Microbial biofilms are commonly referred to as the microbial cities. Because the microbes living inside the biofilms are not only protected from the host defense mechanisms but also from the effects of antibiotics and here the microbe take the advantage of living in a well-developed community. Microbes living inside the biofilm are 1000 times more resistant to the effect of antibiotics as compared to planktonic cells. That is why biofilm formation is considered as an important resistance mechanism. It is pertinent to mention here that 70% of the pathogens have the tendency to form biofilm. Conventional antibiotics fail to eradicate biofilms because of their inability to penetrate protective barrier (Extracellular polymeric substance) that surrounds them. Therefore, it is essential to develop such a treatment that has the tendency to inhibit biofilm formation or that may enhance penetration of antibiotics inside the biofilm. Metallic nanoparticles (NPs) have already displayed their tendency to shred biofilms. However, their utilization is limited because of potential toxicity. Therefore, the objective of the present study was to develop biodegradable and nontoxic nanoparticles with antimicrobial and antibiofilm potential. For this purpose, chitosan NPs were developed and cefotaxime was incorporated in it. Antibiotics loaded chitosan NPs were characterized and their antimicrobial potential by (broth dilution method) and antibiofilm potential by (micro titer plate) was determined. It was observed by Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM) analyses that both empty and drug loaded chitosan nanoparticles were less than 100nm in size and zeta potential analysis revealed that they were positively charged. Drug loaded NPs were tested against various multi drug resistant pathogens and were found to be highly effective against all tested pathogens. They also displayed broad spectrum antibiofilm activity as well.
**Abstract:**

**Background:** *Pseudomonas aeruginosa* is opportunistic pathogen, which easily forms biofilms on various types of surfaces. The biofilm phenotype is characterized by an increased resistance to environmental influences including resistance to antibiotics and other disinfectants. Considerable effort is therefore devoted to finding suitable, in practice useful, tools to inhibit cell adhesion, or eradicate mature biofilm. In this respect, the antibiofilm potential of various natural substances is often tested. One of the promising substances can be chitosan. Its biological activity is given by the presence of amino groups in the structure. Cationic nature enables chitosan to interact with negatively charged compounds. The antimicrobial activity of chitosan was confirmed many times, but information about its interaction with biofilm are relatively scarce. **Methods:** The cultivations were carried out in 100-well microtiter plates using Bioscreen C analyzer. The cell adhesion and biofilm stability under different concentrations and application methods of chitosan were quantified by crystal violet assay. Metabolic activity of the cells in biofilm was measured using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) colorimetric test based on the reduction of MTT into formazan by the dehydrogenase system of living cells. Concentration of biomass in medium above biofilm was determined as OD$_{600}$nm. Activity of N-acyl homoserine lactones (AHLs) involved in the regulation of biofilm formation was determined using *Agrobacterium tumefaciens* strain harboring a traG::lacZ/traR reporter gene responsive to AHLs. SYTO 13 and propidium iodide staining and spinning disc confocal microscopy (SDCM) were used for visualization of biofilm. **Results:** *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC 15442 formed biofilm at the bottom of the microtiter plates after 30 min cultivation. Very low chitosan concentration ranging from 2 to 6 mg / l increased the amount of biomass in the biofilm and metabolic activity of the cells. However, the biofilm formation of both strains was significantly suppressed after 24 h cultivation when the chitosan concentration exceeded 20 mg / l. Application of chitosan at a concentration of 30 mg / l or higher enabled effective eradication of mature biofilm. The resulting effect of chitosan on biofilm depended also on the time of application and the duration of interaction with biofilm. The presence of chitosan in the medium slightly suppressed the level of AHLs regulatory molecules. **Conclusions:** We have proved that chitosan has a considerable antibiofilm potential. It significantly decreased cell adhesion and biofilm formation of *P. aeruginosa*, as well as eradication of the mature biofilm at practically usable concentrations.
**Session:** Poster Session 2  
**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 003  
**Topic:** Antibiofilm Strategies  
**Abstract:** Anti-Infective Potential of a Polyherbal Wound-Care Formulation (Herboheal) against planktonic and Biofilm Forms of Selected Pathogenic Bacteria  
**Title:** plithe title  
**Author:** P. Patel, C. Joshi, V. Kothari;  
**Block:** Nirma University, Ahmedabad, INDIA.

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

**Methods:** Parameters on which effect of Herboheal Formulation (HF) was investigated in vitro include: bacterial growth and biofilm formation, pigment production, cell surface hydrophobicity, susceptibility to serum and antibiotics, heamolytic activity, etc. Biofilm quantification was done by crystal violet assay, whereas its viability was evaluated using MTT assay. In vivo efficacy of HF was assayed using the nematode *Caenorhabditis elegans* as the model host. Molecular targets of the HF in *S. aureus* were elucidated through gene expression analysis at the whole transcriptome level. Additionally, effect of HF on mixed species biofilm of *P. aeruginosa* and *S. aureus* was also investigated.  

**Results:** HF(0.1-0.5%v/v) inhibited bacterial biofilm formation up to 40-55%. It had no effect on pre-formed *P. aeruginosa* biofilm, but could eradicate pre-formed *S. aureus* biofilm by ~63%. Mixed species biofilm formation by these two bacteria was inhibited by 34% in face of HF-challenge. CSH of *P. aeruginosa* and *S. aureus* was marginally decreased and increased under the influence of HF. HF-treated bacteria exerted significantly lesser virulence towards *C. elegans*. Transcriptome analysis revealed that several genes associated with adhesion (*icaR, icaB, icaA*), biofilm formation (*sigB*), determinants of CSH (teichoic/ lipoteichoic acid syntheses: *tagB, tagX, SAFDA_2230, SAFDA_0051*), capsular polysaccharide biosynthesis protein *capA_2*, etc. were differentially expressed in HF-treated *S. aureus*.  

**Conclusions:** This study validates the traditional use of *Herboheal* formulation in wound-care by proving its efficacy against gram-negative as well as gram-positive bacteria, most commonly involved in wound infections.
**Abstract:**

**Background:** Shiga-toxin producing *Escherichia coli* (STEC) is one of the *E. coli* pathotypes that causes haemorrhagic colitis and diarrhea that could lead to fatal haemorrhagic uremic syndrome (HUS). Approximately 2.8 million STEC infections occur annually and is a leading cause of death in children below the age of four years. The incidence of STEC infection has been on a rise and occur both in developed and underdeveloped countries. Antibiotic treatment is not recommended to treat STEC infections as this could lead to increased shiga toxin production and release. Moreover, emergence of antibiotic resistant STEC has added to managing STEC infections. Alternative treatment options for STEC treatment include use of phytochemicals. Garlic (*Allium sativum*) is known to exhibit antimicrobial activity due to presence of organosulfides. As biofilm play an important role in STEC survival and spread through food, we investigated antibacterial and anti-biofilm activity of garlic against STEC isolates from various food sources and patient samples.

**Methods:** Isolation of STEC was performed using selective and differential culture media from food and patient samples of Central India. Fresh garlic extract (FGE) and antibiotics (eight) was used to perform susceptibility of isolated strains by Kirby-Bauer disc/well diffusion assays as well as minimum inhibitory concentration (MIC) of FGE against all isolates was determined using dilution method. Biofilm formation assay and anti-biofilm activity of garlic was tested using 96 well plate crystal violet staining biofilm assays and biofilm visualization was performed through scanning electron microscopy (SEM). The changes in the carbohydrate content of the biofilm after treatment with FGE was determined by phenol-sulphuric acid method.

**Results:** Eight STEC isolates were obtained from various food and patient samples. Antibiotic resistance study indicated that seven strains were resistant to all antibiotics except for rifampicin and one strains was sensitive to both ciprofloxacin and rifampicin designating them as multi drug resistant (MDR) isolates. Well diffusion assay indicated that all strains exhibited dose dependent sensitivity towards FGE with diameter of zone ranging from 7-24 mm with 15 µl of fresh garlic extract. MIC of FGE for isolates ranged from 30-140 µl/ml. The reduction in biofilm formation ranged from 35 to 59% in the presence of 4% of FGE and SEM analysis confirmed these observations. The total carbohydrate content of biofilm treated with 1% FGE was reduced by 42%. **Conclusion:** The study exhibits potent antibacterial activity of garlic against MDR STEC isolates. In addition, garlic also exhibited potent anti-biofilm activity mostly by reducing the carbohydrate content. The results suggest that garlic can not only be used to treat STEC infections but also to control contamination of food sources with STEC by preventing biofilm formation.
Background: *Candida* species cause infections in both immunocompromised and immunocompetent individuals, ranging from relatively benign superficial infections to life-threatening systemic infections. *Candida auris* is an emerging, multidrug resistant yeast that can cause invasive infections and has been frequently associated with healthcare outbreaks throughout the world. *Candida* spp. are commonly encountered wound and bloodstream pathogens and have been known to form monomicrobial and polymicrobial biofilms, especially in combination with *S. aureus*. This work evaluates the effectiveness of novel biofilm-disrupting technology in topical gel, wound wash, and surface disinfectant forms vs chlorhexidine and super-oxygenated water on *C. auris*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, and on polymicrobial biofilms composed of either *C. auris* or *C. albicans/S. aureus* or *C. glabrata* or *C. parapsilosis/S. aureus*. Methods: Prevention of *Candida* spp. biofilm growth is determined by zone of inhibition (ZOI) testing, whereby the relevant organism is seeded onto agar plates and the treatment applied to prevent growth. Efficacy is determined by the size of the zone created. Biofilm disinfection efficacy versus *Candida* spp. is determined by treating biofilm which has been pre-grown on SD agar plates. Treated area of the plates are excised and biofilm enumeration is determined by vortexing, serial dilution, and regrowth to obtain CFU values. Polymicrobial biofilms are created by growing *Candida* spp. and *S. aureus* on the bottom of 96-well microtiter plates. Monomicrobial biofilms of *Candida* spp. and *S. aureus* are used as controls. After biofilm growth, plates are washed with PBS to remove non-adherent cells and then the treatments are applied for 24 hours. Biofilms are resuspended and then sonicated to facilitate biofilm disruption and ten-fold dilutions are prepared for enumeration. The Student’s t-test (two-tailed, unequal variance) will be used to analyze the significance of difference between two experimental groups, with a P-value of 0.05 or less being considered significant. Results: Initial testing of the gel version of the biofilm disruption technology at a 50% dilution yielded multi-log reductions to the *Candida* biofilms in 24 hours of treatment (*C. auris*, 4.1 log; *C. albicans*, 3.9 log; *C. glabrata*, 4.1 log; *C. parapsilosis*, 4.4 log). 50% dilutions of the gel product have also demonstrated multi-log efficacy in treating mixed-species biofilms in 24 hours (*C. auris* + *P. aeruginosa*, 4.0 log; *C. auris/S. aureus*, 4.3 log). Additional data will be presented at the conference. Conclusions: The use of a novel biofilm disrupting technology has demonstrated efficacy in treating *Candida* biofilms and multi-species biofilms of *C. auris* and bacteria.
Antibiofilm Strategies

**Title:** Antibiofilm Effects of a Novel Silver Gelling Fiber Dressing on *Pseudomonas aeruginosa* in Porcine Wound Infection Model

**Abstract:**

The wound environment is a fertile ground for biofilm-forming pathogens. Once biofilms form within wounds, they become very challenging to eradicate and contribute to recurrent infections and inhibition of healing. The purpose of this study was to examine the effect of a novel gelling fiber dressing with silver using a well-established *in vivo* porcine wound biofilm model. Deep partial thickness wounds were inoculated with *Pseudomonas aeruginosa* ATCC 27312 and covered with a polyurethane film dressing to promote biofilm formation. Wounds were then divided into treatment groups: gelling fiber dressing with silver, gelling fiber dressing without silver; hydrofiber dressing with silver, benzethonium chloride and ethylenediaminetetraacetic acid and compared to untreated control. Microbiological, biofilm and histological wound assessments were performed from 104 wounds on days 3, 5 and 7 post infection. Treatment with gelling fiber dressing with silver resulted in significant reduction of *P. aeruginosa* biofilm when compared to all other treatment groups on every assessment time point (p < 0.05). In addition gelling fiber dressing with silver treatment resulted in detachment of biofilm from the wound, while wounds treated with gelling fiber dressing with and without silver showed more granulation tissue formation on day 3. Our data show that a new gelling fiber dressing with silver was effective in reducing biofilm associated *P. aeruginosa in vivo*. This study may have important clinical implications especially for wounds highly colonized with Gram-negative biofilm-forming bacteria.
**Abstract**

**Background:** *Pseudomonas aeruginosa* has a large arsenal of virulence factors that are expressed in a coordinated and cell density-dependent manner by quorum sensing (QS). In addition, most antibiotics are not effective to chronic *P. aeruginosa* infections due to the high antibiotic resistance mediated by biofilm formation. Therefore, to cope with *P. aeruginosa* infection, there is a need for a substance capable of effectively controlling these two activities of *P. aeruginosa*, QS and biofilm formation. In this study, we tested a number of novel synthetic compounds for anti-QS and anti-biofilm activities.

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

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**Abstract:**

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

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

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

**Conclusions.** The NAC enhances the potential of ciprofloxacin and cefazolin against biofilms formed on catheters in pH dependent.
**Background:** Staphylococcus aureus is associated with recurring respiratory tract infections, such as chronic rhinosinusitis and cystic fibrosis. S. aureus has the capacity to form biofilms and small colony variants (SCVs), which are pathogenic subpopulations with a preferred intracellular lifestyle. S. aureus biofilms and SCVs are linked to antibiotic tolerance and resistance, and are challenging to eradicate. Despite aggressive antimicrobial therapies and surgery, infections often recur causing ongoing morbidity and significant healthcare costs.

**Aim:** Preclinical validation of an antibiofilm and anti-SCV treatment targeting bacterial iron metabolism.

**Methods:** The iron-chelator deferiprone (Def) and the haem-analogue gallium-protoporphyrin (GaPP), in solution and incorporated in a surgical wound gel, were tested for antibacterial activity using multidrug-resistant S. aureus SCVs in an intracellular infection model. The antibiofilm activity was assessed in vitro in the colony biofilm model and an artificial wound model, as well as in an in vivo infection model in nematodes (Caenorhabditis elegans).

**Results:** While Def alone failed to show substantial antibacterial activity, GaPP and the combination of Def-GaPP demonstrated concentration- and strain-dependent antibacterial properties. Specifically, the Def-GaPP combination significantly reduced the bacterial load in an artificial wound model (1.4 log10 reduction) and increased the survival of S. aureus SCV infected nematodes (86% survival of infected, treated worms vs. 25% survival of infected, untreated worms over 3 days). When Def-GaPP were combined with ciprofloxacin (Cip) or gentamicin (Gent), the triple combinations exceeded the antibiofilm activity of the individual compounds in the colony biofilm model against Cip- and Gent-resistant strains (5.4 log10 reduction for Def-GaPP-Cip and 3.4 log10 reduction for Def-GaPP-Gent). Moreover, Def-GaPP-Gent eradicated intracellular SCVs in human bronchial epithelial cells.

**Summary:** Def-GaPP showed significant activity against S. aureus biofilms and SCVs and potentiated the activity of Cip and Gent against resistant strains. Delivered in a wound healing gel, Def-GaPP progressed to an ongoing first-in-human pilot study (ACTRN12618000577213) for the treatment of chronic rhinosinusitis.
Abstract:
The control of biofilm formation on implants and orthopedic prostheses is still one of the major challenges concerning infection related to devices in the health field. The objective of this research was to investigate biomaterials with applicability in orthopedics, aiming for advances and facing challenges in the infectology area. The in vitro biofilm formation according to biomaterials (titanium and titanium coated with F18 bioglass), microorganisms (Staphylococcus epidermidis and Candida albicans) and incubation times (2, 4 and 8 hours) was evaluated by fluorescence microscopy. S. epidermidis (ATCC 12228) and C. albicans (ATCC 90028) biofilms were formed on proof bodies of titanium and titanium coated with F18 bioglass after 2, 4 and 8 hours of incubation at 37°C under orbital shaking. The image areas of proof bodies, in percentage, coated with biofilm (living cells) were evaluated by fluorescence microscopy. The data collected were submitted to statistical analysis using normality tests Shapiro Wilk, U from Mann-Whitney and t from Student through IBM SPSS Statistics (version 25) software and significance level α=5%. There was less biofilm formation by S. epidermidis and C. albicans (p<0.001) on titanium coated with F18 bioglass than on titanium, after 8 hours of incubation. However, there was more biofilm formation by S. epidermidis and C. albicans after 8 hours than in 2 hours of incubation, both on titanium and on titanium coated with F18 bioglass (p<0.05). In sum, titanium coated with F18 bioglass presented better antibiofilm activity in comparison with titanium, and the incubation times of 2 to 8 hours increased biofilm formation on both materials. Besides, future studies about F18 bioglass based on physicochemical, biochemical and microbiological aspects are important for the elucidation of action mechanisms related to biofilm control.
Antibiotic resistance is spreading at an alarming pace, and the risk that we fall into a new, pre-antibiotic era is real. Some pathogens are resistant to all known antibiotics. Attractive approaches that interfere with bacterial chemical communication (known as quorum sensing (QS)), have the potential to control pathogens without killing commensal bacteria. Numerous bacterial pathogens produce and utilize acyl homoserine lactones (AHLs) as chemical signal molecules to coordinate, in a cell density dependent manner, pathogen-critical bacterial behaviors including virulence and biofilm formation. Consequently, hydrolysis of AHLs with enzymatic quenchers, termed lactonases, inhibit pathogenicity. The main cause of morbidity and mortality in patients with cystic fibrosis (CF) is chronic lung infection. The most prevalent pathogen is *Pseudomonas aeruginosa* (up to 80% of adult CF patients). Many clinically relevant pathogens utilize quorum sensing for virulence, biofilm formation, and colonization. Lung colonization by *P. aeruginosa* is difficult to fight because of biofilm formation, even more so in cases of multidrug resistant infections. Therefore, new strategies to inhibit biofilm formation, fight or control pathogens are much needed. We investigated the ability of enzymatic quenchers to inhibit both virulence and biofilm formation of CF clinical isolates of *P. aeruginosa*. We used two enzymes, which have been engineered to be highly stable and highly active against AHLs. Because *P. aeruginosa* is known to utilize two QS circuits, one using 3-oxo C12 AHL, the other C4 AHL, we used two enzymes with different substrate specificity. For instance, we used an enzyme that disrupts only the 3-oxo C12 AHL based circuit (Ssopox-W263I) and a second enzyme that disrupts both circuits (GcL) used by *P. aeruginosa* for QS. We tested these enzymes on 39 clinical isolates of *P. aeruginosa*, and demonstrated that Ssopox treatment reduced biofilm formation in 23% of the strains by up to 88%, elastase in 54% of the clinical isolates by up to 63%, protease activity in 28% of the strains by up to 69% and pyocyanin production in 33% of strains. These results are consistent with recently published data using Ssopox-W263I on *P. aeruginosa* isolated from diabetic ulcers. Interestingly, as the enzymes target specific AHLs, we saw a separate pattern of quenching with treatment using an alternative lactonase, GcL. This study demonstrates the ability of these engineered enzymes to disrupt the complex communication system utilized by *P. aeruginosa* so as to reduce virulence and biofilm formation. With future studies on mixed species communities and combination therapy with antibiotics, we expect to provide the key data to assess the potential of signal disruption to treat and prevent infections relevant to CF patients, transition to animal model studies, and delineate the importance of signaling in chronic lung infections.
Title: Discovery of Novel Anti-MRSA Agents by High Throughput Screening of Pre-Metabolized FDA-Approved Drug Library

Abstract:

FDA approved drug screening has become a common strategy for drug repurposing. Drug metabolites frequently have distinct biological activities, as exemplified by the discovery of sulfanilamide as the active metabolite of Protosil. In this study, we described and evaluated the use of microsome metabolized FDA approved drug library screening for the discovery of novel antibacterial agents. Screens were performed against a gram-positive pathogen - methicillin-resistant staphylococcus aureus (MRSA). Initial efforts were hampered by the presences of substantial levels of bacterial contamination in microsomal preparations from several vendors, but an effective library metabolization protocol was developed which eliminated this problem. These screens reveal a number of known FDA approved drugs with enhanced antibacterial activity after metabolism. One of these - capecitabine - was fractionated after a scaled up metabolism reaction, which identified 5-fluoro-5’deoxyuridine (Doxifluridine) as its active metabolite.
Abstract:

**Background:** Biofilms are aggregates of microbial cells, which attach to biotic and abiotic surfaces as a mode of survival. Biofilm formation causes increased tolerance to antimicrobials and are responsible for a large amount of persistent human infections. With the growing problem of antibiotic resistance, there is a need to identify alternative antimicrobials to tackle biofilm-associated infections. Essential oils (EOs) are natural plant products, which have known antimicrobial properties. The focus of this research was to investigate the antibiofilm activity of cinnamon essential oil against *Pseudomonas aeruginosa* biofilms. **Methods:** *Pseudomonas aeruginosa* PAO1 in planktonic cultures were first challenged with cinnamon (*Cinnamomum zeylanicum*) EO using agar disk diffusion and broth microdilution methods to determine minimum inhibitory (MIC) and bactericidal (MBC) concentrations. A time-kill assay was used to assess contact time required for different concentrations of the oil to inhibit the bacteria and imaging using scanning electron microscope (SEM) employed to elucidate possible mode of action. Static biofilms grown in microtitre plates were treated with cinnamon EO to determine minimum biofilm inhibition (MBIC) and eradication (MBEC) concentrations. In addition, using the CDC Biofilm reactor model, mature PAO1 biofilms were challenged with different concentrations of the EO to determine antibiofilm activity. **Results:** Inhibition and killing of planktonic *P. aeruginosa* was achieved by cinnamon EO at concentrations of 0.125% (v/v). Results from the time-kill assay indicate inhibition can be achieved in as little as 2 minutes of contact time when treated with cinnamon EO at 2% (v/v), and electron micrographs indicate cell damage and loss of turgor. The MBIC and MBEC found using microtitre plate growth methods were 0.125% (v/v) and 2% (v/v), respectively. This MBEC was also reflected in CDC reactor-grown biofilms when challenged with EO after only 10 min of contact time, resulting in a reduction of biofilm bacteria by log7 colony-forming units per mL. **Conclusions:** Cinnamon EO is an effective antimicrobial against *P. aeruginosa* PAO1, capable of rapid killing at low concentrations. Its effects against biofilms when in liquid phase is very promising, and this study provides a sound basis for further investigation of the potential of cinnamon essential oil as an alternative to conventional antimicrobial agents.
Abstract: Compounds of natural origin still provide a high number of interesting structures, even in this era of combinatorial chemistry. Essential oils (EOs) represent a group of antimicrobial agents which are complex mixtures of volatile secondary metabolites. EOs show antimicrobial and antifungal properties and are also largely used in various cultures for medical and health purposes. Within the same variety, the EO composition can vary according to geographical region and seasonality. EOs from a variety of plants are also endowed with antibacterial activities as well as anti-inflammatory and antioxidant properties. In this work we investigate the antibacterial and anti-biofilm effect of EOs from three different Mediterranean plants against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. 

