *E. coli* (gram-negative) on hydrophilic substrates functionalized with positively charged amines were observed while lysis in *S. aureus* (gram-positive) were not observed because of the existence of a thicker cell wall and lack of an external surface membrane. The gained knowledge is significant in the context of identifying surfaces with greater risk of bacterial contamination and guiding the development of novel surfaces and coatings with superior bacterial antifouling characteristics for the hygienic design of the surfaces for industry.
Abstract:

Escherichia coli & related enteric bacteria form communities called biofilms that are resistant to host immune defenses & antibiotic treatment. Therefore, alternative approaches to conventional antibiotic therapy are urgently needed to treat biofilm related infections. The biofilm matrix is composed of exopolysaccharides, extracellular DNA & protein polymers commonly known as amyloid fibrils. Curli are amyloid fibrils produced in E. coli. The major subunit of the curli is CsgA protein that is capable of forming amyloid fibrils under in vitro conditions. An innovative strategy to prevent biofilm formation is to inhibit amyloid aggregation on the cell surface. The Chapman Lab has identified a periplasmic protein called CsgC that keeps CsgA in soluble form & assist its efficient secretion to the cell surface where it assembles into curli amyloid fibers. CsgC is structurally similar to the human amyloid precursor protein: transthyretin (TTR). TTR and CsgC are both stably-folded, β-sheet rich proteins. We hypothesized that TTR can prevent bacterial amyloid formation & thus could reduce amyloid-associated biofilm & bacterial colonization inside the human host. In the present study, found that human wild-type tetrameric TTR (WT-TTR) and an engineered monomer (F87M/L110M) [MTTR], can inhibit amyloid formation by CsgA. The biological significance of the TTR-CsgA interaction was demonstrated by the ability of TTR to inhibit amyloid-dependent biofilm formation by E. coli & B. subtilis with no apparent bactericidal or bacteriostatic effects (Jain et al PNAS 2017). These findings prompted us to investigate the anti-amyloid characteristics of other structural homologs of TTR & CsgC. We found that β2-microglobulin, a human protein & structural homolog of TTR, is also capable of inhibiting biofilms without killing the bacteria. The observations suggest that TTR & its homologs can act as a broad spectrum anti-amyloid agents & might be utilized to enhance antibiotic efficacy in infections associated with significant biofilm formation.
Session: **Poster Session 1**
Date & Time: Monday, October 8, 2018, 4:00 pm - 6:00 pm
Poster Board #: 011
Topic: Antibiofilm Strategies
Abstract Title: Evaluation of Antifouling Coatings to Reduce Bacterial Biocorrosion of Steel
Author Block: 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 compared 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.
Mannich Base Limits *Candida albicans* Virulence by Inactivating Ras-cAMP-PKA Pathway

S. Rajasekharan¹, S. Kannapan Mohanvel², C. Kamalanathan¹, V. Ravichandran¹, A. Kumar Ray³, A. Satish⁵;

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.
**Abstract:**

**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 biofilm that were analyzed either immediately or after 24 hours were reduced by 2.5-4 log. 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. 30m-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.
**Abstract:**

**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 spectroscopy, 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.
Antibodies against the Nthi Type IV Pilus Disperse M. CATARRHALIS from a Dual-Species Biofilm

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.

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
Abstract: Enzymatic Depletion of Pyruvate as an Anti-biofilm Treatment Strategy for *P. aeruginosa* and *S. aureus* Biofilm Infections of Burn Wounds

**Author** J. S. Goodwine¹, J. Gil², A. Doiron³, J. Valdes², M. Solis², A. Higa², S. Davis², K. Sauer¹;

¹Department of Biological Sciences, Binghamton University, Binghamton, NY, ²Department of Dermatology & Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, ³Department of Biomedical Engineering, Binghamton University, Binghamton, NY.

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 preparations 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

**Author:** J. Ruiz¹, C. Ortiz¹, A. Pimienta², R. Torres²;

¹Escuela de Microbiología, Facultad de Salud, Universidad Industrial de Santander, Bucaramanga, COLOMBIA, ²Centro de Innovación y Tecnología ICP - Ecopetrol S.A., Bucaramanga, COLOMBIA.

**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 3.87x10⁷ cells per cm² while SRB-3129 formed biofilm with 1.4x10⁶ 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.
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:

**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). The biofilm removal was carried out varying enzymes concentrations, exposure time and temperature conditions and was evaluated through quantification of residual biomass (violet crystal) and cell viability (tetrazolium salt), measured by optical density in a spectrophotometer.