**Methods:** *S. aureus* 6538P and 25923, *S. epidermidis* RP62A and O-47, *P. aeruginosa* PaO1 strains were used. EOs were obtained from plants harvested in different seasons and conditions so as to obtain a total of 89 different samples. The chemical composition of each EO was determined by GC-MS. Determination of MIC for each EO on the bacterial strains was carried out. The action on biofilm formation was assessed by crystal. **Results:** Reported results demonstrate that EOs with different antimicrobial and anti-biofilm features were selected. Some of them inhibit bacterial growth at high concentration and possess good anti-biofilm activity at very low sub-MIC concentration. Other EOs were able to destabilize biofilm structure without killing cells. Furthermore, quantitative activity-composition relationships (QCAR) were developed through machine learning classification approaches with objective of discovery the chemical components mainly responsible for the anti-biofilm activity. 

**Conclusions:** Biofilm growth of *P. aeruginosa* and staphylococci is influenced by the presence of EOs extracted from plants harvested in different seasons. Application of an in-house python based machine learning protocol led to definition of a classification model able to discriminate EOs in active and inactive at a cut-off value of 50% of biofilm formation. Investigation of the most important components by means of feature importance and partial dependence plots seems to indicate estragole and phellandral as the chemical components mostly related to biofilm inhibition, while d-limonene, pulegone, and chrysanthenone seem to be related to biofilm production. The classification model is an example showing machine learning as tool to investigate complex chemical mixtures. Results obtained could enable the identification of blends of EOs with strong anti-biofilm efficacy applicable in many fields: airborne decontamination, products for dermatological and respiratory tract infections, etc.
Abstract:
Extracellular Polymeric Substances (EPS) producing marine actinobacterium has been isolated from a marine natural biofilm and identified as *Streptomyces* sp. CuOff24 through 16S rDNA sequencing. Approximately 450 mg L\(^{-1}\) EPS were produced and major content of carbohydrate followed by protein, nucleic acid and unidentified compounds (68.6%, 12.3%, 10.4% and 8.7% respectively) were quantified. The spectroscopy study also confirmed the presence of carbohydrate functional groups on the EPS surface and GCMS showed that the EPS comprised of mannose (45.2%), glucose (28.8%) and arabinose (26%). The EPS exhibited antibiofilm activity against a multi-drug resistance pathogenic strain of *Staphylococcus aureus* and *Klebsiella pneumoniae*. The high Carbohydrate compounds in marine actinobacterial EPS and their antibiofilm sensitivity would be make it suitable for prospective therapeutic and industrial applications.
Abstract: Synthetic Amphiphilic Arsenal to Combat MRSA Biofilm: Exploring Metal Complexation and Self-Assembling Attribute

**Background:** The dearth of therapeutic antibiotics to counter staphylococci biofilm underscores a critical demand for antibiofilm therapeutics. The present study reports the antibiofilm prospect of zinc complexing salicaldehyde- (C1) and napthaldehyde-based (C2) synthetic amphiphiles and illustrates the development of antibiotic-loaded micelles based on self-assembly of C1 that foster synergy of two warheads and inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm formation on surgical suture. **Methods:** C1, C2 and their zinc complexes were characterized by analytical techniques. Orthopaedic stainless-steel wire (SS wire) was coated with C1 and surgical silk suture was coated with rifampicin-loaded (C1m-R) and vancomycin-loaded (C1m-V) C1 micelles. Bactericidal activity against clinical *S. aureus* MRSA 100 was determined by MIC, cFDA-SE leakage assay and microscopy. Antibiofilm activity was ascertained by MTT assay, crystal violet assay and microscopy. Cytotoxicity on human embryonic kidney (HEK 293) cells was determined by MTT assay. All experiments were performed in triplicates. **Results:** The MIC of C1 and C2 against MRSA was 40 µM and 320 µM, which was also corroborated by the higher membrane-targeting activity of C1. The MBIC50 of C1 and C2 against MRSA was 40 μM and 160 μM, while a reduction in cFDA and congo red staining validated a dose-dependent biofilm inhibition. Amphiphile-zinc complex was visualized by microscopy and characterized by ESI-MS, FTIR, ITC and EDX. At doses equivalent to MBIC50 of C1 and C2, MRSA biofilm growth was nearly 80% and 110%, respectively, upon addition of 50 µM Cl2O8Zn, indicating the relevance of zinc-complexation of amphiphiles in biofilm inhibition. SS wire coated with 0.3% - 5.0% C1 decreased MRSA biofilm viability from 97% to 9% and was non-toxic to HEK 293 cells. MRSA biofilm grown on collagen in presence of 80 µM C1 exhibited only 15% metabolic activity and 25% biomass, highlighting the prospect of C1 as an antibiofilm agent for potential wound site application. Driven by self-assembly, C1m-R and C1m-V having a particle size of 182 nm and 381 nm were generated. The relative anti-MRSA activity of C1m-R was 12-fold and 18-fold higher and that of C1m-V was 8-fold and 5-fold higher following 3 h and 6 h interaction with the cells, in comparison to free antibiotic. FESEM analysis indicated prevention of MRSA biofilm formation on C1m-R coated silk surgical suture, while eluates from C1m-R and C1m-V coated sutures were non-toxic to HEK 293 cells (nearly 85% viability), indicating the biocompatibility of the coated sutures. **Conclusions:** In the light of limited therapeutic options, the prudently designed synthetic amphiphiles that deter biofilm formation by zinc complexation and also facilitate generation of antibiotic-replete micelles by self-assembly emerge as potentially therapeutic material with enhanced capabilities against MRSA biofilm.
**Abstract:** Antibiotic Loaded Bead Spacing is Important in Controlling *Pseudomonas aeruginosa* and *Staphylococcus aureus* Biofilms in Periprosthetic Infections: an *In vitro* Study

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

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**Background:** Antibiotic-loaded calcium sulfate beads (CS-B) are used in the management of periprosthetic joint infections (PJI). PJI is one of the most devastating and costly complications following total joint arthroplasty surgeries. In quiescent areas of the bone joint space, diffusion will be limited and will curtail the spread of antibiotics; therefore both the quantity and distribution of beads are important to ensure optimal antimicrobial concentration and coverage at these quiescent areas to reduce infection. Methods: Lawn biofilms of bioluminescent strains of *Pseudomonas aeruginosa* (PA) Xen41 and *Staphylococcus aureus* (SA) SAP231 were grown on Tryptic Soy Agar (TSA) and Brain Heart Infusion (BHI) agar respectively for 24 h. CS-B (10cc) were loaded with vancomycin (1000 mg) and tobramycin (240 mg). Based on the clinical relevance, beads with vancomycin + tobramycin and tobramycin alone were used against lawn biofilms of PA and vancomycin + tobramycin against SA. CS-B were placed in different configurations on a 9-cm petri dish: as a single bead in the center; as 16 beads clustered in the center; 4 groups of 4 clusters or 16 beads equally spaced arranged in a circle and beads distributed hexagonally considering the optimal distance of the bead that provides a zone of inhibition. Bioluminescence was used to measure metabolic activity over time using an *in vivo* imaging system (IVIS) and replica plating was used to assess the bacterial viability by plating onto a sterile TSA or BHI petri-dishes. Results: After 24h of placing the antibiotic beads on the lawn biofilms of PA and SA, the lawn was cleared and inhibition zones appeared around the beads. The cleared distance was similar for both single and clusters of beads. The cleared area continued to grow outwards to the edge of the petridishes with all cluster arrangements. After 3 days of CS-B placement, resistant colonies appeared within the cleared zone in the case of PA with tobramycin and tobramycin + vancomycin beads. No resistant colonies were observed on SA petridishes. There was no colony growth within 1 cm of the beads suggesting high concentration of antibiotic close to the beads. Replica plating of these petri dishes suggested that this area was sterile. The beads placed in a circle killed more of the biofilm present within the petri dishes than when concentrated in clusters. Beads placed hexagonally completely killed the lawn biofilms. Conclusions: This *in vitro* study suggests that i) antibiotic resistance may arise in *in vitro* lawn biofilms of PA after treatment with tobramycin or combinations of vancomycin + tobramycin and ii) the spacing of antibiotic-loaded CS-B may be an important consideration during the treatment of biofilm associated infections at the surgical site.
Abstract:
Engineered nanoparticles (NPs) for antimicrobial delivery to biofilms is an area of increased interest, particularly in order to increase the effectiveness of such antimicrobials. Numerous studies over the past decade have demonstrated the advantages and challenges of the approach\(^1\). However, the role of the biofilm EPS matrix in NP transport is still poorly understood. A major reason for this is the physical and chemical complexity of the biofilm matrix and its temporal and spatial variations. A wide range of physicochemical parameters influence the uptake and retention of nanoparticles within the biofilm matrix. These include nanoparticle size and charge properties, biofilm topography and EPS composition\(^2\). These aspects must be taken into account when studying biofilm - nanoparticle interactions. In order to gain a better understanding of these interactions, a series of experiments were undertaken using two genetically modified bacterial strains: mCherry-expressing *Pseudomonas fluorescens* and GFP-expressing *Pseudomonas putida*. The biofilms were cultured in both microtiter plates and on glass slides for 24 - 72 hours followed by exposure to fluorescently labelled silica NPs with different size and surface charge properties. Using high throughput fluorescent intensity measurements and confocal laser scanning microscopy, it was possible to investigate the uptake of silica NPs by the two bacterial strains and gain valuable understanding of biofilm - nanoparticle interactions. It was observed that positively charged silica NPs showed a significant increase in uptake compared to negatively charged particles. The increased uptake was most likely caused by the stronger binding of the positively charged amine groups on the surface of the NPs with negatively charged components within the EPS. The NPs also demonstrated different attachment distributions at the biofilm-liquid interface as observed through confocal laser scanning microscopy, where the positively charged NPs were seen to form larger aggregates at the biofilm-liquid interface whereas the negatively charged NPs demonstrated a more evenly distributed profile within the biofilm with reduced aggregation. The findings of this research will help with the future design of nanoparticles for antimicrobial delivery with specific modes of action towards components in the EPS. 1. Ramos M, Da Silva PB, Sposito L, De Toledo LG, Bonifacio BV, Rodero CF, Dos Santos KC, Chorilli M, Bauab TM. 2018. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. *International Journal of Nanomedicine* **13**:1179-1213. 2. Nevius BA, Chen YP, Ferry JL, Decho AW. 2012. Surface-functionalization effects on uptake of fluorescent polystyrene nanoparticles by model biofilms. *Ecotoxicology* **21**:2205-2213.
**Abstract:**

10-undecynoic Acid is a New Agent Killing Biofilm of *Streptococcus mutans*

10-undecynoic Acid is known as a key pathogen to cause oral caries preventing in plaque biofilms as well. Although, numerous synthetic and non-synthetic agents have been evaluated as potential treatments for oral pathologies, a few have shown the clinical applicability. Therefore, development of new effective strategies is still highly desirable. To address the need for novel agents operating against this widespread oral pathogen, we have focused our attention on 10-undecynoic acid as the representative of the acetylenic fatty acids. Using macro-broth susceptibility testing method we established MIC value. The MBC value was determined from broth dilution minimum inhibitory concentration test by sub-culturing it to BHI agar plates that do not contain the test agent. Anti-biofilm efficacy was tested in 96-well coated with saliva plates using BHI broth supplemented with 1% sucrose as a standard approach. Based on obtained results MIC value for 10-undecynoic acid was established to be 2.5 mg/ml and the MBC value to be 5.0 mg/ml. The minimal biofilm inhibitory concentration that prevents biofilm formation in 90% revealed to be the same as MBC value and also showed to kill mature biofilm, causing at the same time approximately 30% eradication of pre-existing biofilm. Thus, we concluded that 10-undecynoic acid might play an important role in the development of alternative or adjunctive anti-caries and anti-biofilm preventive and/or therapeutic approaches.
Biofilms and biofouling causes major problems in several water-intensive industrial sectors (incl. food and beverage). In addition, in hospital environments contamination and biofilm transferred infections are of major concern. Prevention of biofilm formation helps operation of industrial processes and diminishes maintenance costs and losses. Microbes are developing tolerance for currently used biocides. Hence, new alternative compounds and control methods are needed to replace biocidal products. We examined efficacy of Finnish plant extracts to prevent quorum sensing and biofilm formation of Chromobacterium violaceum, Pseudomonas aeruginosa and Escherichia coli strains. Adhesion of microbes and formation of biofouling was determined with crystal violet staining and microscopy. In addition, efficacy of plant extracts to weaken and destabilize outer membrane of Gram-negative bacteria was examined by a fluorescence assay (NPN uptake). Finnish berry extracts (incl. cloudberry extracts) were shown to inhibit quorum sensing and biofilm formation of Gram-negative bacteria. In addition, phenolic plant extracts destabilized outer membrane of P. aeruginosa and E. coli.
Background: Dental caries and periodontal disease are pathologies associated with plaque formation, as a complex microbial community organized in biofilm. This consortium of microorganisms adhered to biotic or abiotic surface covered by matrix polysaccharides, produced by themselves, confers several adaptive advantages, such as greater resistance to the action of antimicrobial agents. Chlorhexidine digluconate (CHX) is considered the gold standard in chemical control of oral biofilm, but several side effects do not indicate for long periods. Peptides with antimicrobial activity (AMP) are molecules that are part of the innate immunity of living beings and represent a new perspective of combat oral biofilms. This work aimed to investigate the effect of the AMP Hylin-a1 (Hy-a1), and synthetic analogue Lys-[Trp6]hy-a1 (Lys-a1), on antimicrobial and antibiofilm activity of chlorhexidine against Streptococcus mutans ATCC 25175.

Methods: Initially, antimicrobial activity (MIC and MBC) of Hy-a1 and Lys-a1 was determined by microdilution in broth against S. mutans. The synergistic activity between the AMP and CHX against the microorganism in question was evaluated by the checkerboard assay. Then, the antibiofilm activity of the Hy-a1/CHX and Lys-a1/CHX combinations was evaluated by treatment, 5 minutes of contact, against preformed S. mutans biofilm (12 h), in 96-well plates. The control was performed with CHX 0.12%. After the treatment, the metabolic activity and biomass from biofilm were analyzed using XTT reduction and crystal violet techniques, respectively. Results: The tested strain presented sensitivity for both peptides, as well as to control test (CHX). The concentrations of MIC were 0.23 μg.mL^{-1} for individual CHX and 15.60 μg.mL^{-1} for the two peptides, Hy-a1 and Lys-a1. The native peptide showed bactericidal effect (MBC) at the same of MIC, while the synthetic analogue presented MBC only at 31.25 μg.mL^{-1}, twice MIC. The bactericidal effect of individual CHX was verified only at 7.80 μg.mL^{-1}. Both AMP showed a synergistic effect with CHX. Hy-a1/CHX reduced the individual antimicrobial activity of CHX up to 30-fold, while Lys-a1/CHX reduced about 17-fold. In addition, the Hy-a1/CHX exhibited high antibiofilm activity, reducing metabolic activity in approximately 60%, and keeping unaltered the biomass of biofilm. Similar results were obtained with control group (CHX 0.12%). According Lys-a1/CHX treated groups, did not showed differ for metabolic activity and biomass from biofilm when compared to untreated group. Conclusion: In the end, the results suggest that the association between Hy-a1/CHX represents a major alternative to control of S. mutans ATCC 25175 biofilm formation than Lys-a1/CHX. This would provide a great reduction of the concentration of CHX used, which could avoid undesirable side effects from it. Keywords: Biofilm. Chlorhexidine. Antimicrobial peptides.
Title: Impact of UV Radiations on Biofilm Community of *Olea europaea*: An In vitro Approach

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Abstract: A variety of bacteria, yeasts, and fungi colonize the aerial parts of plants termed as phyllosphere. Study of phyllosphere has gained considerable attention focusing on new non-invasive methods and bio control agents of plant-associated microorganisms. Bacterial colonization as community called as biofilms has been reported to cause many beneficial as well as harmful effects. This study was designed to isolate microbes from phyllosphere of *Olea europaea* (olive plant species) and assess their biofilm forming ability. Further, correlation between biofilm formation and UV radiation was observed. In total, four strains L1, L2, L3 and L4 were screened and purified from phyllosphere of *O. europaea*. Biochemical characterization indicated that all strains were catalase positive with no H$_2$S production. 16srRNA sequencing identified these strains as *Erwinia herbicola*, *Micrococcus luteus*, *Pseudomonas syringae* and *Bacillus subtilis*. Physiological characterization revealed that strains exhibited optimum growth at 37°C temperature and pH 7. Biofilm formation revealed *B. subtilis* as the strongest biofilm former. Afterwards UV radiation at various time intervals i.e., 3, 4, and 15 minutes indicated that bacterial biofilm was resistant upto 10 minutes UV exposure. A significant decrease (p≤0.01) in biofilm formation of mutant strains compared to wild type was observed after 15 minutes UV exposure thus pointing towards significant reduction of the activity of the DNA repair gene. This study provided solid evidence that environments with extreme conditions including desiccation and/ ambient UV radiations could principally select for highly resistant microbial biofilms organisms to ionizing radiations.
Background: The resistance of clinical infections associated with biofilms is one of the fastest-growing issues in the medical field today. It is estimated that up to 80% of infections have some biofilm-related aspect to them. A biofilm can be defined as the multicellular phase that is not recognized in complex bacterial lifecycles. The biofilm is characterized by the bacteria’s production of a complex and remodelable extracellular matrix composed of polysaccharides, proteins, and DNA/RNA. It was previously believed that the high antibiotic resistance of biofilms was due to low penetration of antibiotics into the matrix; this, however, was demonstrated to be incorrect. The antibiotic recalcitrance of biofilms has since been shown to be an active metabolic process (Nguyen et al. 2011). The relA gene product plays a key role in this active antibiotic resistance. RelA and other RSH enzymes (RelA/SpoT homologs) are ATP:GTP (GDP) pyrophosphate transferases. When activated by amino acid starvation, RelA/RSH produces the stringent response, which activates “magic spot” alarmones. These “magic spot” alarmones are hyperphosphorylated guanosine compounds (guanosine (penta)tetrathosphate, collectively dubbed [(p)ppGpp]). The formation of these alarmones causes a major change in cellular metabolism, turning cells into a persister state. These persister cells upregulate some genes (such as chaperones, toxin/antitoxin systems, and oxidative stress protection) and downregulate other genes involved in cell wall synthesis, translation, and DNA replication. Essentially, all antibiotics function by inducing oxidative stress. A persister cell’s upregulation of oxidative stress defense enzymes allows persister cell to be resistant to over 1000x the concentration of antibiotics in comparison to their planktonic counterparts. Methods: Using the recently published cryo-EM structure of RelA, in silico docking experiments were performed with a library >10^6 compounds. These compounds have yielded many hits, which were then run through our pipeline assay system for validation of RelA inhibitors and their anti-biofilm properties as a standalone treatment and in synergy with antibiotics. This pipeline validation system includes in vitro and in vivo (p)ppGpp quantification, direct binding studies using surface plasmon resonance, and novel approaches to biofilm quantifications. Conclusions: These compounds have been successfully tested and validated for their ability to interrupt the stringent response and re-potentiate antibiotics for the treatment of infectious bacterial biofilms. A library of small molecule lead inhibitors has been discovered for optimization by means of medicinal chemistry. These compounds will now be used to perform structural activity relationships to better understand the RelA/RSH enzymes, and further, develop compounds for the treatment of biofilm infections.
**Abstract:**

**Background:** Consumers’ demand for nutrient-rich natural products has led to an increased consumption of fruit and vegetable-based beverages that are used as liquid meals. For sensory and microbial quality assurance, industrial production comprises a short thermal treatment, followed by a fast cooling stage that is maintained during the filling process right until consumption. However, re-contaminations of the product have been reported, leading to subsequent economic losses and potential risk for consumers. Bacterial biofilms have been indicated as responsible for persistent contaminations in the beverage industry, as bacterial cells are able to survive thermal treatment within biofilms. Deterioration of the surfaces on the processing machinery is a source of recurrent contamination. It facilitates biofilm development by exposing surfaces and creating sheltered microenvironments where cleaning process become ineffective, allowing bacterial cells to attach and grow, even under very poor conditions. Thus, it is mandatory to develop protocols that mimic industrial processes to assess bacterial strains’ abilities to survive and re-contaminate. Here, industrial cleaning conditions were simulated and, as a proof of concept, we propose a pipeline for standardizing the evaluation of bacterial survival in biofilm contamination in liquid food industry. **Methods:** Being a raising concern in the food industry, *Listeria monocytogenes* CECT 4032 was used as a model organism for its ability to form biofilms. Subject to standard processing conditions followed by thermal treatment, survival of *L. monocytogenes* CECT 4032 was evaluated under starvation conditions in diluted nutrient broth. Bacterial cells that grew under starvation were exposed to acidic and caustic cleaning to simulate an industrial cleaning process, after which the samples were grown again on diluted nutrient broth and growth curves were measured on a bioscreen C. After the incubation, selected wells were plated on PCA to calculate the bacterial population (CFU/mL). **Results:** Results show that, when in biofilm, survival curves of *L. monocytogenes* CECT 4032 exhibit tailing phenomena. After thermal treatment, the probability of survival of at least one cell was high. Heat treated cells of *L. monocytogenes* had the ability to survive at 1:100 optimum nutrient dilution. The cells that survived thermal treatment and went through harsh acidic and caustic treatments were able to grow (4.67 CFU/mL). Final starvation conditions stage highlighted that after the whole treatment, cells of *L. monocytogenes* were still able to survive (3.88 CFU/mL). **Conclusions:** Our results show that this evaluation pipeline is effective in predicting the ability of *L. monocytogenes* CECT 4032 to survive after undergoing the standard industrial cleaning process even with low nutrient concentration, and therefore, becoming a source of recurrent contamination.
Abstract:

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

Enterococcus faecalis is a gram-positive, gastrointestinal commensal and a leading cause of healthcare-associated infections. E. faecalis infections are difficult to treat because the organism forms biofilms and is resistant to many antimicrobial agents, including the antimicrobial enzyme lysozyme. Lysozyme is a muramidase that catalyzes the hydrolysis of the linkages between the N-acetylmuramic acid and N-acetylglucosamine subunits that comprise the backbone of peptidoglycan. E. faecalis lysozyme resistance is stimulated through a signal transduction cascade that involves activation of an alternative sigma factor via cleavage of the associated anti-sigma factor by the transmembrane metalloprotease Eep. Under planktonic conditions, strains lacking eep are more sensitive than wild-type strains to growth inhibition by lysozyme. Since bacteria in biofilms gain resistance to high concentrations of antimicrobials through biofilm-specific mechanisms, we investigated whether E. faecalis Δeep biofilms would remain differentially susceptible to lysozyme as compared to biofilms of the isogenic wild-type strain (E. faecalis OG1RF). Unexpectedly, we found that a three-hour treatment with chicken egg white lysozyme was associated with an increase in stained biomass of equal magnitude for both strains and concurrent decreased biofilm cell viability of 99.8% and 99.9% for OG1RF and Δeep, respectively. The bactericidal effect of lysozyme on E. faecalis biofilms of both strains was even more pronounced when recombinant purified human lysozyme was used. In contrast, three-hour treatment with the cell wall-targeting antibiotic ampicillin caused no changes in stainable biomass or cell viability of either strain. LIVE/DEAD florescence staining showed a higher percentage of dead cells in lysozyme-treated OG1RF and Δeep biofilms relative to biofilms treated with buffer alone. These data demonstrate that E. faecalis biofilms are susceptible to killing by lysozyme in a manner that is independent of Eep protease. In addition, these results suggest that E. faecalis biofilm cells lyse following treatment with lysozyme, and the increased biofilm staining observed following lysozyme treatment may be due to the release of DNA from the lysed cells. Consistent with this, ~3-fold more extracellular DNA was measured in association with lysozyme-treated biofilms than with biofilms treated with buffer alone. Finally, we found that lysozyme was effective in reducing the number of viable cells in biofilms of several other laboratory and clinical E. faecalis strains, including a vancomycin-resistant strain. In conclusion, lysozyme has the potential to be developed into a new therapeutic that can reduce the number of E. faecalis cells at the site of an infection where a biofilm has formed.
Abstract: Catalytic Mechanism of the Diadenylate Cyclase CdaA from *Staphylococcus aureus* and Identification of a Potential Allosteric Site

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**Background:** Microorganisms, specially bacteria, integrate signaling pathways mediated by distinct nucleotide-based second messengers, including (p)ppGpp, cAMP, cGMP, c-di-GMP, c-di-AMP and c-AMP-GMP. In this context, cyclic dimeric adenosine monophosphate, c-di-AMP, emerged as a key signaling molecule modulating several cellular processes, e.g. DNA integrity, sporulation and carbon metabolism. C-di-AMP synthesis and degradation are mediated by specific enzymes bearing diadenylate cyclase (DAC) or phosphodiesterase (GdpP, PgpH) domains, respectively. The first characterized DAC-enzyme, the DNA scanning protein DisA, revealed the basic mechanism of c-di-AMP synthesis. Within DisA octameric assembly, four face-to-face DAC-domain dimers are properly oriented to conduct metal-assisted cyclization of ATP bound to each protomer. When DisA N-terminal DNA-binding domain recognizes branched DNA, DAC-domain undergoes conformational changes, inhibiting c-di-AMP synthesis and cell division progression. Besides DisA, DAC-containing membrane-attached, CdaA, and sporulation specific, CdaS, cyclases are widely spread in Gram-positive bacteria. CdaS contains a regulatory N-terminal YojJ domain and forms hexameric rings mediated by YojJ-YojJ and DAC-DAC interfaces. Although the same conserved DAC-DAC interface shared by CdaA and CdaS homologs is found in DisA, this is distinct from the one mediating catalytic competent face-to-face DAC-domain dimers. CdaA activity regulates c-di-AMP levels, being the single diadenylate cyclase encoded by several pathogens, such as *S. aureus* and is observed in almost all microorganisms with c-di-AMP signaling pathways. Therefore, the importance of CdaA makes it an attractive molecular target for the development of new antibiotics. **Methods:** X-ray crystallography, biochemical and biophysical experiments performed with wild-type and site-directed mutants were integrated to propose a molecular mechanism of c-di-AMP synthesis. Using compound libraries, we applied high-throughput X-ray crystallography and luminescence-based screenings to identify CdaA inhibitors. Those were further characterized through biochemical assays. **Results:** CdaA DAC-domain structures showed a catalytic incompetent dimer, where the active site of each protomer faces opposite directions. Enzymatic and oligomerization experiments confirmed that the interface observed in the crystal structures is essential for the enzymatic activity. Moreover, the binary complex ATP+Mn2+ was found to concomitantly bind at the active site. The fragment screening assays revealed a hit molecule that binds specifically at the dimeric interface. **Conclusions:** Our results sheds light on the mechanisms underlying c-di-AMP synthesis and the dimeric interface was found to be a novel target site for the development of new antibiotics, especially against *S. aureus*. 
Biofilm Formation in *Burkholderia thailandensis*

**Title:** Biofilm Formation in *Burkholderia thailandensis*

**Author Block:**

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Bacteria form biofilms to promote their survival, adhesion and persistence in the environmental niches that they occupy. In the model organism *Burkholderia thailandensis* (a model for the virulent organism to *Burkholderia pseudomallei*), the bacteria can be induced to form biofilms and this production can be measured by various assays. We are seeking novel ways to interfere with biofilm production in this organism, including antimicrobial peptides, antimicrobial compounds and anti-microbial or anti-biofilm surface chemistries. Our long-term goal is to develop anti-biofilm approaches that will be effective against the biothreat agent *Burkholderia pseudomallei*. Here, we demonstrate that *B. thailandensis* can reliably form biofilm using one particular growth medium (MVBM) and that biofilm is not significantly produced using other growth media such as LB or Nutrient Broth, while bacterial replication demonstrates the opposite pattern. We demonstrate that this biofilm production can be quantitatively measured using crystal violet as well as other dyes for staining and quantitation. Anti-biofilm approaches such as antimicrobial peptides or antimicrobial compounds can inhibit biofilm production. We screened a large number of antimicrobial peptides and found that an antimicrobial peptide from our library of peptides has anti-biofilm effects against *B. thailandensis* in both the D- and L-enantiomeric conformation. In addition, we have screened a library of FDA-approved drugs and identified several compounds that have significant anti-biofilm activity against this organism. These compounds were found to inhibit biofilm production without significantly inhibiting bacterial growth. Methods for testing coupons/discs of materials with potential anti-biofilm or antimicrobial surfaces were developed to quantitatively assess their anti-biofilm potential, compared to untreated coupons/discs. We are also attaching antibiofilm peptides to surfaces to make the surface have enhanced antibiofilm properties. Overall, we are taking multiple approaches to combat biofilm formation in *B. thailandensis*, in order to potentially develop an anti-biofilm approach that could eventually work against *B. pseudomallei*. This project was supported by a grant from 4VA to Ducker and van Hoek.
**Session:** Poster Session 2  
**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 029  
**Topic:** Antibiofilm Strategies  
**Abstract:** NX-AS-911 Modulates Quorum Sensing to Attenuate *P. aeruginosa* and *S. aureus* Biofilms and Virulence  
**Author Block:** D. Neef, D. Williams, H. Taleb, M. Williams, J. Preece, A. Bernard, W. Nichols, G. Dixon; Neem Biotech, England, UNITED KINGDOM.  

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

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

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

**Conclusion:** NX-AS-911 inhibits quorum sensing in, and the formation and maintenance of stable biofilms by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. NX-AS-911 re-sensitizes bacteria in biofilms to antibacterial agents and reduces secretion of virulence factors.
Backgrounds: Bacteria are well known to form complex multicellular structures on solid surfaces, known as biofilms, to survive in harsh environments. A hallmark characteristic of mature biofilms is the high-level antibiotic tolerance (up to 1000 times) compared to planktonic cells. However, the antibiotic susceptibility of the sessile cells during early-stage biofilm formation is not fully understood, hindering the development of new technologies for effective biofilm control. This study was motivated to systematically investigate the role of cell growth, extracellular matrix production, and cell-cell interaction in antibiotic susceptibility of attached cells.

Method: To investigate the change in bacterial antibiotic susceptibility during early-stage biofilm formation, Escherichia coli cells were harvested every 30 min during the first 7 h biofilm formation in Lysogeny Broth (LB) and subjected to 1 h treatment with 200 µg/mL ampicillin or 5 µg/mL ofloxacin at 37°C. Antibiotic susceptibility of sessile or detached biofilm cells was quantified using colony forming units (CFU) and LIVE/DEAD™ BacLight bacterial viability staining, and compared with that of planktonic cells in static biofilm cultures. Patterned biofilms were used to quantitatively study the role of cell-cell interaction in bacterial antibiotic susceptibility during early-stage biofilm formation.

Results: We found that biofilm cells were not always more tolerant to antibiotics than planktonic cells in the same culture. During the first 2.5 h after inoculation, the antibiotic susceptibility of initially attached biofilm cells increased over time and was higher than that of planktonic cells due to active cell-surface and cell-cell interactions. After reaching the peak at 2.5 h, biofilm antibiotic susceptibility decreased when the attached cells started to produce biofilm matrix. However, the embedded biofilm cells remained active and thus more sensitive to antibiotics compared to planktonic cells if dispersed from the surface. By controlling the cell cluster size using patterned E. coli biofilm formation, cells involved in the interaction between cell clusters were found to be more susceptible to antibiotics than cells embedded in clusters.

Conclusions: Collectively, the results from this study indicate that biofilm formation involves active cellular activities in adaption to the attached life form and interaction between cell clusters to build the complex structure of biofilms, which render these cells more susceptible to antibiotics. These results demonstrate that biofilm cells are not always more tolerant to antibiotics than planktonic cells in the same culture, which may help design better materials and surfaces to reduce biofilm formation and eradicate colonized bacterial cells.
Abstract:

**Background:** Bacterial Biofilm production is the major cause for the relapse and recurrence of many bacterial infections including urinary tract infections. The biofilm producing bacteria are prone to multidrug resistance. The aim of this study is to isolate bacteria from patients suspected of urinary tract infections and correlate the biofilm production, multidrug resistance (MDR), and metallo beta lactamase (MBL) production. **Methods:** The urine samples were collected from different pathology laboratory in Pokhara, Nepal requested for the urine culture. The samples were processed by semi quantitative culture technique. Antibiotic susceptibility tests were performed by Modified Kirby-Bauer Disk Diffusion Method using Mueller Hinton Agar following Clinical and Laboratory Standards Institute Guidelines 2015. Biofilm production was detected by Congo Red Agar Method and production of metallo-beta lactamase was detected by Modified Hudge Test, Double Disk Diffusion Test, and Combined Disk Assay. WHONET-2018, SPSS-17.0 and GraphPad Prism-6 were used for antibiotic class identification and statistical analysis respectively. **Results:** A total of 507 urine samples were incorporated in the study, among which 89(17.55%) showed significant growth. Among significant growth, 69(77.53%) produced biofilm, 76(85.39%) were found to be MDR, and 10(11.24%) produced MBL. In the study, 44 (49.44%) was isolated as *E. coli* of which 40(90.90%) were MDR, 34(77.27%) produced biofilm and 4(9.09%) produced MBL. 10 (11.24%) were isolated as *Klebsiella pneumonia*, of which 10(100%) were MDR, 6(60%) produced biofilm and 3(30%) produced MBL. 7(7.86%) were isolated as *Klebsiella oxytoca*, of which 6(85.71%) were MDR as well as biofilm producers. *Pseudomonas aeruginosa* was found to be 10(11.23%) among which 5(50%) were MDR, 6(60%) produced biofilm and 3(30%) produced MBL. *Proteus species* were found to be 12(13.48%) among which 11(91.67%) were MDR and 9(75%) produced biofilm. Other minor species (*Staphylococcus aureus, Enterobacter cloacae, and Citrobacter diversus*) were found to be 6(6.74%) among which 4(66.67%) were MDR. **Conclusion:** The higher rate of antibiotic resistance was detected in biofilm producing uro-pathogens. Significant associations were not seen between biofilm producers and metallo beta lactamase producing bacteria. Imipenem, Meropenem, Gentamicin, and Amikacin were found to be a more susceptible antibiotic for enterobacteriaceae. Colistin was found to be effective among *Pseudomonas species* and some enterobacteriaceae producing metallo beta lactamase. The higher rate of biofilm and MBL producers warrants the detection of biofilm and MBL in the routine clinical laboratory to limit the spread of MDR bacteria.
Abstract:
The majority of microorganisms in nature, including those responsible for hospital-acquired infections and fouling of industrial processing equipment, live in association with surfaces as biofilms. Due to the secretion of proteins, extracellular DNA and lipopolysaccharides, biofilm communities are encased in a robust matrix which reduces their susceptibility to antimicrobial agents. The long term goal of my research project is to develop efficient, prodrug antimicrobial reagents that are able to permeate the biofilm matrix, as well as the cell membrane in order to eradicate biofilm colonization. This began with the literature review of simple phenolic essential oils that exhibit antimicrobial activity, followed by functionalization to increase potency. The minimum inhibitory concentration and biofilm eradication concentration for each essential oil and each derivative were then evaluated. Upon functionalization with allyl appendages, potency towards planktonic cells increased but decreased towards biofilms. Although it was found that trichloromethyl sulfonyl ester appendages did increase potency towards both planktonic cells and biofilm formations. A prodrug is a compound that is biologically inactive until metabolized. This design will serve two functions for these compounds; to increase permeability towards the biofilm matrix as well as the cell membrane and to achieve cellular retention of the compound. Ester appendages are being placed on select compounds to implement this prodrug design. Ester groups are commonly used in cellular dyes such as Calcein AM, where once inside the cell the ester groups are cleaved, and the resulting dye is trapped in the cell. Similarly after esterase cleavage, the antimicrobial compound will be negatively charged and become concentrated within the cell. A number of structurally diverse ester functional groups are being applied to selected compounds which have shown varying amounts of increased or decreased potency when compared to parent compounds. It is our hypothesize that the efficacy of antimicrobial agents towards biofilms will be efficiently restored through this strategic design and synthesis of derivatives with modulated polarity that are engineered to have high levels of cellular retention upon undergoing a cleavage event in the cell. This new class of prodrugs presents a wide array of potential applications, from the control of biologically induced corrosion to the incorporation into household cleaning products.
Abstract:
The food industry is severely impacted by biofilm communities. Biofilm development by foodborne pathogens on food contact surfaces and processing facilities jeopardizes a safe food supply. Mitigation of bacterial biofilms is challenging because bacterial biofilms are comprised of a diversity of cellular phenotypes, such as slow growing or transient filamentous states with a decreased susceptibility to sanitizers. The current study evaluated several dye-retention methods to characterize the dynamics of biofilm formation by foodborne pathogens. The same staining techniques were employed to quantify bacterial biofilm mitigation by commonly used industrial sanitizers, peroxyacetic acid (PAA) and quaternary ammonium compounds (QAC). Laser scanning confocal microscopy (LSCM) was used to characterize phenotypes and exopolymeric substance (EPS) presence. It was observed that crystal violet staining is not representative of the quantity of EPS in *Listeria monocytogenes* and *Salmonella enterica* and does not correlate with the population of cells embedded in the biofilm matrix. PAA was significantly (P≤0.05) more efficient in controlling *L. monocytogenes* biofilms regardless of the strain origin, clinical, food or environmental. In *Salmonella enterica*, transient mono- and multicellular phenotypes were observed within the biofilm matrix, indicating different outcomes upon release from the biofilm matrix. LSCM analysis revealed the formation of multilayer cellular conglomerates embedded into EPS and not attached to surfaces. This observation indicates the ability of foodborne pathogens to form unbound pellicle-type biofilms that are free-floating in the fluid. Furthermore, the ability of enteric pathogens to form untethered biofilms represents considerable contamination risk for food processing facilities.
Abstract:
The Cell Wall Binding Domains of PlyG and PlyL are Highly Specific for *Bacillus Anthracis*

Endolysins are enzymes used by bacteriophages to digest the peptidoglycan and release their newly packed phage particles to infect other cells. These enzymes have two domains; the Enzymatic active domain (EAD) that cleaves the peptidoglycan and the cell wall binding domain (CBD) that attaches to the receptors on the cell surface. In most cases, the CBDs bind to the receptors independently of their EAD. In this study, phage lysin G and L (PlyG and PlyL) CBDs have shown tight and specific binding to the bacterial surface of *Bacillus anthracis*, a Gram-positive bacteria which is the etiological cause for Anthrax. Because of their specificity and high activity towards bacteria, endolysins have a high potential as the next generation of antimicrobials.
**Poster Session 2**

**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm

**Poster Board #:** 035

**Topic:** Biofilm Antimicrobial Tolerance

**Abstract**
Nutriotional Composition, Biochemical Characteristics & Antimicrobial Analysis of Plant Seed and Nut Oils

**Title:** Nutritional Composition, Biochemical Characteristics & Antimicrobial Analysis of Plant Seed and Nut Oils

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

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**ABSTRACT**

Plant oils such as walnut oil, cashew oil and pumpkin oil with anti-microbial potentials are attractive agents that could help curb the menace of *S. aureus*. In this study we evaluated the activities of walnut oil, cashew oil and pumpkin oil on *S. aureus* ATCC 6538 (methicillin Susceptible) and MRSA 252 (methicillin resistant Strain (MRSA)). Minimum Biofilm Inhibitory Concentration (MBIC) of walnut oil, cashew oil and pumpkin oil were determined using microtiter plate assay. Checker board method was used to evaluate the synergistic effect of walnut oil, cashew oil and pumpkin oil with conventional antibiotics. This assay was used to study the effect of the oils on *S. aureus* biofilms. Minimum Biofilm Eradication Concentration was determined using the MBEC assay. The antibiotic susceptibility test was performed against *S. aureus* strains by the disc diffusion method. Walnut oil & pumpkin oil significantly inhibited biofilm growth of *S. aureus* ATCC 6538 and MRSA 252 in a concentration-dependent manner more effectively than cashew oil. Walnut oil inhibited the growth of *S. aureus* biofilm formation at a concentration of 3.125 mg /ml. Pumpkin oil inhibited the growth of *S. aureus* biofilm formation at a concentration of 6.25 mg /ml. Walnut oil & pumpkin oil at 3×MIC effectively eradicated the established biofilm of ATCC 6538 and MRSA 252. Antibiotic susceptibility test result showed that *S. aureus* ATCC 6538 and MRSA 252 are susceptible to pure oil samples of walnut & pumpkin as well most conventional antibiotics tested. A clear zone of 20.02 ± 0.05 and 25.02 ± 0.01 was obtained for pure walnut oil. Antibiotic susceptibility test result showed that *S. aureus* ATCC 6538 and MRSA 252 are susceptible to pure oil samples of walnut and pumpkin as well most conventional antibiotics tested. These oils could be used as alternatives to conventional antibiotics for the treatment of *S. aureus* infections.
Many bacteria live as populations on surfaces forming three-dimensional structure, called biofilms. Biofilms are bacterial populations covered by self-produced matrix of extracellular polymeric substances (EPS), and show different characteristics compared to the planktonic cells. Inside biofilms, microenvironments are formed that leads to the heterogenic gene expression and physiology of the cells. In addition, it has been reported that various spontaneous mutants can frequently be isolated from biofilms. Spontaneous mutations can be problematic by giving rise to multidrug-resistant bacteria in clinical settings. In this study, we focused on elucidating the emergence of spontaneous mutants from biofilms by using imaging analysis. In *Pseudomonas aeruginosa*, it has been reported that RecA is required for the spontaneous mutation in biofilms. However, the spatiotemporal pattern of this event is largely unknown. Here we used recA promoter reporter plasmid to detect the onset of DNA damage that would lead to mutation. *P. aeruginosa* PAO1 carrying the recA promoter reporter plasmid was observed in a flow cell system using a confocal laser microscope. Our results show that recA-expressing cells are mainly present in the bottom of biofilm. Since recA is induced by DNA damage, our data suggest that cells are more exposed to genotoxic stress at the bottom of the biofilm compared to the upper part. Oxygen gradient and accumulation of metabolite may cause these genotoxic stress. Future work will aim to elucidate this mechanism. We also show that that the recA-expressing cells can be sorted form the rest of the population by using a cell-sorter. This will allow us to further characterize the cells and elucidate the consequence of the heterogeneous gene expression.
A hallmark of biofilms is their heightened tolerance to antimicrobial agents. While the nature of this tolerance has been deemed multifactorial and linked to biofilm biomass accumulation, recent findings suggest a role of c-di-GMP. However, no c-di-GMP modulating enzyme(s) contributing to the drug tolerance phenotype of biofilms has been identified. Here, we made use of RNA-seq to identify genes encoding diguanylate cyclases that are expressed in a biofilm-specific manner. Transcript abundance was confirmed by qRT-PCR. Strains inactivated in diguanylate cyclases were analyzed for biofilm formation, antibiotic susceptibility, and cellular levels of c-di-GMP. Using RNA-seq combined with biofilm formation and drug susceptibility assays, we identified SicA as an active diguanylate cyclase, with sicA inactivation correlating with significantly reduced c-di-GMP levels present in P. aeruginosa biofilms, and biofilms being rendered biofilms susceptible to antimicrobial agents. SicA was found to contribute to biofilm tolerance in a manner dependent on SagS, BrlR, and a small stress-related protein. While SicA contributed to biofilm drug tolerance, sicA inactivation had no effect on attachment and biofilm formation. Our findings suggest biofilm drug tolerance to be linked to a specific c-di-GMP modulating enzyme, SicA, with the pool of SicA-generated c-di-GMP only contributing to biofilm drug tolerance but not to biofilm formation. Moreover, our findings suggest SicA to be SagS-dependent.
**Background:** Nontypeable *Haemophilus influenzae* (NTHi) is a predominant pathogen of otitis media (OM) globally, and the most common cause of chronic and recurrent OM. NTHi chronicity is due to the formation of highly recalcitrant bacterial biofilms. Upon entry into the middle ear, NTHi encounters a pH 7 climate; however, as the infection progresses, the conditions become pH 9. To adapt to changes in microenvironmental conditions, NTHi uses a novel genetic system, termed the phasevarion (phase variable regulon), to adapt to external factors. The NTHi phasevarion acts as a reversible, ON/OFF switch that regulates the expression of multiple genes. Previously, we demonstrated that the ModA2 phasevarion of NTHi strain 723 regulated biofilm formation based on microenvironmental conditions relevant to OM. Due to the known antibiotic resistant nature of biofilms, we hypothesized that the ModA2 phasevarion also influences antibiotic resistance.