**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.
Abstract:

**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 multi-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.
Modulation of Cross-Kingdom Quorum Sensing by Halophilic Archaea

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 lactonase 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. Archaeal 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 archaeal and cross-kingdom signalling, and may be a source of novel antibiofilm activities.

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 caldoxylolysilitiicus. 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.
**Abstract:**

**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 \( \mu \text{g/ml} \) 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.
**Abstract**

**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.
**Title:** Influence of the Synthetic Peptide K-aurein on the Antimicrobial Activity of Ciprofloxacin against *Pseudomonas aeruginosa* ATCC 9027

**Abstract:**

**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*.  

**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, 62.25 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 antibiofilm 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.
**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*.
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. 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. Among different phenotypic assay used for detection of biofilm formation, tissue culture method detected biofilm production in 22.1% isolates followed by tube method and congo red agar method which detected only 16.8% and 5.3% respectively. On further verification, PCR detected ica genes in 23% of staphylococcal isolates thus revealing the biofilm formation in these samples. In-vitro persister assay is undergoing to study the causal link between persister cells and treatment failures in these isolates. 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.
**Abstract:** 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:** 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 underlies 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 (H2O2) 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 H2O2 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 H2O2 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 O2-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 H2O2-treated biofilms had higher σB activity than the planktonic cells (p=0.0157). The σB activities in H2O2-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: **Poster Session 1**  
Date & Time: Monday, October 8, 2018, 4:00 pm - 6:00 pm  
Poster Board #: 032  
Topic: Biofilm Antimicrobial Tolerance  
Abstract: **Prevalence of Biofilms & Enterotoxins Produced by Staphylococcus aureus-Inducing**  
Title: *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.
Abstract: 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 inceptive 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.
Abstract:

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.
Abstract: Bacterial biofilms are highly complex communities, composed of highly structured extracellular polymers and subpopulations of differentiated cells, such as persisters. These contribute to the resistance of bacterial biofilms to antibiotics, creating a significant issue in the treatment of infections and resulting in elevated levels of mortality and morbidity. Here, we use a 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:

Development of biofilms on different surfaces are formed due to varies 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*, *rfa*L, *rfa*I, *rfa*F, and *rfa*G 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.
Session: 
Poster Session 1
Date & Time: 
Monday, October 8, 2018, 4:00 pm - 6:00 pm
Poster Board #: 037
Topic: Biofilm Antimicrobial Tolerance
Abstract Title: Sphingosine and Staphylococcus epidermidis Modulate Growth of Staphylococcus aureus Biofilms
Author Block: M. Kim, G. K. German, C. N. Marques; Binghamton University, Binghamton, NY.

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:** Poster Session 1  
**Date & Time:** Monday, October 8, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 038  
**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. Lamarche\(^1\), Y. Abassi\(^1\), A. Mira\(^2\), M. D. Ferrer\(^2\);\(^\ 1\)ACEA Biosciences, San Diego, CA, \(^2\)Center for Advanced Research in Public Health, Valencia, SPAIN.

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\(^{\circledR}\) 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.

**Abstract:** 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 evaluable 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.
**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.
Abstract:

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).
**Abstract:**

*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

A. Wang, S. Keten; Northwestern University, Evanston, IL.

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 Mon-mucoid *Pseudomonas aeruginosa* Isolates

**Abstract:**

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 *P. 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.
**Abstract:**

**Objective:** As polyester polyurethanes are degraded by microorganisms they release aliphatic and aromatic diols, carboxylates, intact polyurethanes 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 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.
Abstract: 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.
Abstract:
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.
Abstract:

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.
Abstract: Investigating Biofilms within Washing Machines: Nanosensor Technology Enables Novel Insights into pH Gradients of *Pseudomonas aeruginosa* Biofilms

Ratiometric fluorescent pH-sensitive nanosensors are novel tools to look at pH changes within the physiological range between pH 8 and pH 3 at a cellular level in real-time. The nanosensors were used to analyse the pH variation throughout biofilms of *Pseudomonas aeruginosa*, an opportunistic human pathogen that is involved in a variety of biofilm related infections and is one of the predominant bacteria in washing machines. The formation of biofilms in washing machines has become a growing concern as survival of bacteria is associated with biofouling and an increased infection risk due to the distribution of pathogens into the environment. Currently, little is known about environmental microniches present in biofilms. The optical nanosensors penetrate biofilms and emit fluorescence in response to variation in pH. Nanoparticles are likely to interact with biofilm components like polysaccharide or extracellular DNA. Therefore, characterisation of nanosensors with different surface charges that are used for the pH analysis within biofilms of *P. aeruginosa* was undertaken. Confocal laser scanning microscopy revealed that the addition of the positively charged polyacrylamide nanosensors to PA01 WT enhances biofilm formation in comparison to no nanosensors or neutral nanosensors. Furthermore, the responsiveness of the nanosensors to pH changes enables calculation of the pH gradient across the thickness of a biofilm. Using time lapse imaging, pH changes can be tracked in real time at a microcolony and single cell level which will ultimately facilitate monitoring of environmental changes induced as biocides penetrate biofilms, underpinning the development of more effective antimicrobials.
Abstract: 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
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.
Abstract:

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 pathogenesis of Group A Streptococcus (GAS), a gram-positive pathogen responsible for a wide range of mild to severe infections with 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.
**Abstract:**

**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 depolymerization of T4P. Polymerization causes pili to elongate and subsequently attach into surfaces. De-polymerization 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, SedFoam (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. **Conclusions:** The model can successfully predict Jeffery orbit of a non-motile rod-shaped bacterium with different aspect ratios in various shear flows. The twitching model can capture experimentally observed common behaviours of twitching bacteria such as upstream twitching of motile rod-shaped bacteria in shear flows. **References:** Jayathilake, P.G., Gupta, P., Li, B.W., Madsen, C., Oyebamiji, O., Gonzalez-Cabaleiro, R., Rushton, S., Bridgens, B., Swailles, D., Allen, B., McGough, A.S., Zuliani, P., Ofiteru, I.D., Wilkinson, D., Chen, J.J., Curtis, T., 2017. A mechanistic Individual-based Model of microbial communities. PLoS One 12. Sun, R., Xiao, H., 2016. SedFoam: A general-purpose, open-source CFD-DEM solver for particle-laden flow with emphasis on sediment transport. Computers & Geosciences 89, 207-219.
Abstract:

**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 (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.

**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.
**Abstract:** 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/ΔrhlI*. 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 (265μg/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.
Abstract:

**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 larvae 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.
Mortality due to lung infection with nontuberculous mycobacteria (NTM) is outpacing tuberculosis (TB) in the US and Europe. *Mycobacterium abscessus* has emerged as an important NTM pathogen in people with chronic inflammatory lung diseases such as chronic obstructive pulmonary disease and cystic fibrosis (CF). Despite regimens as prolonged as for multi-drug resistant-TB, antibiotic therapy often fails to eradicate *M. abscessus* infection. While our understanding of mycobacterial biofilms is limited, cell wall glycopeptidolipids (GPLs) have been implicated as key determinants in biofilm formation in mycobacteria. *M. abscessus* exhibits a smooth colony morphology variant that expresses GPLs and a rough colony variant lacking GPLs and previous studies with a clinical *M. abscessus* isolate indicated that the smooth variant forms biofilms, but the rough does not. This paradigm suggests that GPL expression promotes a colonizing, biofilm-forming phenotype, whereas a lack of GPL promotes a non-biofilm forming, invasive phenotype. Using isogenic variants from the sequenced *M. abscessus* reference strain, we show that the rough variant, which lacks GPL expression, is hyper-aggregative and that each morphotypic variant develops loosely adherent biofilm aggregates, which are significantly more tolerant than planktonic variants to acidic pH, hydrogen peroxide, and antibiotic treatment (p<0.001). These studies suggest that contrary to the current paradigm, each *M. abscessus* variant forms biofilm aggregates and that biofilm formation is not restricted to the GPL locus. Further studies suggest that interactions with phagocytic cells are different. While the rough variant is not more invasive than smooth in primary human macrophages, it is more resistant to innate immune cell effectors such as reactive oxygen species once inside macrophages. Notably, despite similar phagocytosis of each variant, the rough variant grows significantly better than the smooth in macrophages from people with CF (p<0.001). We are investigating how biofilm development and the interaction of *M. abscessus* variants with innate immune phagocytic cells may contribute to chronic infection in the human respiratory tract.
**Title:** Association of *P. gingivalis* and *P. intermedia* Provoke High Levels of Bone Resorption in a Murine Experimental Periodontitis Model