**Methods:** Biofilms formed by NTHi strain 723 modA2 ON or OFF variants at 37°C/pH 7 or 37°C/pH 9 were treated with 1000 times the planktonic MIC of ampicillin, cefdinir, or amoxicillin/clavulanic acid, all of which are broad spectrum antibiotics commonly used to treat OM. We determined the relative antibiotic sensitivity of planktonic bacteria, bacteria resident within the biofilm and newly released bacteria disrupted by antibodies against a bacterial DNABII-binding protein, integration host factor (α-IHF), or dispersed by antibodies that target the NTHi type IV pilus (α-rsPilA). We examined biofilm architecture and relative percentage of live bacteria via viability stain.

**Results:** Antibiotic treatment of 24 hr biofilms formed by NTHi strain 723 revealed phasevarion-dependent differences in relative antibiotic sensitivity between modA2 ON and modA2 OFF variants. To assay the relative sensitivity of newly released NTHi, we determined the time point at which either α-IHF or α-rsPilA mediated disruption occurred at pH 7 or pH 9 for the ON and OFF variants. Ongoing work examines relative antibiotic sensitivity of ON and OFF variants post-biofilm disruption by either α-IHF or α-rsPilA.

**Conclusions:** Biofilm contributes to antibiotic resistance; however, the role of the phasevarion in this resistant phenotype has yet to be determined. Our data demonstrates the importance of the ModA2 phasevarion in relative antibiotic resistance of planktonic versus biofilm-resident bacteria in ON and OFF variants. We are currently assessing the relative antibiotic sensitivity of ON versus OFF variants that have been newly released from a biofilm by the action of α-IHF or α-rsPilA given that newly released NTHi reveal markedly increased sensitivity to antibiotics. The work presented herein is designed to give insight into the role of the phasevarion in adaptation to environmental changes induced by exposure to broad spectrum antibiotics. Support: NIH/NIDCD R01DC015688
Abstract: Surface-attached bacterial communities called biofilms display a diversity of morphologies. Biological components required for biofilm formation are known, however, not if or how these essential constituents promote biofilm morphological features. Here, we combine mechanical measurements, mechanical modeling, quantitative image analyses, surface energy characterizations, and mutagenesis, to show that mechanical instabilities, including wrinkling and delamination, underlie the morphogenesis program of growing biofilms using *Vibrio cholerae* as our model system. We discover that interfacial energy is the key driving force for mechanomorphogenesis because it dictates the generation/annihilation of new/existing interfaces. Finally, we find that feedback exists between mechanomorphogenesis and biofilm expansion, which promotes an ordered set of sequential steps that yield the morphological pattern and shape the overall biofilm contour.
Session: **Poster Session 2**
Date & Time: Tuesday, October 9, 2018, 4:00 pm - 6:00 pm
Poster Board #: 040
Topic: Biofilm Mechanics
Title: Large Amplitude Oscillatory Shear Reveals Distinct Rheological Fingerprints of Bacterial Biofilms
Author: S. Jana, S. Charlton, T. P. Curtis, J. Chen; Newcastle University, Newcastle Upon Tyne, UNITED KINGDOM.
Block: Newcastle University, Newcastle Upon Tyne, UNITED KINGDOM.

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

Understanding the material and mechanical properties of biofilms is crucial to developing future treatments for persistent chronic infections. Particle-tracking passive microrheology using individual bacteria as tracers was employed to investigate the viscoelasticity of *Staphylococcus aureus* biofilms grown in microfluidic cells that were subject to increasing hydrodynamic shears, specific biofilm-degrading enzymes and reduced-nutrient media. In this study, we have found that biofilm creep compliance (related to the inverse of viscosity) increases with height from the attachment surface and decreases universally in the presence of hydrodynamic shear. The addition of Proteinase K to the culture media substantially softened the biofilm, whereas DNase 1 showed no significant effects over the first 6 hours of growth. Spatial analysis of bacteria within the biofilm using Ripley’s K-function revealed biofilms under flow tend to spatial randomness with maturation at all heights, compared to static biofilms that remain statistically ‘clustered’. Reduced-nutrient media suggests a structural response by the biofilm to produce amyloid fibres, which have been imaged using super-resolution Stochastic Optical Reconstruction Microscopy (STORM).
Abstract: Mechanical and Adhesive Properties of Cross-linked Semi-flexible Polymer Network

Abstract: 

Semi-flexible polymer networks have been emerging as promising bio-inspired materials in the application of underwater adhesives, biosensors, structural materials, and scaffolds for tissue engineering. Although the mechanics of semi-flexible networks has been investigated, their underlying adhesion mechanisms are not well understood. Understanding the structure-property relationship of semi-flexible polymer networks is important to advance the design of biomaterials based on semi-flexible polymer networks. A coarse-grained model of cross-linked semi-flexible polymer networks is developed to investigate the network mechanical and adhesive properties and this model is characterized by tunable crosslink density, polymer Young’s modulus and bending rigidity (persistence length). Our simulations reveal that an increase in network stiffness caused by a larger polymer persistence length or a larger crosslink density leads to a decrease in the interfacial energy between the network and the surface. While the work of adhesion (energetic cost to detach a material from a surface) of stiff materials such as glassy polymers is almost equal to the interfacial energy, the work of adhesion of deformable cross-linked polymer networks has to overcome not only the interfacial energy but also considerable absorbed strain energy. Networks with larger polymer persistence lengths leads to desirable lighter mass density and larger pore size, but at the expense of interfacial energy and work of adhesion. There is an optimal polymer persistence length to reach maximum work of adhesion normalized by network mass density. While network mass density and interfacial energy are insensitive to the change in crosslink density, networks with fewer crosslinks exhibit a smaller elastic modulus, absorb more strain energy during desorption and possess larger work of adhesion. However, if the crosslink density is lower than a critical limit, networks cannot maintain structural integrity under uniaxial stretching, despite that they exhibit slight variation in storage modulus under dynamic mechanical analysis. Our finding provides physical insight into the adhesive mechanisms of biofilm and shed light on the design of biomaterials based on semi-flexible polymer networks.
Abstract: Biological technology used to treat flue gas is useful to replace conventional treatment, but there is sulfide inhibition. However, no sulfide toxicity effect was observed in haloalkaliphilic bioreactors. The performance of the ethanol-fed bioreactor was better than that of lactate-, glucose-, and formate-fed bioreactor, respectively. To support this result strongly, Illumina MiSeq paired-end sequencing of 16S rRNA gene was applied to investigate the bacterial communities in the membranes. A total of 389,971 effective sequences were obtained and all of them were assigned to 10,220 operational taxonomic units (OTUs) at a 97% similarity. Bacterial communities in the glucose-fed bioreactor showed the greatest richness and evenness. The highest relative abundance of sulfate-reducing bacteria (SRB) was found in the ethanol-fed bioreactor, which can explain why the performance of the ethanol-fed bioreactor was the best. Different types of SRB, sulfur-oxidizing bacteria, and sulfur-reducing bacteria were detected, indicating that sulfur may be cycled among these microorganisms. Because high-throughput 16S rRNA gene paired-end sequencing has improved resolution of bacterial community analysis, many rare microorganisms were detected, such as Halanaerobium, Halothiobacillus, Desulfonatronum, Syntrophobacter, and Fusibacter. 16S rRNA gene sequencing of these bacteria would provide more functional and phylogenetic information about the bacterial communities. Keywords: Haloalkaliphilic, Sulfate reduction, MiSeq, Bacterial community
Bacteria have a tendency to assemble in a matrix-encapsulated multicellular community called a biofilm. Biofilms form when planktonic (free-swimming) cells attach to a surface (living or inert) and secrete a polymeric substance known as the exopolymeric matrix. Biofilm bacteria are more resistant to antibiotics and the immune response of a host compared to planktonic (free-swimming) bacteria. Biofilms thus contribute to making pathogenic bacteria more persistent and threatening. Many studies have shown that biofilm development is affected by nitric oxide (NO), a diatomic gas molecule with well-understood signaling roles in mammalian cells. Data from our laboratory suggest that a novel NO sensing hemoprotein (NosP) initiates the biochemical signaling pathway that eventually leads to biofilm dispersal in many bacteria. While NosP exhibits similar biochemistry to other, better-studied hemoproteins, the structure of the protein, including details of the heme-binding site, is still unknown. We seek to gain knowledge of the structure of the protein using Raman spectroscopy and X-ray crystallography. This study will yield insight into the structural changes in NosP that occur during NO signaling.
**Session:** Poster Session 2  
**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 046  
**Topic:** Biofilm Metabolism  
**Abstract Title:** Light- and Temperature-controlled Behavioral Patterns in *Pseudomonas aeruginosa* biofilms  
**Author Block:** L. J. Kahl\(^1\), D. K. Morales\(^2\), L. Dietrich\(^3\); \(^1\)Columbia University, New York, NY, \(^2\)Weill Cornell Medical College, New York, NY.

**Background:** Most organisms are subject to Earth’s 24h day-night cycle. Rhythmic behaviors that anticipate daily changes in light and temperature are prevalent among both phototrophic and non-phototrophic organisms, but have not been described for non-phototrophic bacteria. We hypothesize that, during infection, pathogens could sense and respond to oscillations in light exposure or temperature in ways that facilitate survival. We are using a biofilm model of the pathogen *Pseudomonas aeruginosa* to probe this theory. The biofilm lifestyle contributes to persistence of *P. aeruginosa* infections. *P. aeruginosa* cells in biofilms employ various metabolic and structural mechanisms to maintain redox homeostasis in the face of limited oxygen availability. We are examining the physiological changes that occur in response to cyclic changes in light exposure and temperature, with a focus on redox metabolism as it relates to survival during biofilm growth. **Methods:** We used the dye triphenyl tetrazolium chloride (TTC) to examine the respiratory activity of growing colony biofilms when exposed to 24h cycles of light/dark switching and a +/- 1°C temperature variation. RNAseq analysis was performed on samples isolated from biofilm growth that occurred during the light or dark intervals of this cycling. A targeted genetic screen was conducted to identify genes required for TTC ring formation. **Results:** Colony biofilm growth on medium containing TTC led to the formation of concentric, alternating rings of TTC reduction and indicated that respiratory activity is higher during growth in the dark (corresponding to a slightly lower temperature) relative to growth in the light. The RNAseq analysis comparing dark (-1°C) and light (+1°C) biofilm growth revealed a higher than 2-fold change in expression for roughly 10% of the genome. Central pathways such as oxidative phosphorylation, purine metabolism and the pentose phosphate pathway showed strong transcriptomic changes, correlated with phases of “light” or “dark” growth. Intriguingly, different terminal oxidase complexes, which allow *P. aeruginosa* to carry out aerobic respiration under specific conditions including low oxygen tension, were differentially regulated in response to the light/dark and temperature cycling. The genetic screen revealed two putative sensors for different wavelengths of the visible spectrum that are important for reduced TTC ring formation under conditions of light/dark cycling. **Conclusions:** Since many aspects of host physiology, including immune activity and body temperature, are regulated by the host’s biological clock, we theorized that *P. aeruginosa* has evolved behaviors that anticipate rhythmic host activities and facilitate survival during infection. The phenotypic responses to cyclic light/dark switching and temperature variation described here could constitute adaptations to the host-associated lifestyle.
Many microbial infections in the body, including dental plaque, involve biofilms. Experiments focused on mimicking the removal of tooth associated biofilms have demonstrate distinct ripple patterns. We use this data to develop and validate theoretical models that describe the basic physics of the process. Additionally, insight gained from analyzing the physical process of rippling can lead to methods to enhance oral biofilm removal. We use a multiphase model that treats the biofilm as a mixture of two materials. Similar models have been used to study detachment, deformation, and structural development within a variety of biofilm systems. By direct numerical simulation we show the fully nonlinear model provides accurately reflects the relationship between the apparent wavelength and the external forcing velocities. We also show how the model can be used to optimize the design of tooth cleaning devices.
Abstract: Microbial biofilms are present on aircraft parts and can contribute to biodegradation of coatings and insulation. Lipases are industrially-relevant enzymes of which a subset contribute to polyurethane degradation of these aircraft coating materials. We devised a strategy to mine for microbial lipases in a cultivation-independent approach by leveraging structural domain homology using Hidden Markov Modeling (HMM). Microbial lipases cluster by sequence similarity and by the presence of catalytic residue motifs and structural lid domains; all of which contribute to function, specificity, and stability. While many lipases have been identified from cultured isolates, not all environmental isolates are easily grown in culture due to nutrient, symbiotic, or other as yet unknown growth requirements. We used lipase query sequences from experimentally verified polyurethane (Impranil®)-degrading Pseudomonas species to bioinformatically interrogate as a proof-of-concept publicly-available environmental metagenomes. From marine metagenomics libraries, we recovered lipase homologs which harbored the key catalytic residues. One of the recovered homologs was from a hydrothermal vent microorganism, suggesting that this particular lipase may be thermostable. The predicted structure for the putative hydrothermal lipase aligned well with the Pseudomonas lipase structure and lid domain, critical to lipase activity. Many of the other recovered lipases were derived from uncultivated bacterial lineages or were annotated as ‘hypothetical’, stressing the value of this agnostic mining approach. We constructed and tested a synthetic plasmid construct that successfully expresses lipases in a Pseudomonas heterologous host. Constructs are tested for polyurethane-degrading activity using biochemical assays developed in our laboratory. Ultimately, our goal is to apply this mining approach to mine aircraft-specific metagenomics and metatranscriptomics libraries. Developing an HMM domain-centric based pipeline offers an optimized approach for assessing aircraft biofilm biodegradation enzyme activity.
Abstract: Understanding biofilm interactions with synthetic polymers under nutrient limited conditions is relevant to many cases of microbiological fouling and degradation of painted surfaces. *P. laurentii* was isolated from a polyurethane painted surface and screening showed high degradation activity of polyester polyurethane. We identified a cutinase as a contributor of enzymatic degradation and determined that *P. laurentii* can metabolize polyesters as a sole carbon source. To further analyze the biological-materials interactions, *P. laurentii* biofibbiofilms were prepared by dropcasting cells onto polyurethane coatings and kept under >95% RH incubation without any additional nutrient sources. Samples were removed for analysis at time points up to 35 days. Infrared spectroscopy confirmed that preferential ester loss occurred in a polyester polyether polyurethane (Irogran) and no detectable polymer changes occurred for the biofilm on a polyether polyurethane. Brightfield microscopy showed slight changes in biofilm coverage on Irogran over time with both expanding and receding regions. The morphology and composition were further mapped down to the sub-cellular level using combined atomic force microscopy/infrared nanospectroscopy (AFM-IR). The deposited cells formed monolayer clusters showing both typical and novel cellular morphologies. Overall biomaterial content of cells was also comparatively reduced under the polymer degradative environment, suggesting that cells were physiologically impacted. Multiple polymer biodegradation processes were identified, consisting of diffusive and highly localized processes, with localized processes the most damaging to the polymer. Aggregation of degradation resistant Irogran components was also observed, a concern regarding release of micro and nanoplastics into the environment.
Model of Ciprofloxacin Killing Enhanced by Hyperbaric Oxygen Treatment in *Pseudomonas aeruginosa* Biofilms

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

Background: NUFEB is a flexible and open source 3D framework for simulating the dynamics of biofilms at the micro-scale. NUFEB is based on the Individual-based Modelling (IbM) approach, where microbes are represented as discrete units and their behaviour changes over time due to different processes. This approach allows us to study population behaviours (e.g., biofilm morphology, detachment) that emerge from the interactions between individuals and their environment. The IbM implemented in NUFEB is constructed by combining the ideas described in [1, 2] and extending them to include hydrodynamics and parallelization.

Methods: Microbes are implemented as rigid spheres. The computational domain is defined as a micro-scale 3D rectangular box with different compartments. Various sub-models are employed for describing Biology, Chemistry and Physics at different temporal scales. The biological sub-model includes microbe growth, decay and reproduction (EPS excretion and cell division). The chemical sub-model introduces pH dynamics and gas-liquid transfer to improve the accuracy of microbial growth. The physical sub-model models three key aspects and their interaction: nutrients, microbes and fluid. Nutrient distributions are governed by a convection-diffusion-reaction equation; Mechanical relaxation describes microbe motion and is carried out by the Discrete Element Method (DEM); An accurate modelling of hydrodynamics is implemented by using the Computational Fluid Dynamics-DEM approach.

Implementation: NUFEB is built on top of the classical molecular dynamics simulator LAMMPS, extended with IbM features. LAMMPS offers a wide range of inter-particle interactions that can be directly applied to biofilm simulation (e.g., contact, adhesive forces). Chemical and biological processes are implemented as a set of ‘fix’ commands which can be invoked at each time step to update field quantities and microbe attributes. Solving hydrodynamics is done by communicating with the external CFD solver OpenFOAM. NUFEB is fully parallelized and allowing for the simulation of large numbers of bacteria (1E8 bacteria and beyond). The parallelization is based on a domain decomposition scheme that divides the domain into multiple sub-domains which are distributed to different processors. NUFEB can also log various biofilm properties into different formats (vtk, hdf5, etc) for post-processing. The tool has been verified and validated with a number of case studies including biofilm BM1, BM2.

Abstract: *Escherichia coli* is a model prokaryote and a key organism for laboratory and industrial applications. *E. coli* C, a restrictionless strain, has been used for bacteriophage studies since the early 50’s. Its genetic map was constructed in 1970. Able to utilize the pentitol sugars ribitol and D-arabitol. *E. coli* C is the last of five strains designated as safe for laboratory research for which genome had not been sequenced. We found that *E. coli* C forms biofilm more efficiently than other laboratory strains. Genome comparison of *E. coli* C to these other strains will reveal differences in gene composition. Transcriptional activity comparison between planktonic and biofilm will reveal which genes play a role in biofilm formation. Methods. Biofilms on microscope slide and 96 well plate were grown and analyzed as described previously (Krol et al. 2013, Krol et al. 2014). DNA for sequencing was isolated using Qiagen Blood and Tissue DNA isolation Kit. Pacific Biosciences DNA sequencing was performed, and sequences were assembled by the HGAP Assembly 2 and annotated by Prokka. Comparison of *E. coli* C, K12, B, W and Crooks genomes was carried out by Roary, Mauve and Artemis. Total RNA were extracted from 4 day-old biofilm and planktonic cells by Trizol method. Ribosomal RNAs were depleted with Illumina RiboZero kit, library construction was completed utilizing Illumina’s TruSeq stranded RNA LT kit, without the poly-A selection. A single 1x75 sequencing was run on Illumina NextSeq Midi. Read alignments and gene feature mapping were performed with Hisat2, and Stringtie respectively. Differential expression analysis was performed with deseq2 in R. Results. *E. coli* C strain forms biofilm more efficiently than other laboratory *E. coli* strains. In minimal medium the difference is 1.5- to 3-fold, while in rich LB medium, biofilm formation is as much as 7.4-fold higher. *E. coli* C shows the highest genome similarity with the K12 strain. The chromosome is 4,617,024 bp and encodes 4,209 ORFs. Out of the 5686 genes, 3603 genes are shared by all 5 strains. Only 33 genes are present in all except *E. coli* C strains, but 177 genes are unique for C strain. Some of these genes i.e. encoding TolA, WzzB1 or fibrial-like adhesion protein might be involved in biofilm formation. Four day-old biofilm and an overnight planktonic cells were used to analyze gene expression profiles resulted in 51.5 million reads (25.7 biofilm and 25.8 control reads). Detailed results of that experiment will be presented and discussed. References. Krol et al., 2013. Plasmid 70; 110 Krol et al., 2014, J. Bacteriol. 196;129
Theoretical Understanding of Free Ammonia Inhibition on Nitrite Oxidizing Bacteria Suppression in Granular Sludge for Wastewater Treatment


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

Methods Tumour cells (N = 9), isolated from patients’ with BIA-ALCL; ALK T-cell breast lymphoma cell lines (TLBR)(n= 3); primary cutaneous (pc)-ALCL cells (n=2); MT-4 cells (immortal T-cell line); and IL-2 stimulated-peripheral blood mononuclear cells (PBMCs) from patients with capsular contracture (CC, n=3) and 3 healthy controls were seeded at a concentration of $10^5$, $10^{5.5}$ and $10^6$ cells per well, and stimulated with PHA, LPS and SEA at 5 or 10 μg/ml, over 72 hours. Mitogen-induced proliferation of lymphocytes was measured using a tetrazolium (MTT) colourimetric assay. Results BIA-ALCL tumour cells and TLBR cells respond significantly more to LPS than PHA ($p < 0.05$). In contrast, pc-ALCL cells and MT-4 cells had higher stimulation index values with the T-cell mitogen PHA ($p < 0.05$). Similarly, IL-2 stimulated-PBMCs from CC and control patients responded strongest to stimulation with PHA, with low proliferative responses to LPS ($p < 0.05$). The SEA response was similar to the PHA response in all cells except control patients who had lower responses. Conclusion These findings suggest a role for Gram-negative bacteria cell wall, of which LPS is a major component, providing further support to our hypothesis that a predominantly Gram-negative microbiome is the inflammatory trigger that leads to T-cell activation and sustained clonal proliferation.
Poster Session 2  
Tuesday, October 9, 2018, 4:00 pm - 6:00 pm  
Poster Board #: 057  
Topic: Biofilms and Infection  
Title: Oral Infection of S. Typhimurium Leads to In vivo Expression of Curli and Generation of Anti-dsDNA Autoantibodies  
A. Miller, N. Medeiros, S. Tursi, R. Wilson, A. White, C. Tukel;  
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Abstract: Amyloids are proteins with a cross-beta sheet structure that fold into a quaternary fibrillar structure. They are found within the organs and tissues throughout the human body and have been linked to the development of a variety of diseases. Like humans, bacteria also produce amyloids. It is estimated that 40% of bacterial species produce amyloids and these proteins are major structural components of biofilms. Members of the Enterobacteriaceae family including Salmonella enterica serovar Typhimurium and Escherichia coli produce a beta amyloid called curli. Curli, encoded by two operons known as csgBAC and csgDEFG. Curli production can be induced in vitro at temperatures lower than 30°C, suggesting that the expression of the fibers are limited to environmental conditions and not in vivo during infection at 37°C. Previous reports suggest that intraperitoneal injection of purified curli or S. Typhimurium, that were triggered to express curli by growing the bacteria at 28°C, lead to the generation of anti-dsDNA and anti-chromatin autoantibodies. However, it is not known whether curli is expressed during natural S. Typhimurium infection, and whether this expression would be enough to trigger autoantibody generation. We used Nramp+CBA and 129/SvJ mice that are resistant to S. Typhimurium infection to investigate curli expression in vivo. We found that curli is expressed in the cecum and colon of mice and this expression led to the generation of anti-dsDNA autoantibodies. In addition, we performed similar experiments in Nramp+ C57BL/6 mice that are susceptible to S. Typhimurium and found in vivo expression of curli throughout the gastrointestinal tract.
**Session:** Poster Session 2  
**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm  
**Poster Board #:** 058  
**Topic:** Biofilms and Infection  
**Abstract**  
**Title:** *Pseudomonas aeruginosa* Evolves wsp Mutations that are Positively Selected for in Response to Chronic Infections  
**Author:** E. S. Gloag¹, C. W. Marshall², D. Snyder³, S. B. Chaney¹, J. S. Harris¹, G. R. Lewin³, M. Whiteley³, V. S. Cooper², D. J. Wozniak¹;  
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**Background:** Chronic infections are persistent, despite extensive and drastic treatment strategies and are established due to the presence of microbial biofilms. The biofilms of *Pseudomonas aeruginosa* are often implicated in chronic infections, specifically in immune-compromised individuals. Complicating chronic infections is the ability of bacteria to adapt to an infection by evolving variants that are more fit and persist. Adapted variants typically display hyperbiofilm phenotypes and increased evasion of the host immune system and tolerance to antimicrobials. Despite our current understanding of the divergent phenotypes of adapted variants, studying their emergence in an infection is challenging. Here we analyzed *P. aeruginosa* evolution in a porcine full-thickness chronic burn wound model, which is a clinically relevant model of chronic infection.  