**Abstract:**

**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
**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 PAO1 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.
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 *csg(D)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.
Abstract:

**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 combination of 10 µl of 50 µg/ml arachidonic acid (sclerosing agent) and 50 µl of a 10^8 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 log_{10} cfu/g of bone or log_{10} 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 log_{10} 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 log_{10} 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 log_{10} cfu/g bone of 5.28 and mean log_{10} 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.
**Title:** Characterization of *Mycobacterium chimaera* Biofilm on Med. Device Materials Under Stationary and Continuous Flow Conditions

**Authors:**
A. Siddam\(^1\), S. Zaslow\(^1\), Y. Wang\(^2\), K. S. Phillips\(^2\), M. Silverman\(^1\), P. Regan\(^1\), J. Amarasinghe\(^1\);
\(^1\)Winchester Engineering and Analytical Center, Office of Regulatory Affairs, Winchester, MA; \(^2\)Center for Devices and Radiological Health, Silver Springs, MD.

**Abstract:**

**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.
**Abstract:**

**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

**Abstract:**

**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.
Abstract: 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 H2O2 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.
**Abstract:** Vibrio Cholerae Combines Individual and Collective Sensing to Trigger Biofilm Dispersal

**Author Block:** P. K. Singh¹, S. Bartalomej¹, R. Hartmann¹, H. Jeckel¹, L. Vidakovic¹, C. D. Nadell², K. Drescher¹; ¹Max Planck Institute for Terrestrial Microbiology, Marburg, GERMANY, ²Department of Biological Sciences, Dartmouth College, Hanover, NH.

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.
Abstract:

**Background:** Biofilm and planktonic bacterial populations experience different stresses, resource requirements, and degrees of population and spatial structure. These differences can, in turn, affect patterns of adaptation to other environmental factors. We focus here on high- and low-carbon environments because variation in resource limitation is an important selective pressure in adaptation both in the environment and in patients. Specifically, we ask whether adaptation to high-carbon and low-carbon environments occurs via the same genes in biofilm- and planktonic-selected conditions. **Methods:** We propagated populations of *Burkholderia cenocepacia* HI2424 in high and low carbon conditions, under selection for either planktonic or biofilm life cycles. The concentration of galactose, the sole carbon source in our experiments, limited population size in all environments. Following 90 days of evolution we sequenced the populations and measured their fitness relative to their ancestor. **Results:** Under high-carbon conditions, both biofilm and planktonic populations evolved mutations in several of the same genes. In fact, 15 of 17 high-carbon populations had mutations in the same nucleotide of an upstream promoter of the iron-storage gene bacterioferritin. In contrast, under low-carbon conditions, no genes were mutated in common between biofilm and planktonic populations. All low-carbon, planktonic populations evolved mutations in the same carbon transporter gene whereas the low-carbon, biofilm populations evolved mutations in a single regulator of carbon metabolism genes. Furthermore, adaptation to low-carbon, biofilm conditions led to reduced competitive success in planktonic environments, whereas adaptation to high-carbon, biofilm environments let to improved competitive success in both biofilm and planktonic environments. **Conclusions:** The degree of parallel evolution (repeated selection of mutations in the same genes) between planktonic and biofilm selected populations was higher in high-carbon than in low-carbon environments. This indicates that mode of growth has a larger effect on evolutionary trajectories in some environments than in others. It may be that there are more trade-offs between biofilm and planktonic adaptation in low-carbon environments because carbon allocated to exopolysaccharide production for biofilm formation reduces the amount of carbon available for bacterial growth.
**Title:** Interesting New Features and Functions of the *Pseudomonas aeruginosa* Biofilm Matrix Protein CdrA

**Abstract:**

*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.
**Abstract:** Infections acquired from clinical settings often involve bacterial colonisation and biofilm formation in water systems. Sensors are used to monitor various water quality parameters aimed at assessing the health of the water system, where the deployment of continuous, *in-situ* monitoring is considered most beneficial. However, the performance of the sensor can be affected over time by biofilm growth, which presents a significant challenge for obtaining reliable long-term measurements. Both synthetic insulating and conducting, boron doped diamond (BDD) are now being explored as materials for long-term sensor deployment because of their superior properties, such as hardness, chemical inertness, and corrosion resistance. Diamond and BDD are also claimed to be low biofouling materials, but this is often cited in relation to biological environments where proteins, neuronal cells *etc.* are considered potential fouling agents. Literature on the interaction of bacterial cells with diamond is sparse, and there have been no extensive studies into the extent of bacterial adherence and biofilm formation on BDD. 