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

**Results:** Rugose small-colony variants (RSCVs) were isolated from punch biopsies at all timepoints, at a frequency of approximately 1% of the *P. aeruginosa* burden. Whole-genome sequencing revealed that RSCVs had driver mutations exclusively in the wsp pathway. The RSCVs had elevated levels of c-di-GMP and displayed increased biofilm formation compared to the ancestor strain. Furthermore representative RSCVs out-competed the ancestor strain under both planktonic and biofilm conditions. Some RSCVs also possessed secondary mutations. Of interest was a 14,299bp deletion in two PA14-derived RSCVs, which removed the remaining psl operon. Another two RSCVs contained a 60bp insertion at the CRISPR-Cas locus, which resulted in these RSCVs being resistant to phage infection from *P. aeruginosa* strains in the initial inoculum pool.  

**Conclusion:** Our data indicates that *P. aeruginosa* experiences strong selective pressures to evolve in chronic infections. Adapted variants were isolated at early stages of infection, which challenges the dogma that variants arise due to chronic adaptation. We only isolated RSCVs with driver mutations in the wsp pathway. This indicates that the Wsp system experiences significant pressure in the wound to evolve adapted variants, despite other pathways being implicated in RSCV formation. RSCVs are routinely isolated from other chronic infections, particularly the pulmonary infections of cystic fibrosis patients. We predict that RSCVs may be an adaptation common to chronic infections.
**Abstract:**

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

**Methods:** Three strains each of Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes were grown in monoculture within a modified drip flow biofilm system using a collagen based growth matrix and simulated wound fluid (n=3 per strain). SIFT-MS in full scan (FS) mode and selected ion mode (SIM; to target specific VCs identified through GC-MS) was used to analyse biofilm headspace gases. Multivariate statistical analysis was employed to determine if bacterial volatile product ion profiles (from the resulting FS mass spectra) and volatile compound concentrations (through use of SIM) could be utilised to discriminate between species.

**Results:** The novel wound biofilm model produced reproducible and steady-state biofilm monocultures (determined by cell viability). The biofilms were analysed using SIFT-MS in both FS and SIM modes and the resultant headspace gas data processed through Ward’s method of Hierarchical Cluster analysis and Principal Component Analysis. These were visualized by constructing dendrograms and scatter plots of principal component scores, with associated eigenvector plots. The resultant data sets show clear species specific differential clustering of microbial biofilms using both product ion (FS) and volatile compound (SIM) data.

**Conclusions:** This study has shown that steady-state biofilm monocultures of *S. aureus*, *P. aeruginosa* and *S. pyogenes* grown within a novel biofilm wound model, can be differentiated based on analysis of headspace gases alone. We have shown that through utilizing SIFT-MS both volatile product ions and specific volatile compounds can be used to discriminate between bacterial species based on the analysis of headspace gases of continuous culture biofilms. This work lays the scientific foundations for development of a non-invasive diagnostic tool, based on recognition of biofilm associated volatile compounds for rapidly identifying bacteria associated with wound infection in the clinic.
Background: Skin is the largest organ of the body and acts as a barrier against external insults. An important part of the barrier is the skin microbiota, which offers protection by outcompeting or modulating the virulence of pathogens. However, a skin injury can disturb microbiota homeostasis, opening the door for colonization by opportunistic pathogens. For immunocompromised patients with healing impairment, colonization can lead to life-threatening chronic wound infections involving antimicrobial resistant, structured communities called biofilms. Furthermore, chronic wound biofilms tend to be polymicrobial, which are difficult to eradicate. Given that increased tolerance to antibiotics is an inherent characteristic of biofilms, new strategies are needed to treat and prevent these types of infection. Understanding the interactions between skin colonising bacteria (commensal and pathogen) to identify targets (potential biomarkers) that promote a healthy microbiota and reduce the likelihood of life-threatening infections will enable treatment to be applied early and provide a model for testing topical application designed to promote healthy skin. The aim of this study was to generate a skin colonization model combining pathogens and commensal bacteria to study microbial-microbial and host-microbial interactions and to provide a platform to test different compounds of interest. Methods: Immortalised human keratinocytes (HaCat) were used as a support for the infection model. Cultures of Staphylococcus epidermidis and Micrococcus luteus (commensals) and Staphylococcus aureus and Pseudomonas aeruginosa (pathogens) were normalised at OD\(_{600nm}\)=0.01 and diluted accordingly. Total colonization time was set to 40h with \(P.\ aeruginosa\) being introduced after 20 h. HaCat cells were stained to observe monolayer’s structure. Confocal Z-stack biofilm images were analysed by COMSTAT and bio-volume, surface area and thickness were quantified. Results: A stable and reproducible polymicrobial colonization model has been generated. The presence of commensals protects the HaCat cells against the pathogens and significantly reduces bio-volume, surface area and thickness of the biofilm formed by \(S.\ aureus\) and \(P.\ aeruginosa\). Conclusions: Preliminary data obtained from our model suggests that the beneficial role of the commensals is during the early stages of infection. Further investigation of the mechanisms involved will enable us to explore new ways to boost the commensals to the detriment of pathogens.
Abstract: Thriving in an Unfriendly Environment: Bactericidal Bile Salt Produced by the Microbiota Induces Biofilm Formation in *Clostridium difficile* 

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

**Methods:** Bacteria were grown in presence of bile salts and biofilm formation was evaluated in 24-well plates and staining with crystal violet. The biofilm matrix was then characterized using fluorescent dyes or lectins, enzymatic digestion and gel electrophoresis. The transcriptome of the induced biofilm was then analyzed and used to identify targets for gene inactivation.

**Results:** We show that DCA induces biofilm formation in the presence of glucose and acidified growth medium. The DCA-induced biofilm matrix is composed of eDNA, protein and an unidentified exopolysaccharide; however, only eDNA is required to maintain biofilm stability. Furthermore, we demonstrate that a member of the intestinal microbiota, *Clostridium scindens*, enhances *C. difficile* biofilm formation by converting cholate into DCA. Our transcriptomic analysis indicates that long term exposure to DCA induces major changes in the metabolic pathways used by *C. difficile*. This was confirmed by reduced biofilm formation by strains lacking master regulators of metabolism (*spo0A, ccpA* or *codY*). Furthermore, our transcriptomic analysis combined with gene inactivation identified a novel lipoprotein required for biofilm formation.

**Conclusions:** Our data indicate that DCA exposure induces biofilm formation in *C. difficile* and is associated with a major metabolic reorganization. Based on these results and co-biofilm experiments, we hypothesize that the resident commensal microbiota can cause *C. difficile* to switch from a symptomatic infectious agent into a silent intruder, increasing the risk of relapse.
**Abstract:** Multi-species Biofilm Bacteria from Human Carotid Arteries Undergo Iron-induced Dispersion and Release Enzymes with the Potential to Degrade Vascular Tissue

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

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

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

**Conclusions:** Bacteria from multi-species biofilms identified in carotid arteries and grown *in vitro* have been shown to disperse and release increased amounts of lipases and proteases compared to untreated controls. Such an event *in vivo* has the potential to influence plaque stability and possibly contribute to plaque rupture.
Abstract: Complex polymicrobial communities are present in the respiratory tract of cystic fibrosis (CF) patients, but chronic *Pseudomonas aeruginosa* (*Pa*) infections account for the majority of mortality. Chronic rhinosinusitis (CRS) is highly prevalent in CF; recent studies suggest the paranasal (PN) sinuses are a site of primary colonization and dissemination of *Pa* into the lungs. Despite a robust host immune response and aggressive antimicrobial treatment, *Pa* adapts via mutations that promote chronic respiratory infections. Respiratory viral infection leads to pulmonary exacerbation and has been linked with the development of *Pa*-dominant chronic infections. Our lab described how viral co-infection promotes colonization of airway epithelial cells (AECs) by *Pa*, through increased iron released from AECs that enhances *Pa* biofilms. However, the impact of viral infections on *Pa* pathoadaptation, and on the respiratory microbiome overall, is unknown. Our goal was to identify causal relationships between viral infection, *Pa* pathoadaptive evolution, sinus microbiota changes, and clinical disease severity. In a 2-year longitudinal study of 33 CF adults, we collected sinus swabs for bacterial culture, virus and metals analysis, and 16S microbiome sequencing at quarterly clinic visits and during exacerbations. We genotyped 140 *Pa* isolates and screened for in vitro phenotypes associated with chronic *Pa* infection. We used Tetrad, a program that searches for causal explanations represented by directed graphs, to construct Bayesian networks with directed edges between variables (nodes) in a combined patient clinical, microbiome, and *Pa* in vitro phenotyping dataset. We searched using both constraint-based (PC, FCI) and search-and-score (FGES) algorithms, then evaluated the networks based on their BIC score, bootstrapping, and biological plausibility. These searches reproducibly identified a causal relationship between respiratory viral infection and levels of iron present in patient sinus samples, in agreement with our previous in vitro and murine infection models. Validating the network with previously published findings, we identified directed edges between swimming motility and polysaccharide overproduction, as well as a loss of swimming motility and increased patient hospitalization. Using the model to generate hypotheses regarding drivers of CF disease progression, we are currently examining directed edges between in vitro phenotypes associated with *Pa* pathoadaptation, such as overproduction of the quinolone HHQ (“lysis and sheen”), and microbiome diversity indices by competing *Pa* with other sinus bacteria in a model of biofilm formation on polarized AECs (CFBE41o-). The long-term goal is to use machine learning to identify biomarkers and novel therapeutic targets for preventing chronic bacterial infections and CF respiratory disease progression.
**Background:** *P. aeruginosa* is an opportunistic pathogen causing biofilm associated infection in immunocompromised patients. There are many internal factors involved in bacterial biofilm formation. The objective of this study was to investigate the natural variation in the antibiotic sensitivity, virulence and biofilm formation among the urinary catheter associated *P. aeruginosa* isolates, we also evaluated the role of host DNA on biofilm expansion by clinical isolates of *P. aeruginosa*. **Methodology:** *P. aeruginosa* isolates collected from urinary tract infections were identified by 16S rRNA gene sequencing. Sensitivity to antibiotics and healthy human serum were measured using standard procedures. Crystal violet staining and confocal laser scanning microscopy and scanning electron microscopy were used for biofilm studies. Ability to induce cell lysis was studied using Human Embryonic Kidney cells (HEK 293T). Association between virulence factors, biofilm and antibiotic resistance among the strains was analysed statistically. The presence of pyocyanin and H$_2$O$_2$ in the culture free supernatant was quantified by colorimetric method. The eDNA was quantified in the culture free supernatant by DNA quantifying fluorescent dye assay. **Results:** We observed 98 cases of CAUTI in 1,266 patients (Male, n=1008, Female, n=258, age: 48.4 ± 21.4), among them 18.3% (n=18) was *P. aeruginosa* infection. 94.4% (17/18) isolates were resistant to antibiotics including carbapenem (73.7%). All the isolates formed biofilm on all the materials tested. Intensity of biofilm on silicone latex material was significantly higher than polystyrene. The strains were highly virulent and could induce significant cytotoxicity to HEK cells (>60% cell killing) and showed very low sensitivity to healthy human serum. Biofilm formation was not associated with antibiotic sensitivity in some isolates. Variation among the factors contributing to biofilm was also observed. The host-DNA showed significant increase in the biofilm intensity by *P. aeruginosa* isolates. **Conclusion:** Present study showed the natural variation among the catheter associated *P. aeruginosa* isolates compared to the standard strains or of their mutants routinely used in the studies. Such diversity and antibiotic resistance among the isolates warrant newer strategies to target this ubiquitous opportunistic pathogen. Present study showed that the host-DNA can increase the biofilm rigidity and expansion during infection. The isolates showed high cytotoxicity on epithelial cells and was resistant to human serum which leads to the need of early detection and treatment for *P. aeruginosa* associated infections.
Abstract:

Background: Pseudomonas aeruginosa is responsible for much of the morbidity and mortality associated with cystic fibrosis (CF); therefore, studying the pathogenesis and immune response to P. aeruginosa is essential. The difficulty of modeling the infections in animals, however, hampers the study of chronic P. aeruginosa infections. In this project, we develop a model of chronic P. aeruginosa infections in ENaC-overexpressing mice to test the hypothesis that these infections produce Th2-biased responses that fail to clear the bacteria. Methods: To study how P. aeruginosa causes chronic infections in CF lungs, we developed a model of CF-associated lung infections in B-ENaC BALB/c mice. B-ENaC mice overexpress ENaC channels in their lungs, causing them to display CF-like mucus plugging, neutrophil infiltration, and airway inflammation. To characterize the immune response to infection, we intranasally infect 6-8 week-old mice with 3x10^6 colony forming units (CFUs) and collect lungs at 3, 7, 10 and 14 days after infection for histologic and cytokine analysis. We section and stain lungs by H&E, and measure the cytokines IL-1B, IL-4, IL-17 and IFNγ, in lung homogenates using flow cytometry. Statistical tests include Fisher’s exact test and log rank tests. Wild type littermates serve as controls. Results: We found that both B-ENaC mice and their wild type littermates clear even large inoculums of planktonic P. aeruginosa within one week of infection. However, if we infect mice with small biofilm aggregates, B-ENaC mice are unable to resolve the infections effectively compared to controls, and most carry at least 10^4 CFUs per lung one week after infection. Bacterial clearance in B-ENaC mice is delayed up to 14 days after infection. At least 90% of control mice survive infection, and 75% of their B-ENaC littermates survive (p = 0.17 by log-rank test). Approximately 90% of surviving B-ENaC mice carry at least 10^3 CFUs per gram of lung one week after infection, whereas only 10% of controls remain infected (p = 0.001, Fisher’s exact test). On examination of H&E stained lung sections, infection with P. aeruginosa is characterized by increased inflammation, mucus production, and inflammatory infiltrate in the airways of B-ENaC mice compared to their wild type littermates. Uninfected B-ENaC mice demonstrate airway narrowing or plugging by mucus, while their uninfected wild type littermates exhibit no pathology. In our preliminary analysis of the cytokines produced in lungs during infection, B-ENaC mice show greater increases in IL-1B, IL-4 and IL-17, and decreases in IFNγ, relative to their wild type littermates. Conclusions: Our results show that B-ENaC mice are more susceptible to infection with biofilm aggregates than planktonic bacteria, and these mice show a delayed clearance of P. aeruginosa. The preliminary cytokine tests suggest that infection might cause a shift from Th1 to Th2/Th17-biased responses.
Abstract: Bacterial contamination during meat processing is a major cause of food borne disease in Nigeria. Bacterial contamination of beef processing surfaces in Nigeria is usually an unavoidable aftermath in the processing of meat for human consumption, especially where there is poor standard of hygiene and sanitation practices. Biofilm formation has been a major source of concern in the food processing and production facilities and industries. It leads to persistent contamination of food, food borne disease and offset of food spoilage. Also, microbial antibiotic resistance has been major health implications both to humans and animals. Biofilms aid bacterial tolerance to antibiotics thereby leading to chronic infections. Microbiological analyses of swabs taken from meat processing table (before and after processing) was carried out and the antibiotic susceptibility of representative isolates determined using Kirby-Bauer disc diffusion assay and results interpreted using CLSI standard, 2016. Biofilm formation was determined using the crystal violet binding assay. Haemolysin, Gelatinase and DNase test were also conducted using standard procedures to determine the virulence properties. The data obtained were subjected to analysis of variance (ANOVA) and p < 0.05 was considered to be statistically significant. Total heterotrophic counts before meat processing ranged from 17.5×10^5 cfu/g to 5.95×10^5 cfu/g while the values after processing ranged 19.3×10^5 cfu/g to 8.85×10^5 cfu/g. A total of sixty-four bacteria isolates was obtained and fifty-two were observed to produce biofilms. The 52 isolates were selected and further identified by biochemical and physiological characterization. The selected bacteria isolates were in the following proportion: *E. coli* (28.9%), *S. aureus* (21.2%), *Pseudomonas* spp. (7.7%), *Bacillus* spp. (15.4%), *K. pneumoniae* (13.5%) and *Proteus* spp. (13.5%). The Gram negative isolates displayed 90.9% resistance to Ampicillin, 28.1% to Cefotaxime, 25% to Ceftaxidime, 28.1% to Cefpodoxime, 38.5% to Sulphomethoxazole, 15.2% to Ciprofloxacin, 96.9% to Tetracycline and 54.5% to Chloramphenicol. The Gram positive isolates showed 57.9% resistance to Ampicillin, 31.6% to Chloramphenicol and Ceftaxidime, 26.3% to Cefotaxime, 36.8% to Cefpodoxime, 84.2% to Vancomycin and 15.8 to Sulphomethoxazole. All Gram positive were susceptible to Ciprofloxacin. All *S. aureus* showed resistance to Vancomycin and Oxacillin. 11 out of 15 *E. coli* showed DNase activity, 7 *K. pneumoniae* isolates produced gelatinase and haemolysin. 50% of the *Bacillus* spp. Showed hemolysis activity while 8 out of 11 *S. aureus* strains showed hemolysis activity. The meat processing surfaces in this study were contaminated with potentially pathogenic and antibiotic resistant bacteria that may constitute public health risk. This study reiterates the need for safe and hygienic practises during meat processing.
**Poster Session 2**

**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm

**Poster Board #:** 067

**Topic:** Biofilms and Infection

**Abstract**: Evaluation of the Interplay Between the Membrane Metalloprotease Eep and the Cellular Host Innate Immune Response in *Enterococcus faecalis* Biofilms

**Title:** Host Innate Immune Response in *Enterococcus faecalis* Biofilms

**Author** S. D. Schaffer, C. N. Rouchon, K. L. Frank;

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

*Enterococcus faecalis* is an opportunistic, healthcare-associated pathogen that causes a range of infections. Biofilm formation is a major factor that contributes to the severity of *E. faecalis* infections. Our lab has shown that Eep, a transmembrane metalloprotease, is associated with biofilm formation in *E. faecalis*. Specifically, transcription of the *eep* promoter was increased in *in vitro*-grown biofilms and in two rabbit models of biofilm-associated infection (infective endocarditis and foreign body abscesses). We also showed that a Δ*ee* strain has an altered cell distribution phenotype during biofilm development. In addition to its role in biofilm formation, Eep processes substrates in planktonic cells that promote resistance to lysozyme and uptake by phagocytic cells, thereby indirectly modulating the host innate immune system. Based on this, we hypothesize that Eep may promote *E. faecalis* resistance to the cellular response of the host innate immune system in biofilm-mode cells. Our approach to test this hypothesis will employ *in vitro* and *in vivo* methods. Our *in vitro* assays will measure the effect of Eep on survival of planktonic- and biofilm-mode cells within macrophages and after exposure to hydrogen peroxide. Our current results indicate that planktonic-mode isogenic wild-type (OG1RF) and Δ*ee* strains survive at identical levels in macrophages over the course of a 96-hour infection, thereby emphasizing the importance of testing both growth forms. Our *in vivo* assays will use flow cytometry to evaluate how the host innate immune response to OG1RF and Δ*ee* changes over time in a rabbit foreign-body abscess biofilm infection model. We are currently optimizing a flow cytometry panel of antibodies to measure macrophage, monocyte, dendritic cell, and granulocyte immune cell populations in abscess samples collected from the biofilm infection model. Together, these methods will provide new insight on the role of Eep in enterococcal host-pathogen interactions during biofilm infection. This may aid in the discovery of improved methods to treat enterococcal infections, particularly those infections complicated by biofilms.
Attachment Behavior as a Predator Avoidance Mechanism in Bacteria - *Pseudomonas aeruginosa* is Not a Polite Victim

N. Shteindel¹, Y. Gerchman²; ¹University of Haifa, Haifa, ISRAEL, ²University of Haifa department of biology at the Oranim college of education, Tivon, ISRAEL.

Microbial predation scenarios develop over timescales of seconds and minutes, a time frame which makes gene expression and protein synthesis impractical. Nonetheless, many effective predator avoidance strategies rely on these mechanisms. A first line solution should be employed to allow gene expression and protein synthesis, bridging the gap between the appearance of a predator and the successful expression of predator avoidance phenotypes. Behavioral strategies based on motility or attachment, which rely on pre-expressed molecular mechanisms, are good candidate solutions for this problem. We observed *Pseudomonas aeruginosa* attachment behavior in the presence of two predators - *Paramecium multimicronucleatum* and *Acanthamoeba castellanii*, in various predator population densities, using a recently developed method which allow real time kinetic measurement of bacterial attachment in a high throughput setting in seconds timescale. *Pseudomonas aeruginosa* goes towards it's predators displaying elevated attachment to in the presence of amoeba and reduced attachment to the presence of paramecium, in correlation to the predator population density in both cases. This may be a form of mobbing behavior, attacking and killing potential predators. These results show a dynamic and interesting aspect of bacterial existence, offering another perspective on the function of attachment, and suggesting that predation avoidance causing short term attachment behavior can be a possible precursor to biofilm development, explaining the prevalence of the biofilm phenomenon.
Abstract:

Composition and Properties of Holdfast Adhesin in the marine bacterium *Hirschia baltica*. The dimorphic α-proteobacterium *Hirschia baltica* is a marine budding bacterium in the Caulobacterales clade. *H. baltica* uses a polar adhesin, the holdfast, located at the cell pole opposite the reproductive stalk for surface attachment and cell-cell adhesion. Holdfast adhesins have been best characterized in *Caulobacter crescentus*, a freshwater member of the Caulobacterales, and little is known about holdfast composition and properties in marine Caulobacterales. Environmental factors, such as ionic strength, pH, temperature, and shear forces affect bacterial adhesion, and therefore marine bacteria must have developed holdfasts with different composition and structures than their freshwater counterparts. Here we use *H. baltica* as a model to characterize holdfast properties in marine Caulobacterales. We show that freshwater and marine Caulobacterales use similar genes in holdfast biogenesis, that these genes are highly conserved among these strains, but that some of their properties are different. We show that *H. baltica* produces larger holdfast than *C. crescentus* and that those holdfasts contain N-acetylglucosamine and galactose monosaccharide residues and proteins, but lack DNA. We also show that *H. baltica* holdfast tolerates higher ionic strength than *C. crescentus* holdfast. We conclude that marine Caulobacterales holdfasts have binding and physicochemical properties that maximize binding at high ionic environments.
Title: Honey, I shrunk the Culture Plate! 3D Interferometric Non-Destructive Imaging Pinpoints Transition from Planktonic Culture to Biofilm

Abstract: The interference of light has been foundational to scientific research since Isaac Newton first studied ring patterns in optical glasses in 1717. On the other hand, the agar culture plate has played a crucial role in bacteriology since the origins of the discipline. In this work, we developed a process to use white-light interferometric (WLI) imaging for enhanced non-destructive characterization of bacterial colonies and biofilms growing on miniaturized agar culture plates. WLI is a 3D surface imaging technique that has not been used extensively in microbiology. It has extremely high vertical resolution (3-5 nm) which is far below the resolution of diffraction-limited imaging techniques even when capturing large fields of view (up to 5 mm). We used WLI to track volumetric changes in hundreds of unlabeled *Pseudomonas fluorescens* and *Bacillus thuringiensis* colonies spread across 5 mm culture disks with volumetric resolution of ~50 zeptoliters (5x10^{-20} l). In the early hours (approximately 1-6 hrs) after plating the culture, it was possible to observe individual cells multiplying into colonies. Tracking colonies as they grew from single cells into biofilms revealed, with high temporal resolution, the emergence of EPS through characteristic changes in surface roughness. We also developed a technique to measure, in parallel, the independent growth rates of several hundred plated colonies and a method to count colony forming units in a culture within 1-2 hrs of plating. The results indicate that WLI is capable of accurate enumeration of CFU counts from agar plates while also enabling accurate quantification of other morphological and phenotypic attributes, such as transition into a biofilm state. The highly sensitive 3D and volume information was also used to identify sub-lethal effects of antibiotics on colonies and biofilms. Finally, we showed that morphological characteristics of groups of bacteria and the way they spread can be used to differentiate some species when co-cultured. Based on these results, bioimaging with WLI was demonstrated as a powerful tool for non-destructive study of the structures that evolve during early stage biofilm development.
Abstract: Characterization of FleN Interaction with the Flagellum Assembly Machinery in *Pseudomonas aeruginosa*

**Background:** *Pseudomonas aeruginosa* is an important human opportunist pathogen, with wide resistance against several known antibiotics. The high *P. aeruginosa* adaptability and its dynamism in switching between planktonic and biofilm lifestyles further lead to intermittent chronic infections. The bacterial enhancer binding protein (bEBP) FleQ is the main transcriptional regulator controlling both the expression of flagellar proteins and the *pel* operon. FleQ controls its target operons in completely different manners but involving the signaling molecule c-di-GMP and FleN. At the flagellar operons, FleQ-mediated gene activation undergoes the classical mechanism of bEBPs, and its activity regulation occurs via interaction with FleN; a MinD-like ATPase encoded by the FleQ-regulated operon *flhFfleN*. FleN acts as an antiactivator of FleQ, generating a feedback regulation in flagella development. Although the molecular mechanisms underlying FleQ activity modulation by FleN are unknown, such regulation is crucial for flagella number control. However, it is not sufficient to fully understand *P. aeruginosa* phenotype of a single, polar flagellum. In *Campylobacter jejuni*, a FleN homolog takes part of the basal flagellum assembly machinery, triggering its polar localization. Although *P. aeruginosa* FleN lacks an N-terminal segment important for interaction and activation of FlhF, it could interact with other components of flagellar machinery. Therefore, this work aimed to expand the knowledge of FleN in the regulation of FleQ activity and involvement in flagellum basal assembly machinery. Thus, we investigated and characterized FleN-FleQ interaction and searched for potential FleN interaction partners within flagellum assembly machinery. **Methods:** The flagellum assembly factors FlhF, FliM, and FliN; FleQ and FleN from *P. aeruginosa* PA14 were employed for the *in vitro* interaction experiments. Accordingly, heterologously expressed proteins were produced in *E. coli* host system. The interactions were evaluated via chemical crosslinking with EGS and further explored with microscale thermophoresis experiments. ATP and GTP were included in the reactions according to the cofactor necessities of each protein. **Results:** Beyond the known interaction with FleQ, FleN and FliM interaction were observed in the *in vitro* experiments. FlhF or FliN did not to interact FleN, unlike the reported for other single flagellum species. Moreover, FleN interaction with FleQ and FliM occurs independent of the presence of ATP, although the cofactor addition altered the interaction affinities. **Conclusions:** Our results indicate that FleN is not only triggering feedback regulation of the flagella assemble but is also engaged itself in the assembly machinery in *P. aeruginosa*. However, further experiments are needed to understand FleN involvement into the assembling of the single flagellum in *P. aeruginosa*. 
Despite the medical, environmental and industrial relevance of biofilms formed by anaerobic bacteria, biofilms have been mostly studied in aerobic conditions using aerobic model bacteria. *Bacteroides thetaiotaomicron*, a Gram-negative, strict anaerobe, is one of the dominant commensal members of human intestinal microbiota. Whereas *B. thetaiotaomicron* adhesion and biofilm formation capacity could be important for colonization and maintenance in the intestine, the molecular basis of biofilm formation in this commensal anaerobe are poorly understood. Moreover, the widely used and genetically amenable reference strain *B. thetaiotaomicron* VPI 5482 only displays poor biofilm forming capacity. Using random transposon mutagenesis and multiple rounds of positive selection for *B. thetaiotaomicron* mutants developing biofilm in continuous-flow biofilm microfermenters, we identified mutations in the operon BT1338-56 leading to increased biofilm formation. This operon corresponds to capsule 4 locus (*cps4*), one of the 8 capsule operons described for this bacterium, suggesting that, when expressed, capsule 4 could mask a putative *B. thetaiotaomicron* adhesion factor. A second round of random transposon mutagenesis in Δ*cps4* mutant screening for loss of biofilm capacity led us to identify a capsule regulator and a putative lipopolysaccharide biosynthesis locus as candidates potentially involved in adhesion in a Δ*cps4* background. This study could contribute to a better understanding of biofilm formation dynamics in *B. thetaiotaomicron*. 
**Abstract:**

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

**Methods:** All test materials were selected of components among water purifier or tubing. Samples were prepared in two shapes of plate and tubing. Thirteen materials were used in this test; NORYL, PP, PET, PTFE, Tritan, LDPE, Stainless steel(SUS), Silicone, TA, TES, PL, PN and TYGON®. The sizes were 100 x 15 mm (Surface area = 1,500 mm²) for plates and Ф4.15 x 100 mm (Surface area = 1,300 mm²) for tubing shape of samples. The experiment was conducted for 5 to 30 days and flow rate was 3 ml/min.

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

**Conclusion:** Through this study, the biofilm formation rates on the surface of various materials were compared. However, it has not yet been determined which material properties affect the biofilm formation of the surface. Thus, further studies are needed to identify factors affecting biofilm formation on the surface and to reduce biofilm in water system.
**Abstract:**

**Background:** With rapidly development of Canadian Oil Sands industry, the third-largest proven crude oil reserve in the world, concerns about adverse impacts of oil sands process-affected water (OSPW) on aquatic resources are magnified. Environmental management of OSPW becomes a pressing challenge for regulators, engineers and researchers. **Methods:** We engineered a novel biological process that is composed of a moving bed biofilm reactor (MBBR), an ozonation unit, a membrane aerated biofilm reactor (MABR) for treating OSPW. In this study, several microsensors were utilized to measure related chemical profiles to illustrate the function of the biofilm inside each reactor. Illumina high-throughput sequencing technology was adopted to explore the bacterial communities in suspended biomass and biofilms of the bioreactors as well as in raw OSPW. **Results:** In MBBR biofilm, DO was gradually consumed and depleted at 550 μm below the biofilm/bulk liquid interface where nitrate vanished accordingly. Hydrogen sulphide was only detected inside MABR biofilm where the ORP was lower than -320 mV (Vs. Ag/AgCl). The diversity in biofilms is more complex than that in suspended biomass and in raw OSPW based on analysis of the 3,608,551 16S rRNA gene sequences obtained. MABR biofilm was revealed possessing the most complex microbial community. **Conclusions:** Due to the nutrients condition, toxicity level and operation conditions, the microbial community in raw OSPW was hugely different from that inside the bioreactors. Nitrification process existed in both biofilms while sulphate reducing process only existed in MABR biofilm. Both bioreactors could select and enrich specific microorganisms belonging to classes such as Pseudomonas, Falvobacterium and Rhodobacter, which showed great resistance to the harsh environment and the capability of degrading carboxylic group of the main carbon chain. Bioaugmentation happening inside MBBR and MABR made the biodegradation of recalcitrant organic chemicals in OSPW faster and the acceleration of end-pit lake reclamation promising.
Methane is converted to methanol by methane monooxygenase of methanotrophs. In this study, a methanotrophic bacterial strain was isolated from a rice paddy soil that has an ability of producing extensive biofilm, characterized by whole genome sequence analysis & named as Methylomonas koyamae LM6. Using this isolate as a whole-cell biocatalyst, optimal bioconversion conditions were investigated: temperature, pH, reaction time, CH₄/air mixing ratio, & reaction mixture volume to headspace ratio were found to be 30°C, 7.0, 24 h, 60/40, & 5:1, respectively. Due to low methane solubility in the reaction mixture, a new strategy was introduced to produce a high methanol concentration by using an organic solvent, 1-octanol. Under optimal condition, methane/air mixture was supplied to 500 mL Erlenmeyer flask, which contained 100 mL nitrate mineral salt (NMS) medium and 2 mL 1-octanol, & the flask was agitated at 180 rpm for 8 h at 30°C. With 1-octanol, more than 1.5 times higher methane was solubilized in the NMS medium. After removal of 1-octanol, 10 mL of methane solubilized NMS medium in tightly sealed 70 mL glass serum vial was treated with not only 0.6 mg dry cells/mL & 80 mM Na-formate (reducing power source), but also 60 mM potassium phosphate, 60 mM MgCl₂, 90 mM NH₄Cl and 3 mM EDTA as methanol dehydrogenase inhibitors, & then incubated at 30°C for 24 h without additional methane. Through this reaction, 53 mM methanol was produced, & this concentration was the highest concentration reported so far. These results demonstrated that 1-octanol acts well as a methane solubilizer without cell damage to convert methane to methanol.
Abstract:

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

**Introduction:** Gingival epithelial cells serve as a physical barrier and also fulfill an active role in innate host defense by secreting different soluble mediators like cytokines. The epithelium is in constant contact with bacteria. It represents the first line of defense against developing biofilms. Host immune responses to biofilms play a key role in periodontal disease pathogenesis. Periodontal diseases occur from a dysregulation between the bacterial biofilm and the immune response. The challenge to oral bacterial biofilms can trigger the release of cytokines and lead up to chronic inflammatory responses. It is known that different bacteria differentially stimulate the epithelial cells and also alter the biofilm composition. In our study we use a co-culture model of mono- and multispecies biofilms in combination with epithelial cells. We aim to investigate the interactions between commensal and pathogenic oral bacteria to the immune response. Additionally, we will examine how the different combinations of bacteria differently influence a multispecies biofilm in composition and depth. **Methods:** Biofilms developed with *Streptococcus sanguinis* (Ss), *Corynebacterium durum* (Cd) and *Porphyromonas gingivalis* (Pg) were cultured in vitro under anaerobic conditions on Thermanox™ coverslips. Oral epithelial cells (OKF4) were co-cultured in vitro with bacterial biofilms for 6h under aerobic and anaerobic conditions. Changes in epithelial cell viability was measured by Calcein AM stain. Biofilms depth was determined via crystal violet stain assay. Culture supernatants were collected for analyses of cytokine content. Gene expression of epithelial cells and the composition of the biofilm was examined via q-PCR. **Results:** The first results of our project revealed, that, although both are commensal bacteria, *S. sanguinis* causes a different immune response of epithelial cells compared to Cd. We also observed differences in biofilm depth after co-culturing of Ss and Cd compared to the respective single species growth. To further improve our setup we will introduce the pathogen Pg. Furthermore, first progress was achieved in establishment of a q-PCR based setup to quantify the species composition of the tested multispecies biofilms. **Conclusion:** Biofilms trigger the immune response of the host which can lead to periodontal diseases. The extent of the host response depends on the composition of the oral polymicrobial biofilm. Due to the high variety of oral bacterial species in such biofilms, it is extremely difficult to study the different interactions and the complex effects to the host immune response. Thus, we selected only a few commensals and one pathogen to develop our biofilm model. Due to its simplicity, we hope to address the big question of how commensal and pathogen bacteria influence the composition of a biofilm and further how they influence the host immune response more easily.
The Interaction between *Pseudomonas aeruginosa* Biofilms and Human Neutrophils

Colonisation and chronic lung infection by the opportunistic pathogen *Pseudomonas aeruginosa* (PA) is the leading cause of morbidity and mortality in cystic fibrosis patients (CF). A critical key determinant of PA pathogenicity is the switch from planktonic to biofilm mode of growth which facilitates chronic infections and makes PA eradication extremely difficult even with antibiotic therapy and robust immune attack. Neutrophils are essential for protection against PA but neutrophil-dominated inflammatory responses can also compromise organ function through tissue damage. The overarching aim of this project is to investigate the interplay between PA and neutrophils and how it is affected by biofilm formation. We characterized the interaction of purified human neutrophils with PA fixed biofilms with regard to morphology, cell-association, phagocytosis, and NETs formation. Preliminary live and confocal microscopy data indicated that when exposed to PA 18hr fixed biofilms, neutrophils increased in size and could clear PA biofilm. Furthermore, biofilm formation impaired neutrophil movement. Future work will involve analysis of how biofilm composition influences neutrophil behavior. Investigation of the interplay between PA and innate immune cells and how it is affected by biofilm formation will give us an insight into novel approaches to combat PA infection.
Abstract: Dietary Fatty Acids Modulate *C. albicans* Biofilms on Biotic and Abiotic Surfaces

**Abstract:**

*Candida albicans* is a major species of the fungal component of the human microbiome; commonly colonizing oral, gastrointestinal and genital niches of the human body. *C. albicans* also forms biofilms on medical materials found in devices such as catheters and dentures. These niches occupied by *C. albicans* can result in a pathogenic interaction with an immunocompromised host. The human diet can modulate immune response, inflammation levels and the human microbiome. The average American diet is high in omega-6 fatty acids but low in omega-3 fatty acids. Here we demonstrate that an omega-3 fatty acid, docosahexaenoic acid (DHA), inhibits switching from yeast to the pathogenic-associated hyphal morphology. Treatment of *C. albicans* with DHA mitigates biofilm formation on abiotic surfaces. Furthermore DHA also prevents binding of *C. albicans* to human oral epithelial cell monolayers. Our results suggest that DHA interferes with *C. albicans* morphogenesis, adhesion to biotic and abiotic surfaces. These findings serve as a basis for future studies to determine whether DHA decreases pathogenic interactions between *C. albicans* and its host.
Abstract:

**Background:** Bacteria can form biofilms, i.e., multicellular communities which are attached to a surface and surrounded by a self-produced extracellular matrix. When grown on LB agar without salt, *Escherichia coli* develops a matrix consisting of proteinaceous curli fimbriae and the exopolysaccharide cellulose (1). Biofilm formation is regulated by the second messenger c-di-GMP. C-di-GMP is synthesized by diguanylate cyclases (DGCs) with GGDEF-domains and degraded by phosphodiesterases (PDEs) via their EAL-domain (2). High c-di-GMP levels promote a sessile lifestyle, while low levels force flagella-dependent motility, important for acute infections. In contrast, biofilms might aid in development and persistence of chronic infections, causing e.g., catheter-associated urinary tract infections (UTI). Thus, it is of pressing interest to study biofilm formation at human body temperature.

**Methods:** Comparative analyses of biofilm formation at 37°C were executed for the probiotic commensal *E. coli* Nissle 1917 (EcN) and uropathogenic *E. coli* (UPEC) derived from an extensive in-house collection to shed light on the underlying regulatory mechanisms and the implications of biofilms for probiotic or virulence traits. Biofilm tests in liquid culture and on agar plate were performed along with motility assays and whole genome sequencing.

**Results and Conclusions:** In many *E. coli*, the transcriptional regulator CsgD is critical for biofilm formation in directly regulating curli synthesis and indirectly cellulose synthesis through the DGC AdrA. However, cellulose expression in EcN is CsgD-independent and again unlike in other *E. coli*, the source of the c-di-GMP needed for allosteric activation of the cellulose synthase BcsA is unknown (3). By comparative analysis of the genome sequences and phenotypic characterization of EcN and spontaneous non-biofilm forming laboratory EcN variants, the DGC YegE was identified as likely c-di-GMP provider for cellulose synthesis. UPEC clinical isolates express a diversity of biofilm phenotypes, reflected by variety regarding presence and conservation of c-di-GMP turnover proteins, possibly allowing each strain to individually fine-tune their biofilm capabilities for improved adaptation to the host environment.

**References:**

Abstract:

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

**Background:** Limited published information regarding the basic aspects of biofilm formation in agriculturally important microorganisms is available, despite the awareness and significance of bacterial-fungal biofilms in clinical and industrial settings. An investigation was undertaken to analyse the interactions between a diazotrophic bacterium (*Azotobacter chroococcum* - Az) and a filamentous fungus (*Trichoderma viride* - Tv), with emphasis on the structural and temporal changes during biofilm formation, nature of EPS, and gene expression.

**Methods:** Aggregation assay was done through centrifugation, and the growth and biofilm formation was monitored through Bioscreen C lab system and Crystal Violet assay respectively. The composition of EPS isolated through ethanol precipitation, was determined both photocolorimetrically and using ATR-FTIR spectroscopy. Mesocosm experiments (with wheat, cotton and chickpea) were conducted at National Phytotron Facility. Soil nutrients, plant defense enzymes and soil biological parameters were quantified following standard methods. Whole transcriptome sequencing was done through Illumina NextSeq500 and the transcriptome was validated through qRT-PCR.

**Results:** Manipulation of growth media led to significant increase in growth, aggregation and biofilm formation. Biofilm EPS showed significantly higher concentrations of proteins, acetyl groups and uronic acids, while planktonic EPS recorded higher amount of carbohydrates. Supplementation of different concentrations of L-amino acids revealed that 40 mmol l⁻¹ was the most effective in enhancing growth and biofilm formation. L-Glu and L-Gln favoured planktonic growth, while L-Trp and L-Thr, enhanced aggregation and biofilm formation. Whole transcriptome sequencing, revealed differential gene expression profiles in the individual (Az, Tv) and biofilm (Tv-Az). Gene expression analyses (Az or Tv vs. Tv-Az biofilm) revealed the up- and down-regulation of a large number of genes, particularly those related to *T. viride*. Pot studies using Tv-Az biofilm revealed significant enhancement in colonisation ability, plant growth, soil parameters and activity of plant defense enzymes as compared to individual inoculations (Az, Tv) in all three crops.