**Methods:** We evaluated the extent of *Pseudomonas aeruginosa* monospecies and multispecies biofilm formation on a variety of materials employed in sensor fabrication (PVC, stainless steel, copper, diamond, BDD, alumina and screen printed carbon). Substrates were characterised to determine their surface hydrophobicity, roughness, and topography, before being exposed to inocula of ten waterborne biofilm producing bacterial strains. Subsequently, these were directly compared for qualitative and quantitative assessment of biofilm formation, using crystal violet staining, scanning electron microscopy (SEM), and confocal laser scanning microscopy. 

**Results:** By using the crystal violet multititer plate assay, we found that the lowest amount of *P. aeruginosa* monospecies biofilm was observed on both BDD and diamond. Qualitative investigation by SEM supported this finding. Correlating the physical properties of the substrates with the degree of biofilm formed, revealed that biofilm formation increased with both increasing surface roughness and hydrophobicity. Focusing on diamond substrates, we were able to systematically change hydrophobicity (whilst keeping roughness constant) and vice versa and examine the extent of biofilm formation. We found that by deliberately increasing the surface roughness and hydrophobicity, the amount of biofilm formed increased. 

**Conclusions:** Overall, our multi-disciplinary study offers insight into the characteristics of a sensor surface that influences bacterial adherence and biofilm formation. We find that smooth, hydrophilic, BDD and diamond surfaces are interfaces that can naturally offer a relatively lower extent of biofilm formation.
Abstract: 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. Overexpression 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.
Abstract:
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 perisplasmic 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 hfsEFGH and hfsDABC. 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.
**Title:** A Large Repetitive RTX-Like Protein as a Link between Surface Adhesion and Pathogen Invasion for the Bacterial Phytopathogen, *Pantoea stewartii subsp. stewartii*

**Authors:** P. Viravathana¹, L. Burbank², M. Roper¹

¹University of California, Riverside, Riverside, CA, ²USDA-ARS, Parlier, CA.

**Abstract:** 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 H₂O₂ and superoxides. The transcription factor OxyR is essential in regulating protection against H₂O₂ 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 (Rcs) 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, ∆rtx2 produces less EPS, indicating a possible link between activation of the Rcs pathway, RTX2 and sensing of ROS via OxyR. RTX2 is a large (249.8 kDa) protein containing 5 putative Ca²⁺-binding domains similar to serralysin, 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 hemagluttinins and to the BAP subfamily of RTX proteins implicated in promoting bacterial-host adhesion during biofilm formation. ∆rtx2 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.
**Abstract:** The Nontypeable *Haemophilus influenzae* Phasevarion Influences Adherence to Human Airway Epithelial Cells: The First Step in Biofilm Formation

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

**Author Block:** S. D. Stacey¹, P. N. Azzari¹, K. L. Brockman¹, J. M. Atack², M. P. Jennings², L. O. Bakaletz¹; ¹The Research Institute at Nationwide Children’s Hospital and The Ohio State University College of Medicine, Columbus, OH, ²Institute for Glycomics, Gold Coast, AUSTRALIA.

**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 (*modA2, 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 (*modA2*), C486 (*modA4*), 477 (*modA5*), and 1209 (*modA9*) locked ON and locked OFF variants for relative adherence to polarized human airway epithelial cells (HAEs) after 1, 3 or 6 hr. Locked variants 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 (*modA2*) or C486 (*modA4*) after 3 hr, there was significance between locked ON and OFF variants for strain 477 (*modA5*). Strain 477 (*modA5*) 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
Abstract: Host suppression of Quorum Sensing During Catheter Associated Urinary Tract Infections

S. J. Cole¹, C. L. Hall¹, M. Schniederberend², J. M. Farrow III¹, J. R. Goodson¹, E. C. Pesci³, B. I. Kazmierczak², 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.
Abstract: 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: Quorum sensing is a cell to cell communication process where bacteria communicate with one another using extracellular signaling molecules called autoinducers which is functional during high cell density. Quorum sensing plays an important role during the colonization process of the bacteria. Adherence is the initial step of colonization in bacterial pathogenesis. Streptococcus pyogenes is a human-restricted pathogen capable of causing a broad spectrum of diseases. This bacteria primarily resides in the oropharynx and the skin of its human host generating illnesses ranging from mild, like pharyngitis and impetigo, to severe and life-threatening, like necrotizing fasciitis and toxic shock syndrome. Quorum sensing tightly regulates the expression of virulence factors exhibited by the bacteria. The role of quorum sensing molecules from gram negative bacteria on pathogenesis of gram positive bacteria is not fully understood. The current study focuses on degree of adherence of S. pyogenes to host epithelial cells in presence of different homoserine lactones. Methods: Adherence of S. pyogenes to FaDu cells were studied in presence of 10µM of homoserine lactones (Oxo-C6, Oxo-C8, Oxo-C10, Oxo-C12). Adhesion assay was performed in 24 well plates. Adherence assay was conducted wherein S. pyogenes culture was incubated on FaDu cells in presence of 10µMof homoserine lactones. To understand the role of homoserine lactones on S. pyogenes adherence, the bacteria were first allowed to grow in 10µM of homoserine lactones and then subjected to adherence assay. A displacement assay was carried out where the S. pyogenes was allowed to adhere to FaDu cells for 2h, and later 10µM of homoserine lactones were added. Results: During the co-incubation study, it was found that Oxo-C10 and Oxo-C12 significantly inhibited the adherence of S. pyogenes to FaDu cells. Similar results were obtained when S. pyogenes were grown in presence of homoserine lactone and then added to FaDu cells. In the displacement assay, it was found that the homoserine lactone could not displace the adhered S. pyogenes. Conclusion: The results indicate that Oxo-C10 and Oxo-C12 homoserine lactones inhibit the adherence of S. pyogenes at initial stages. This data suggests that during the colonization process of S. pyogenes, microbes in the vicinity are capable of secreting homoserine lactones with fatty acid side chain of ≥ 10 carbon atoms which in turn inhibits the adherence of S. pyogenes to its host epithelial cells. The study on effect of Oxo-C10 and Oxo-C12 homoserine lactone on invasion of S. pyogenes is under process. Keywords: Streptococcus pyogenes, adherence, acyl homoserine lactone.
**Abstract:**

**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)
Session: Poster Session 1
Date & Time: Monday, October 8, 2018, 4:00 pm - 6:00 pm
Poster Board #: 079
Topic: Regulation of Biofilm Development
Abstract: Molecular Regulation of Adhesion and Biofilm Formation In Bacillus Licheniformis high and Low
Title: Biofilm Producers Using RNA Seq
Author: F. A. Sadiq;
Block: Jiangnan University, Wuxi, CHINA.

Bacillus licheniformis is a dominant spore-forming microbial contaminant of the dairy industry throughout the world. A variety of studies suggest that the ability of this species to form biofilms is the key reason for its widespread prevalence. However, despite its apparent prevalence and spoilage potential, there is no information regarding the molecular determinants involved in biofilm formation by B. licheniformis. We used the transcriptome sequencing (RNA-seq) technology to reveal over- and underrepresented transcripts in the transition from the planktonic (logarithmic culture) to the biofilm state (24 h old) in a high and low biofilm forming B. licheniformis dairy strains. Genes, representing 28.3 % and 36.3 % of the whole B. licheniformis gene content were upregulated in the biofilm state as compared to the planktonic state in high and low biofilm producers, respectively. The gene expression patterns of several groups of genes differed significantly (P<0.05) under the two growth conditions. The genes related to chemotaxis proteins (CheA, CheB, CheC, CheD, CheV, CheW, CheY, MotA, MotB) and flagellar assembly were exclusively upregulated in the planktonic phenotype as compared to biofilms in both strains. In terms of Kyoto Encyclopedia of Genes and Genomes (KEGG) based pathways, metabolic pathways were significantly (P < 0.05; Q-values < 0.05) downregulated in the planktonic state as compared to the biofilm state in both strains which shows that biofilm formation is an energetically expensive process. Lipid and sugar metabolism seemed to play an important role in the matrix production. Overall, several genes involved in adhesion, matrix production and matrix coating were either absent or less expressed in the biofilm state of low biofilm producer as compared to the high biofilm producer. Interestingly, genes related to sporulation and the production of extracellular polymeric substances were concomitantly expressed in the biofilm state of both strains which suggests that sporulation is coupled with the biofilm formation in this species similar to B. subtilis. Collectively, our results provide a comprehensive insight into biofilm formation in B. licheniformis that will be helpful for future research into mechanisms and targets.
PqsE and RhlR are an Autoinducer Synthase-Receptor Pair that Controls Virulence and Biofilm Dev. in Pseudomonas aeruginosa

S. Mukherjee, D. Moustafa, V. Stergioula, C. Smith, J. Goldberg, B. Bassler; 1Princeton University, Princeton, NJ, 2Emory University School of Medicine, Atlanta, GA.