**Conclusions:** Our study illustrates the potential of Tv-Az biofilm as a promising biofertilizing option in agriculture, for enhancing rhizosphere colonisation of applied bioinoculants leading to improved plant growth and availability of soil nutrients.
Abstract:
Quorum Sensing (QS) is a bacterial cell to cell communication mechanism, which is responsible for regulation of gene expression that mediates the production of virulence factors in a cell density-dependent manner. Inactivation of quorum sensing signal molecules of pathogenic bacteria has been proposed as a novel biotherapeutic method to fight against bacterial diseases. In this study, potential quorum quenching property of virulence factor production of shrimp pathogen *V. harveyi* by Bacillus spp. was investigated. 118 isolates of Bacillus spp. from aquaculture ponds and mangrove soil samples were screened for their ability to degrade synthetic AHLs; C4-HSL, C6-HSL, C8-HSL, C10-HSL and C12-HSL. Based on their potential to degrade all the five synthetic AHLs tested, seventeen Bacillus isolates were selected for further study. The AHL degradation potential was highest in *B. subtilis* MFB10, *B. lentus* MFB2 and *B. firmus* MFB7 and among these *B. subtilis* MFB10 exhibited maximum activity (78%). The supernatant of the three isolates of Bacillus spp. tested suppressed the production of various virulence factors of *V. harveyi* VH201 such as protease, lipase, phospholipase, caseinase and gelatinase and also the isolates led to a reduction of 75%, 73%, 70% and 65% in the biofilm formation of *V. harveyi* VH201. Also, the reduction in the expression of virulence genes such as metalloprotease, serine protease and haemolysin was confirmed by PCR analysis. Thus the present study suggests the potential of AHLs degrading Bacillus spp. as an alternative to antibiotics in shrimp hatcheries for controlling the luminescent Vibriosis.
**Abstract:**

Streptococcus pneumoniae (pneumococcus) is an opportunistic pathogen that causes otitis media, sinusitis, pneumonia, meningitis and sepsis. The progression to this pathogenic lifestyle is preceded by asymptomatic colonization of the nasopharynx. This colonization is associated with biofilm formation; the competence pathway influences the structure and stability of biofilms. However, the molecules that link the competence pathway to biofilm formation are unknown. Here, we describe a new competence-induced gene, called *briC*, and demonstrate that its product promotes biofilm development and stimulates colonization in a murine model. We show that expression of *briC* is induced by the master regulator of competence, ComE. Whereas *briC* does not substantially influence early biofilm development on abiotic surfaces, it significantly impacts later stages of biofilm development. Specifically, *briC* expression leads to increases in biofilm biomass and thickness at 72h.

Consistent with the role of biofilms in colonization, *briC* promotes nasopharyngeal colonization in the murine model. The function of BriC appears to be conserved across pneumococci, as comparative genomics reveal that *briC* is widespread across isolates. Surprisingly, many isolates, including strains from clinically important PMEN1 and PMEN14 lineages, which are widely associated with colonization, encode a long *briC* promoter. This long form captures an instance of genomic plasticity and functions as a competence-independent expression enhancer that may serve as a precocious point of entry into this otherwise competence-regulated pathway. Moreover, overexpression of *briC* by the long promoter fully rescues the *comE*-deletion induced biofilm defect *in vitro*, and partially *in vivo*. These findings indicate that BriC may bypass the influence of competence in biofilm development and that such a pathway may be active in a subset of pneumococcal lineages. In conclusion, BriC is a part of the complex molecular network that connects signaling of the competence pathway to biofilm development and colonization.
**Abstract:** Filamentous phage impact biofilm development, stress tolerance, virulence, biofilm dispersal, and colony variants. Previously, we identified 137 *Pseudomonas aeruginosa* PA14 mutants with more than 3-fold enhanced and 88 mutants with more than 10-fold reduced biofilm formation by screening 5850 transposon mutants (*PLoS Pathogens* 5: e1000483, 2009). Here, we characterized the function of one of these 225 mutations, *dppA1* (PA14_58350), in regard to biofilm formation. DppA1 is a substrate-binding protein involved in peptide utilization via the DppBCDF ABC transporter system. We show that compared to the wild-type strain, inactivating *dppA1* led to 68-fold less biofilm formation in a static model and abolished biofilm formation in flow cells. Moreover, the *dppA1* mutant had a delay in swarming and produced 20-fold less small-colony variants, and both biofilm formation and swarming were complemented by producing DppA1. A whole-transcriptome analysis showed that only 10 bacteriophage Pf5 genes were significantly induced in the biofilm cells of the *dppA1* mutant compared to the wild-type strain, and inactivation of *dppA1* resulted in a 600-fold increase in Pf5 excision and a million-fold increase in phage production. As expected, inactivating Pf5 genes PA0720 and PA0723 increased biofilm formation substantially. Inactivation of DppA1 also reduced growth (due to cell lysis). Hence, DppA1 increases biofilm formation by repressing Pf5 prophage (*Frontiers Microbiol.* 9:30, 2018).
Abstract: Agrobacterium tumefaciens is a plant pathogen that attaches to diverse surfaces via a single cellular pole using a polysaccharide adhesin, the unipolar polysaccharide (UPP). UPP production is strictly surface-contact dependent, but this can be bypassed by artificially elevating cytoplasmic levels of cyclic diguanylate monophosphate (cdGMP). One of the primary regulators of UPP production is the dual function diguanylate cyclase (DGC)-phosphodiesterase (PDE) protein we have designated DcpA, which has two transmembrane segments and a large periplasmic domain, in addition to its cytoplasmic catalytic domains. Null mutations in dcpA result in elevated cdGMP, loss of surface-contact dependence, increased attachment, and aggregation due to overproduction of UPP and cellulose. Under laboratory conditions DcpA is maintained in a PDE-biased state, governing low UPP production under non-inducing conditions. In contrast, DcpA exhibits strong DGC activity in null mutants of pruR, encoded immediately upstream of dcpA, and pruA, unlinked to the pruR-dcpA operon. PruA is a pteridine reductase that reduces the small co-factor 1,2-dihydromonapterin to 1,2,3,4-tetrahydromonapterin. PruR is homologous to molybdopterin-binding proteins in the SUOX family, but lacks the conserved cysteine that conjugates molybdenum. Null mutations in pruA or pruR result in strong elevation of DcpA DGC activity, and ectopic expression of pruR complements the pruR mutation, but does not rescue the pruA mutant. Chromatographic and mass spectrometric analyses reveal that a hexahistidinyl-tagged version of PruR expressed in A. tumefaciens, co-purifies with a monapterin. PruR is a periplasmic protein and is targeted to the periplasm, via an N-terminal signal sequence. Whole-cell crosslinking experiments reveal that PruR and DcpA form a complex. Thus, a tentative model is that periplasmic PruR binds to a monapterin co-factor (synthesized via PruA activity), and through direct interaction with the DcpA periplasmic domain, pterin-associated PruR regulates the balance between PDE and DGC activity. PruR homologues are found in diverse proteobacteria and all share the N-terminal signal sequence, the absence of the Cys residues and the presence of several additional uniformly conserved residues. Strikingly, DcpA-type proteins (dual transmembrane domain DGC-PDEs) are found immediately downstream of most of these pruR homologues, suggesting widespread functionality of the pterin-dependent cdGMP regulatory circuit.
**Abstract:**

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

**Methods:** A determination of the minimal inhibitory concentration in polymyxin B, a standard antimicrobial peptide, has been performed. After verification of the viability of the bacteria using flow cytometry analysis and growth curves coupled with colony forming unit counting, we determined the work concentration of polymyxin B as ¼ of the minimal inhibitory concentration. Then, standard crystal violet quantification of the biofilm, soft agar motility test, electronic microscopy and western blotting analysis were performed. **Results:** After 24h we surprisingly observed a drastic significant drop from 20 to 70% - depending on the *V. cholerae* strain considered - in biofilm formation in presence of polymyxin B. Similarly, a significant decrease of 20 to 35% in motility was also observed after 24h. A western-blot analysis demonstrated that the quantity of flagellin attached to the cells decreased in presence of polymyxin B associated with an increase in the free flagellin in the supernatant, suggesting a detachment of the flagella from the cells. Observation in electronic microscopy confirmed that result. We could also observe that a bulb appears at the tip of the flagella that remain attached to the cells. To our knowledge, a similar flagellar structure with bulb has only been observed in *Campylobacter* and never in *V. cholerae*. **Conclusions:** The presence of polymyxin B at sub-lethal concentration induces a decrease in *V. cholerae* motility probably due to a modification of the flagellar structure leading to the detachment of the flagella. In addition, polymyxin B also induces a drastic drop in biofilm formation. Whether the two phenotypes are linked or not is currently under investigation. In parallel, we are also investigating if the biofilm regulation pathway are involved in the decreased biofilm formation observed in presence of polymyxin B.
Yeast of the genus Candida are the most common cause of hospital-acquired fungal infections worldwide. Candida parapsilosis is the second or third most common Candida pathogen, depending on geographic location, and predominantly affects the very old and the very young. Biofilm formation is a key virulence factor in Candida species, which are frequently isolated from device-associated infections among hospitalised patients. A key step in biofilm formation is adhesion to a surface which requires the expression of cell surface proteins like adhesins. Although putative adhesins have been identified in the C. parapsilosis genome, their mechanism and regulation is poorly understood. In this study, we screen a library of transcriptional regulator deletion mutants to identify proteins that are involved in the regulation of adhesion to a plastic surface. We found four genes (RFX1, RFX2, SFL2 and NRG1) that are required to regulate adhesion on multiple abiotic surfaces in vitro. Deletion any of these genes significantly increased the adhesion capacity of C. parapsilosis CLIB214 in vitro. Extensive transcriptomic analyses focusing on NRG1-mutant strains suggests a role for Nrg1 in the regulation of several adhesin-like proteins and proteins involved in GlcNAc metabolism. Finally, we use of a CRISPR-based approach to demonstrate the varying phenotypic effects of Nrg1-depletion in different clinical isolates of C. parapsilosis.
Abstract:

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

**Background:** River biofilms dominated by *Phormidium* (filamentous cyanobacteria) are receiving increased attention worldwide because of a recent expansion in their distribution and their ability to produce anatoxins, potent neurotoxins leading to animal mortalities. While planktonic cyanobacterial blooms have been the subject of numerous studies, mainly due to their detrimental effects on the ecosystems, only a limited number of studies have focused on their benthic counterparts. Consequently, there is limited data on the ecology and biodiversity of cyanobacterial biofilms. **Methods:** In order to identify the environmental conditions that favour their development and potential toxicity, the composition and structure of bacterial communities (BCs) associated with *Phormidium* was investigated. Biofilms were collected during the summer season of 2013 and 2014 in the Tarn River (France) and in eight rivers across New Zealand. Biofilms biomass and cover was quantified as well as biodiversity and anatoxin concentrations using high-throughput sequencing and mass spectrometry respectively. **Results:** This study revealed that *Phormidium* biofilm development was exclusively detected in riffle areas. In the Tarn River *Phormidium* cover was positively correlated with water temperature, depth, and anatoxin concentrations. The highest anatoxin concentrations were recorded at the upstream sites of the river and at the end of the summer period. The structure of the BCs from both countries was well conserved at the order level and 28% of the OTUs containing 90% of the reads were shared by these BCs. A strong and significant distance-decay relationship was found in BCs from New Zealand rivers but the Bray-Curtis dissimilarities between French and New Zealand BCs were in the same order of magnitude as those found between New Zealand BCs. **Conclusions:** The spatio-temporal pattern of anatoxin production in the Tarn River highlights the importance of biofilm maturity and the local environmental conditions on anatoxin variability. The conserved structure of BCs suggests that micro-environmental conditions occurring within *Phormidium* biofilms strongly shape the associated communities. Local environmental conditions seem to have more impact on BCs than dispersal capacities of bacteria.
**Abstract:**

*Xylella fastidiosa* (*Xf*), a gram-negative bacterium that causes numerous severe diseases in economically important crops, resides in the host xylem and in the mouthparts of its insect vectors where it produces exopolysaccharides (EPS) and forms robust biofilms. We investigated the role of a β-1,4-endoglucanase (EngXCA2) secreted by *Xf* subsp. *fastidiosa*, the causal agent of Pierce’s disease of grapevine, in phenotypes associated with biofilm development: surface attachment, cell-cell aggregation, EPS production, and 3D biofilm architecture. ∆engXCA2, an EngXCA2-deficient mutant strain, was impaired in cell-cell aggregation and produced significantly more EPS than the wild-type strain *in vitro*. In addition, ∆engXCA2 was significantly compromised in attachment to glass and was unable to develop a full biofilm relative to the wild-type strain. Furthermore, confocal laser scanning microscopy images of 4-day-old biofilms show that ∆engXCA2 biofilms are thinner than wild-type biofilms, which was confirmed by measuring average and maximum biofilm thickness. Therefore, we hypothesize that EngXCA2 facilitates the turnover of EPS during the *Xf* biofilm developmental cycle by dismantling the predicted β-1,4-glucan backbone of EPS.
**Background:** *Vibrio fischeri* expresses a complex set of two-component regulators to control biofilm formation, a critical event in the symbiotic colonization by *V. fischeri* of the Hawaiian squid, *Euprymna scolopes*. Overexpression of the sensor kinase *rscS* induces biofilm formation dependent on the symbiosis polysaccharide (*syp*) locus, and results in the formation of wrinkled colonies, pellicles in static liquid cultures, and enhanced symbiotic biofilms. Activated RscS is thought to donate a phosphoryl group to the Hpt domain of hybrid sensor kinase SypF, which promotes phosphorylation of the response regulator and transcriptional activator SypG. Recently identified sensor kinase BinK is a negative regulator of biofilm formation, also acting at the level of *syp* transcription. Numerous regulators are involved in biofilm formation, but until recently, no environmental signals were known to control biofilm formation by *V. fischeri*. **Results:** We recently reported the identification of calcium as a signal that promotes biofilm formation by biofilm-competent strains, including *rscS* overexpressing cells and *binK* mutant cells. Under shaking liquid growth, calcium induced both *syp*-dependent biofilms, resulting in cohesive cellular clumps, and bacterial cellulose synthesis (*bcs*)-dependent biofilms, resulting in surface attached rings. Calcium also induced formation of *syp*-dependent cohesive wrinkled colonies, and significantly increased transcription of both *syp* and *bcs* in liquid culture. In the absence of BinK, calcium-induced biofilms were shown to have three independent inputs- RscS, SypF, and newly identified sensor kinase HahK, all dependent on the Hpt domain of SypF. Deletion of these regulators resulted in disruptions to biofilm formation. HahK is in an operon downstream of nitric oxide-responsive sensor kinase HnoX. Investigation of HnoX revealed that it decreases *syp*-dependent biofilm formation through inhibition of HahK. Finally, we determined that inhibition by HnoX was dependent on nitric oxide, a result that reveals nitric oxide as a second environmental signal controlling biofilm formation in *V. fischeri*. **Conclusions:** This work identifies calcium and nitric oxide as the first known environmental signals to control *V. fischeri* biofilm formation. Calcium induces two distinct types of biofilms, dependent on SYP and cellulose polysaccharide, while nitric oxide inhibits *syp*-dependent biofilm formation. Effects of calcium and nitric oxide both occur, at least in part, at the level of transcription. Furthermore, this work reveals two new regulators, sensor kinases HahK and HnoX, as additional components of this complex pathway. The roles of both calcium and nitric oxide in biofilm formation by *V. fischeri* appear to be distinct from other *Vibrio* species, highlighting the diverse mechanisms by which these ubiquitous molecules can control bacterial behavior.
A core regulatory pathway that directs developmental transitions and cellular asymmetries in *Agrobacterium tumefaciens* involves two overlapping, integrated phosphorelays. One of these phosphorelays putatively includes four histidine sensor kinase homologues, DivJ, PleC, PdhS1, and PdhS2, and two response regulators, DivK and PleD. In several different alphaproteobacteria, this pathway influences a conserved downstream phosphorelay that ultimately controls the phosphorylation state of the CtrA master response regulator. The PdhS2 sensor kinase reciprocally regulates biofilm formation and swimming motility. In the current study the mechanisms by which the *A. tumefaciens* sensor kinase PdhS2 directs this regulation are delineated. PdhS2 lacking a key residue implicated in phosphatase activity is markedly deficient in proper control of attachment and motility phenotypes, whereas a kinase-deficient PdhS2 mutant is only modestly affected. Genetic interactions between the DivK/PleD response regulator pair and PdhS2 are revealed, unmasking one of several connections between PdhS2-dependent phenotypes and transcriptional control by CtrA. Epistasis experiments suggest that PdhS2 may function independently of the CckA sensor kinase, the cognate sensor kinase for CtrA which is inhibited by the DivK/PleD response regulator pair. Gene expression analyses support PdhS2 regulating both motility and levels of the intracellular signal cyclic diguanylate monophosphate (cdGMP) through CtrA transcriptional control, thereby affecting production of adhesive polysaccharides and attachment. We hypothesize that in *A. tumefaciens* the CtrA regulatory circuit has expanded to include additional inputs through addition of PdhS-type sensor kinases, likely fine-tuning the response of this organism to the soil microenvironment.
**Abstract:**

*Lactobacillus reuteri* ZJ625 mechanisms of cell-cell communication were investigated in this study when co-cultured with either *Lactobacillus reuteri* VB4 or *Lactobacillus salivarius* ZJ614. Almost 80% of *Lactobacillus reuteri* ZJ625 cells were killed when co-cultured with either *Lactobacillus reuteri* VB4 or *Lactobacillus salivarius* ZJ614 as stationary phase of growth was reached. Almost the same proteins were induced in all co-cultures at the mid-exponential phase of growth, and the number of induced proteins significantly increased at stationary phase of growth in all co-cultures. In all, ten proteins were over expressed in the co-cultures; these proteins have been reported to play critical roles in stress response mechanisms. The LuxS-mediated signalling was involved in the regulation of most of these proteins in this study. QPCR showed that the expression of luxS gene decreased as the cultures approached stationery phase of growth in co-cultures. It could be inferred from the study that: the type of microbial co-cultures dictates the synthesis of proteins, also two compounds: 2(3H) dihydrofuranone-5ethyl and 2(3H) dihydrofuranone-5pentyl were identified as putative signalling molecules when *Lactobacillus reuteri* ZJ625 cells were co-cultured with either *Lactobacillus reuteri* VB4 or *Lactobacillus salivarius* ZJ614

**Keywords:** Proteomics; Porcine; Cell-cell communication; Co-cultured; LuxS; Microbial interaction; Lactic acid bacteria
Effects of Substrate Geometry on Tolerance Dev. of *S. aureus* Biofilms to Antibiotic Treatment

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Abstract:

Biomaterials designed to heal bone fractures often serve as a nidus for chronic infection. The primary barriers to alleviating these infections are the development of biofilms that are tolerant to systemic antibiotics. Cell-cell communication through the quorum sensing (QS) accessory gene regulatory (*agr*) pathway has been shown to contribute to biofilm regulation. While some *agr* inhibitors have shown promise, the large diversity in peptide pheromones that drive QS presents a challenge. Thus, it is necessary to develop materials that delay the *agr*-mediated biofilm development. In this work, we investigated (1) the effect of surface geometry of an implanted device on the kinetics of tolerance development in *S. aureus* and (2) the relationship between the quorum sensing accessory gene regulatory (*agr*) system and tolerance development across different surface geometries. Finally, we determine the ideal implant structure capable of delaying tolerance development on medical implants. Convex substrates were 3D printed using PLA extrusion. Concave scaffolds were synthesized by casting a settable poly(ester urethane) (PEUR) within the 3D convex structure and extracting the PLA. Inoculations of *S. aureus* UAMS-1 WT and *agr* knockout strains were seeded on the 3D structures and allowed to develop biofilm communities for 6, 24, and 48 hours before 24-hour treatment with vancomycin. Cells surviving vancomycin treatment were quantified and classified as tolerant cells. *S. aureus* cells seeded on 3D concave morphologies demonstrated minimal decrease in surface density following vancomycin treatment after 24 hours of biofilm establishment. Conversely, cells seeded on a convex 3D geometry exhibited a more susceptible phenotype after 24h of biofilm development despite having a similar surface density to 3D concave morphologies when left untreated. *S. aureus* cells on both morphologies demonstrated similar tolerance profiles when allowed 48h of biofilm development, suggesting that a tolerant community will eventually develop on both surfaces. *S. aureus agr* knockout cells grown on concave substrates demonstrated a significantly smaller community of tolerant cells compared to the WT control, while no difference was seen between the WT and *agr* knockout strains on convex surfaces. Thus, substrate morphology was shown to influence the kinetic development profile of biofilms on a surface, and convex morphologies have shown capabilities of delaying biofilm treatment recalcitrance by interrupting QS-mediated cell tolerance development.
Abstract:

Bacteria in chronic lung infections alternate between surface-attached and planktonic lifestyles. Adaptation in such a dynamic environment can select for ecological diversity that can increase survival and fitness of the population. To understand the molecular mechanisms underlying this adaptive diversity, we propagated *Burkholderia cenocepacia*, a pathogen associated with chronic lung infections, in biofilm conditions for >1000 generations. Multiple coexisting lineages that evolved within biofilm populations acquired single nonsynonymous mutations affecting different domains of RpfR, a key modulator of cyclic-di-GMP (cdG) levels in various proteobacteria. RpfR has both GGDEF and EAL domains which function as diguanylate cyclase and phosphodiesterase, respectively. Thus, RpfR can synthesize as well as degrade cdG. High cdG generally promotes exopolysaccharide production and inhibits motility and in *B. cenocepacia* it binds to the RpfR-GtrR complex, repressing the influence of RpfR on GtrR-regulated genes such as fucose-binding lectins. Further, the RpfR PAS domain interacts with the diffusible signal factor cis-2-dodecenoic acid, which stimulates phosphodiesterase activity and degrades cdG. We ask if the different mutations selected in *rpfR* regulate these cdG-mediated processes differently. Specifically, what functional advantage do these mutations provide that results in their selection and maintenance in the population? To achieve this, we used phenotypic and transcriptomic assays to quantify the growth, biofilm productivity, polysaccharide composition and gene expression differences in various mutants. We find that evolved mutants have higher fitness in biofilms, vary in their cdG concentrations as well as display structural differences in biofilm architecture. The RNAseq results display downregulation of genes conferring motility and fucose binding lectins, which coincide with an increased presence of fucose in the matrix of evolved mutants. When grown in cocultures, certain isogenic mutants can invade each other when rare, which implies that they could stably coexist. Moreover, the increased biofilm productivity of mixed cultures suggests complementary interactions and a potential display of division of labor between the genotypes. Taken together, we demonstrate that distinct genotypic changes in one gene- *rpfR*, result in diverse ecological roles contributing to the evolution of synergistic biofilm assembly. These results not only reveal RpfR is at the core of biofilm regulation in this bacterium but also indicate that modifying the regulation of RpfR mediated pathways can generate adaptive diversification.
**Background:** Horizontal gene transfer via conjugation is a very important mechanism of gene spreading in bacterial populations, and is of particular concern for the emergence of antibiotic resistant bacteria. While transmission of conjugative plasmids were described as being particularly efficient in biofilms, the molecular mechanisms underlying this enhanced transfer is not fully elucidated. Additionally, nothing is currently known on the transfer of genome-encoded integrative and conjugative elements (ICEs) in these multicellular structures. *B. subtilis* is host to ICEBs1, a well-characterized ICE that is thoroughly characterized. This Gram-positive bacterium is also known to form robust biofilms under the appropriate conditions, which allows us to precisely evaluate the impact of biofilm and extracellular matrix on conjugation.