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 ΔrhlR and ΔrhlI mutants have radically different biofilm phenotypes and the ΔrhlI mutant displays full virulence in animals whereas the ΔrhlR mutant is attenuated. The ΔrhlI 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 C4-HSL 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 Rhl 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.
**Background:** *Pseudomonas aeruginosa*, an opportunistic human pathogen is of great interest because of their remarkable metabolic versatility and ability to colonize a variety of habitats. *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 *Pseudomonas* quinolone signal (PQS), and metabolized by anthranilate dioxygenase complex (*antABC* gene products) via TCA cycle. Recently, anthranilate has been reported to be an inducer of the *antABC* expression and cause biofilm dispersal in various bacteria by deteriorating the biofilm structure. **Methods:** We measured the production and accumulation levels of anthranilate during long-term culture of *P. aeruginosa*. We also measured the expression level of *antABC* that encodes the anthranilate-degrading enzymes throughout the growth of *P. aeruginosa*. To figure out the relation between the anthranilate and biofilm formation, we traced how the level of anthranilate changes as *P. aeruginosa* grows and at what point the biofilm forms. **Results:** The production and secretion of anthranilate remain very low until *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 *antABC* function, because the high-level accumulation of anthranilate lasted in *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 *antABC*. **Conclusions:** In *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 *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.
**Abstract:**

**Title:** Effect of Nano-Silver, Nano-Copper, Deconex Andbenzalkonium Chloride on Biofilm Formation Andexpression of Transcription Regulatory Quorum Sensing Gene (Rh1R) In Drug-Resistance *Pseudomonas aeruginosa* Burn Isolates

**Author:** M. -. Shakibaie;

**Block:** 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.
Abstract:

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.
**Title:** The Role of SAWR in Regulating Virulence and Biofilm Formation in *Pseudomonas aeruginosa*

**Author:** Y. Ben-David, I. Zander, E. Banin; Bar-Ilan, Ramat Gan, ISRAEL.

**Abstract:**
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.
**Session:** Poster Session 1  
**Date & Time:** Monday, October 8, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 087  
**Topic:** Regulation of Biofilm Development  
**Abstract Title:** Protein Interaction Network Connects a c-di-GMP Signaling Core with Multiple Cellular Processes and Guides Functional Analysis of Proteins Complexes Involved in Biofilm Formation in *Pseudomonas fluorescens*  
**Author Block:** M. Gros, R. Wilton, S. Forrester, S. Flores, P. Larsen, P. H. Noirot; Argonne National Laboratory, Lemont, IL.

**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 diguanosine 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:

*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 cyanobacterial 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 pili. 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:**

**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 in 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.
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 member 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.
**Abstract:**

*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 AB5075. 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 asses 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.
Session: Poster Session 1
Date & Time: Monday, October 8, 2018, 4:00 pm - 6:00 pm
Poster Board #: 096
Topic: Regulation of Biofilm Development
Title: Post-translational Modification via Acetylation may Regulate Chitinase Activity and Lead to Alterations in Francisella Biofilm Production

Author Block: M. L. Van Hoek1, E. S. Marakasova1, A. II1, K. T. Nelson2;
1George Mason University, Manassas, VA, 2Virginia Commonwealth University, Richmond, VA.

Abstract: 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 hydrolases (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.
Abstract:
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.
Abstract:

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 perio-pathogens *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

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.

**Abstract:**

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

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

**Author:** D. Sirias, K. A. Gibbs;

**Block:** 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)XK 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.
Abstract: 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
Abstract:
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 violent 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. aeruginosa alginate-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:** Poster Session 1  
**Date & Time:** Monday, October 8, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 106  
**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, P. Azadi, C. Heiss, J. M. Troutman, B. Scarbrough, A. Fiebig, S. Crosson;  
1University of Chicago, Chicago, IL, 2Complex Carbohydrate Research Center, Athens, GA, 3UNC 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.
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.
**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.
Abstract:
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.