**Methods:** For this study, the non-domesticated NCIB3610 *B. subtilis* bearing an antibiotic resistance marker on ICEBs1 (donors) or cured of ICEBs1 (recipient) were used. Conjugation assays were performed using solid and liquid media differing in their capacity to induce biofilm, and several deletion mutants of biofilm-related genes allowed us to determine the importance of the extracellular matrix. Excision rates were evaluated using a qPCR approach.

**Results:** Our results clearly demonstrate that biofilm formation increases ICEBs1 transfer efficiency by at least a 100-fold. This efficient transfer is maintained even at low donor:recipient ratios. Evaluation of transfer rates through time shows a clear temporal correlation between formation of the biofilm and ICEBs1 transfer. However, biofilm formation does not trigger an increase of ICEBs1 activity, since excision levels in the donor cells remains constant through time. This result suggests that the biophysical context of biofilms favours ICEBs1 conjugative transfer. Indeed, cells constitutively secreting component of the extracellular matrix can transfer ICEBs1 very efficiently in conditions that normally do not induce biofilm formation. Additionally, production of the extracellular matrix, particularly from the recipient cells, is essential for efficient ICEBs1 transfer in biofilms. Presence of exopolysaccharides in the matrix was slightly more important than amyloid-like fibers in favouring conjugation.

**Conclusions:** Our study shows that biofilms favour high efficiency conjugative transfer of ICEBs1 between cells due to the presence of extracellular matrix. These results provide a new perspective on the transfer of Gram-positive ICEs, many of which exhibit inefficient transfer *in vitro* but are nevertheless harboured by biofilm-prone bacteria. Our work provides important insights on horizontal gene transfer in biofilms, a widely preponderant bacterial lifestyle.
Session: Poster Session 2
Date & Time: Tuesday, October 9, 2018, 4:00 pm - 6:00 pm
Poster Board #: 099
Topic: Social and Asocial Interactions in Biofilms
Abstract: Physical and Chemical Interactions between Biofilm Evolved Morphotypes of Pseudomonas aeruginosa
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Abstract:
Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects cystic fibrosis (CF) lungs and chronic wounds by forming biofilms. Emergence of phenotypically diverse isolates within P. aeruginosa biofilms has been already reported. However, the dynamics between isolates evolved within biofilms and the possible effects on the progression of chronic infection and treatment outcomes are poorly understood. Here we tested how the P. aeruginosa strain PAO1 evolves in biofilms over 50 days using a bead biofilm model in combination with synthetic sputum medium. Our evolution experiment showed that within biofilms, the emergence of distinct P. aeruginosa morphotypes occurs. We studied interactions between selected evolved morphotypes to understand how these impact upon the phenotype of diverse populations. We observed varying levels of cooperation and conflict between certain morphotypes in biofilms. We also tested the antibiotic tolerance of evolved populations and individual morphotypes, and observed a population level increase in tolerance to certain antibiotics, despite no previous antibiotic exposure. We used MALDI-TOF analysis and confocal imaging to observe both physical and chemical interactions between morphotypes. A greater understanding of interactions between diverse morphotypes within populations could form the basis of new strategies towards treatment and control of biofilms, and also provide explanations as to how and why phenotypic diversity and antibiotic resistance evolves during long term chronic infection.
Bacteria use complex regulatory networks to cope with stress, including antibiotics, but the function of these networks in natural habitats is poorly understood. The competition sensing hypothesis states that bacterial stress response systems can serve to sense ecological competition but studying regulatory responses in mixed culture is challenging. Here we solve this problem by using differential fluorescence induction to screen the *Salmonella* Typhimurium genome for loci that respond, at the single-cell level, to life in a competitive biofilm community. This reveals the presence of competing strains drives up the expression of genes involved in biofilm matrix production (CsgD pathway), epithelial invasion (SPI1 invasion system), and antibiotic tolerance (TolC efflux pump; AadA aminoglycoside 3'-adenyltransferase) and the associated phenotypes. We study mutants in key regulatory pathways and link these competitive responses to major stress responses, as predicted by the competition sensing hypothesis. Moreover, inactivation of the Type VI secretion system (T6SS) of a competitor annuls the responses to competition, indicating that T6SS-derived cell damage activates these stress response systems. Our work provides critical support for the competition sensing hypothesis by showing that bacterial regulatory networks detect and respond to competition in a manner important for biofilm formation, virulence and antibiotic tolerance. This also reveals microbial consortia can become tolerant to antibiotics and other stresses because bacteria are defending themselves from competitors, not because they are cooperating with one another.
When growing in a natural biofilm microbial species are rarely found in solitude, instead existing in complex multi-species communities termed a microbiome. One facet of a microbiome that can influence composition and functional diversity is the exchange of genetic material across species, known as horizontal gene transfer (HGT). We recently demonstrated through a bioinformatics approach that HGT is widespread in microbial species isolated from the biofilm of aged cheese, known as the cheese rind, and identified over 200 putative horizontally transferred genomic regions containing 4,844 protein-coding genes. These include proteins putatively involved in processes such as iron-siderophore transport & lactate utilization. We hypothesize that these genes can confer a selective advantage in the iron deplete and lactate rich cheese medium. Here, we focus on a widespread genetic element termed RUSTI (iRon Uptake and Siderophore Transport Island). Our data suggest this element is encoded on an active Integrative and Conjugative Element (ICE). By tracking this ICE during the initial colonization and aging of the cheese medium, investigating the regulation of the RUSTI genes, and assaying the effect RUSTI confers on the fitness of the recipient bacteria, these data provide insights into how HGT can influence microbial communities including during the colonization of a new environment.
The emergent behavior of microbes in multispecies communities can lead to robust biofilm formation, a phenomenon that causes major problems in many segments of our society, including in the clinic. As many as 80% of infections in the human body are associated with biofilms[1], which often include more than one microbial species. Despite the prevalence of multispecies communities in disease and medical device infections, we lack robust in vitro models and metrics to standardize multispecies biofilm measurements and facilitate reproducible, comparable experiments. Our objective was to evaluate the potential of metabolic profiling to establish reproducibility criteria for biofilm function, by measuring repeatability of intracellular and extracellular metabolomes for a clinically relevant in vitro coculture model. Pseudomonas aeruginosa and Staphylococcus aureus are pathogens often co-located in human disease states including wound infections and chronic lung infections of cystic fibrosis (CF) patients. Coinfection involving these species typically worsens patient outcomes and can lead to antibiotic resistance as compared to monoculture infections. The two species were grown as mono- or co-cultures for 24 h in artificial sputum medium to mimic nutrients in CF lung infections and improve clinical relevance versus typical in vitro growth media. We integrated and optimized protocols for microbial culturing and quantification, quenching, and metabolite extraction. Global metabolite profiles were analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS). Principal component analysis indicated that profiles of P. aeruginosa, S. aureus, and the co-culture are statistically differentiable (n = 9) and highly repeatable across all three levels (day, biological and technical replicate). In addition, we found 575 features unique to the co-culture, which are under identification analysis. Overall, our results suggest that LC-MS/MS metabolic analysis is a promising approach to monitor reproducibility of multispecies biofilms. Our approach enables comparison and combination of results across laboratories toward a collective improved understanding of interspecies interactions, their role in disease, and novel therapeutics to target these systems.
Abstract:
Multicellular bacterial communities, known as biofilms, have been thought to be held together solely by a self-produced organic extracellular matrix. Our study of two phylogenetically distinct bacteria: *Bacillus subtilis* and *Mycobacterium smegmatis*, identified a novel mechanism maintaining biofilms - an active production of crystalline calcite scaffolds. We demonstrated the existence of calcite scaffolds in bacterial biofilms and their roles in stabilizing biofilms, and in limiting penetration of small molecule solutes as antibiotics. In addition, we demonstrated that calcite mineral scaffolds play a conserved role in the assembly of complex communities. Inhibiting enzymes promoting biomineralization, hampered biofilm formation and restored diffusion of small molecules into the biofilm (Keren-Paz et al., 2018; Oppenheimer-Shaanan et al., 2016). Furthermore, our recent results demonstrate that both *in vitro* and in clinical scenarios, biofilms formed by the Gram-negative pathogen *Pseudomonas aeruginosa* are held together by biogenic calcite minerals. To identify the cellular pathways involved in biomineralization, we performed an unbiased transcriptome analysis of the biofilm cells responding to a soluble calcium source. The transcriptome architecture suggested a role for cell envelope synthesis and remodeling during biomineralization, as well as for increased calcium storage within the cells. These results support the hypothesis that crystal formation initiates intracellularly. Consistently, high resolution Scanning Transmission Electron Microscopy (STEM) of the biofilm cells indicated that the minerals are produced within discrete foci in the periplasm of dedicated cells, released from the cell, and finally mature by interactions with the organic extracellular matrix. Our work provides insights into the relationship between the behavior of single cells, community structure and biofilm function.
Pseudomonas aeruginosa Pel Biosynthesis Defines a New Class of Cyclic Diguanylate Regulated Polymerization and Transport Systems


Abstract:
Biofilm formation by the opportunistic pathogen Pseudomonas aeruginosa involves the secretion of polysaccharides or other polymers that structure the extracellular matrix and protect bacteria from antimicrobials and host immunity. The Pel polysaccharide is important for biofilm development by P. aeruginosa, yet very little is known about the initial stages of Pel biosynthesis and export. In this study, we elucidate a complex of the proteins PelDEFG that is key for the earliest steps of exopolysaccharide production. By using a combination of genetic dissection, cellular fractionation, co-immunoprecipitation, and bacterial two-hybrid analyses, we demonstrate that these proteins associate, and bring together in a single molecular machine all the functionalities necessary for Pel polymerization and its transport across the cytoplasmic membrane. We find that binding of the bacterial secondary messenger cyclic diguanylate (c-di-GMP) to the receptor PelD modulates interactions within this complex, suggesting a means to post-translationally regulate Pel biosynthesis in response to changing intracellular c-di-GMP levels. Further, we identify direct interactions between PelD and c-di-GMP metabolizing enzymes, including the phosphodiesterase BifA, which implies a mechanism for maintaining specific control over Pel biosynthesis independently of other c-di-GMP binding effectors in the cell. Overall, the mechanism for Pel biosynthesis inferred from these findings deviates from established exopolysaccharide secretion mechanisms in Gram-negative bacteria, leading us to propose that the Pel apparatus defines a new mechanistic sub-class of the synthase-dependent exopolysaccharide secretion pathway. This work also expands our fundamental understanding of how c-di-GMP signaling specificity can be attained in the unrestricted environment of a bacterial cell, as well as how c-di-GMP can allosterically regulate the functionalities of membrane-associated protein complexes.
**Background:** Bacteria adopt a biofilm state that represents multicellular microbial communities adherent to each other as well as to an abiotic or biotic surface. Bacteria in a biofilm are surrounded by extracellular polymeric substances, primarily comprised of exopolysaccharides, extracellular DNA (eDNA) and proteins. eDNA is ubiquitous and a pivotal component to maintain the structural integrity of bacterial biofilms. We have shown previously that eDNA in biofilms formed by multiple bacterial species is organized into a lattice-like structure that is stabilized by DNABII proteins. DNABII proteins are a family of DNA binding proteins that exhibit high affinity to pre-bent DNA. Sequestration of these proteins from the bacterial biofilm matrix with a DNABII-specific antibody (α-DNABII) destabilizes the matrix and thereby results in disruption of the biofilm structure. Since the cross strand organization of eDNA bears a striking resemblance to Holliday-junction (HJ) DNA and the DNABII proteins naturally bind to HJs, we hypothesized that eDNA lattice in bacterial biofilms are structurally related to HJ recombination intermediates.

**Methods:** To incorporate RuvA, we added RuvA (450 nM) to pre-formed biofilms at 16h and 24h in the presence of α-DNABII. RuvA dependent helicase RuvB (1130 nM), HJ-specific endonucleases RuvC (100 nM) or RusA (350 nM) were added at 24h. At 40h, the biofilms were stained with LIVE/DEAD® or labeled with appropriate antibody for immunofluorescence and analyzed using confocal laser scanning microscopy. **Results:** The addition of the prototypic HJ-specific DNA binding protein RuvA, both readily incorporated within the biofilm matrix and prevented α-DNABII-mediated disruption of bacterial biofilms formed by uropathogenic *E. coli*, nontypeable *Haemophilus influenzae* (NTHI), and *Staphylococcus epidermidis*. Next, we assembled the HJ-specific endonuclease complex RuvABC at the RuvA-bound HJ DNA sites, which resulted in collapse of the biofilm structure. Additionally, treatment of bacterial biofilms with another HJ-specific endonuclease RusA, also resulted in total collapse of the biofilm structure of multiple bacterial species. Addition of RusA also prevented the formation of the complex web-like eDNA lattice structure in biofilms formed by NTHI. As a final confirmation for the presence of HJ DNA structure within the biofilm matrix, we labeled bacterial biofilms with a monoclonal antibody directed against cruciform DNA that recognizes HJ DNA and observed uniform distribution of HJ DNA throughout the biofilm matrix. Addition of RusA to biofilms at seeding also dramatically decreased the cruciform DNA within the biofilm matrix. **Conclusion:** Collectively, these data indicated that the eDNA lattice of bacterial biofilms are structurally related to HJ recombination intermediates and are critical to the structural integrity of bacterial biofilms.
Abstract:

Introduction: Acetogenic bacteria utilize the Wood-Ljungdahl pathway to convert hydrogen, carbon dioxide, carbon monoxide, and a wide range of sugars to volatile fatty acids (VFA) and alcohols, especially acetate and ethanol. VFA production using syngas (H₂, CO₂ and CO) has been widely reported; however, gas hydrophobicity has limited the production rate of this process. The Membrane Biofilm Reactor (MBfR) is a unique technology that delivers syngas directly to microorganisms that live as a biofilm on the outer surface of gas-transfer membranes. Direct transfer to the biofilm overcomes interfacial gas-liquid mass-transfer limitations. Today, the MBfR is used for waste treatment, especially for oxidized contaminants such as perchlorate, uranium and chromate. Here, we develop an MBfR capable of producing VFAs using the H₂ and CO present in syngas as electron donors and CO₂, CO, and bicarbonate as carbon sources.

Methods: We operated three 120-cm³ glass MBfRs with a continuous medium flow containing 120 mM of bicarbonate. Digester sludge was the inoculum. Reactors 1 and 2 (R1 and R2) had 130 cm² of composite hollow fibers (polyurethane-polylethylene) and hydraulic retention times (HRT) of 40 h and 14 h, respectively. Reactor 3 (R3) had 2.15 cm² of asymmetric Matrimid®-based fibers and an HRT of 14 h; this fiber was synthesized specially for this project by our collaborators at Georgia Institute of Technology, and it can achieve >10000-fold faster gas delivery than the composite fiber. Syngas pressure inside the membranes was 5 psig. We tracked production/consumption of H₂, CH₄, CO, and CO₂ by gas chromatography, measured bicarbonate concentration by ion chromatography, and measured VFA concentrations by high performance liquid chromatography. Gas and liquid samples were taken daily. We also sequenced the 16S ribosomal RNA gene in biofilm samples and performed qPCR to assay total 16S ribosomal RNA gene for all bacteria, mcrA gene for methanogens, and the FTHFS gene for homoacetogens. Sequencing results were analyzed using Quantitative Insights Into Microbial Ecology -Quiime®.

Results and Discussion: All reactors achieved steady state and substantial VFA concentrations. Notably, R1 generated products up to 7 carbons chain length, while R3 had the highest acetate concentration, 67 mM. The longer HRT of R1 seemed to play a key role in chain elongation. Gas chromatography showed that H₂ was below 5% and CH₄ was negligible in the gas output in all reactors. Although general bacteria decreased from about 2x10¹¹ to 8x10⁷ gene copies/cm² and methanogens decreased to undetectable, acetogens increased from negligible to 5x10⁶ gene copies/cm². This enrichment of the biofilm with homoacetogens is desired. Conclusions: This study proves that the syngas-based MBfR is a viable platform for VFA production and enrichment of acetogens in a biofilm. Reaching steady state is promising for large-scale applications.
**Poster Session 2**

**Date & Time:** Tuesday, October 9, 2018, 4:00 pm - 6:00 pm

**Poster Board #:** 108

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

**Abstract:**

**Title:** Ecological Analysis of Mucoid Variant in *Pseudomonas aeruginosa* Biofilm

**Author Block:** K. Itagaki, N. Obana, M. Toyofuku, N. Nomura, A. S. Utada; Univ. Tsukuba, Tsukuba, JAPAN.

The Gram-negative bacterium *Pseudomonas aeruginosa* is found ubiquitously and is an opportunistic human pathogen in people with weakened immune-system. Due to its ubiquity, abundance, pathogenicity, and ability to form biofilms it is often used as a model organism to study biofilm development. The biofilm lifestyle enables efficient resources sharing, protection from predation and changing environmental conditions. Biofilm bacteria often have an increased resistance to antibiotics. People with the genetic disease Cystic fibrosis (CF) are often afflicted with chronic infections of *P. aeruginosa*. The prognosis of CF patients becomes markedly worse with the appearance of an alginate over-producing phenotype, called the mucoid variant, in the late stages of infection. This mutant greatly increases the environmental viscosity and the alginate encapsulated cells also have increased resistance to environmental stresses; these factors contribute to the deterioration of the patient’s health.

It is reported that the *P. aeruginosa* mucoid variant and non-mucoid wild type coexist in *in vivo* biofilms. However, there is little understanding of the spatio-temporal appearance of the mucoid mutant and localization of alginate within those biofilms. In this study we use the *P. aeruginosa* PAO1 mucoid variant to clarify the ecology of the mucoid variant in 3-dimensional biofilms starting at the single cell level. We utilize flow-cell channels to observe the development of the mucoid-variant biofilms and image the localization of alginate using immuno-staining and confocal microscopy. We find that alginate secretion increases with the elapsed time: in the early stages of biofilm formation, we observe a heterogeneous distribution of filamentous alginate strands decorating small microcolonies, while at later times the alginate completely covers the mature biofilm. Interestingly, we observe the appearance of different phenotypic populations from the edges of plated mucoid colony biofilms. These phenotypically different cells exhibit surface motility patterns similar to WT suggesting that a reversion variant appears under certain conditions present in a mucoid biofilm. To visualize this change, we constructed a revertant variant reporter strain in the PAO1 mucoid variant background that expresses DsRed when overproducing alginate or sfGFP when the reversion mutation has occurred. We imaged the appearance of this reversion mutant at the base of a mature mucoid colony using this reporter strain and confocal microscopy. We aim to observe biofilm formation using this reporter mutant to clarify the localization of alginate production and, simultaneously, the appearance of revertant variant in biofilms. We hope that these results will contribute to development of a deeper understanding of the ecological implications of the appearance of the mucoid phenotype.
Abstract: A Multidisciplinary Approach to Study the Role of Lipoprotein SslE in the Escherichia coli Biofilm

Biofilms are groups of microorganisms that adhere to one another on a surface, through a self-produced matrix of extracellular polymeric substance. This mesh provides protection against environmental pressures, host immune responses and antimicrobial agents. As almost all bacteria establish biofilms as a strategy for survival and persistence, understanding how they form is key to the development of new compounds to combat antibacterial resistance. Biofilm growth by virulent strains of Escherichia coli is a major contributor to the establishment of diseases such as hemorrhagic colitis, neonatal meningitis, urinary tract infections, pneumonia and sepsis. Matrix components such as SslE have been identified as fundamental for biofilm maturation; however, the actual interactions that this molecule mediates are not well understood. SslE is a ~165-kDa protein found on the bacterial surface and within outer membrane vesicles but details of its structural features are unclear. It does, however, possess a C-terminal zinc-metalloprotease-like domain, which influences colonization of the small intestine through degradation of mucins. This facilitates bacterial penetration of the mucus layer, adhesion to host receptors and delivery of toxins. In this work we are developing an interdisciplinary approach to identify interactions that are essential for the establishment of biofilms in Escherichia coli. We are using the non-pathogenic Escherichia coli strain W as a model system to (a) determine the structure of the lipoprotein SslE, (b) understand its role in establishing colonization and (c) why it is required for biofilm maturation. We are using structural biology approaches such as crystallography and NMR to solve SslE structure and observe its interaction with substrates. We have developed mucin degradation assays to observe and compare the mucinase activity of the full-length protein and its isolated domains. We have built a biofilm flow-cell system to observe difference in phenotypes of the wild type vs SslE mutants using confocal microscopy. We have also developed a static biofilm plate assay where we are being able to observe biofilm maturation, when according to literature, this would not be possible for our system. Altogether we are using microbiology and biochemistry techniques to validate and complement each other on our quest to understand SslE function in establishing a mature biofilm and its importance in degrading mucin. In the future our findings will be further tested in the enterotoxigenic E. coli strain H10407.
**Background:** CsgA, also known as a major curli component, is a secreted protein ubiquitous in biofilms of gram-negative bacteria (Zhou et al., 2012a). Thanks to its ability to create durable fibers, CsgA is a dominant proteinaceous scaffold of biofilms. In fact, CsgA belongs to amyloids, proteins that form fibers during a spontaneous aggregation. CsgC, a sequence-specific inhibitor, is able to arrest aggregation of CsgA in periplasmic space (Evans et al., 2015). The presence of pre-formed amyloid fibers can accelerate aggregation of other amyloids. This process is known as cross-seeding. It is extremely sequence specific and can be restricted by a difference in a single amino acid (Zhou et al., 2012b). CsgA can be in vivo cross-seeded by its nucleator protein, CsgB, but also other CsgA fibrils. **Methods:** We recently published AmyloGram (Burdukiewicz et al., 2017). It is an efficient tool for prediction of amyloid proteins as CsgA and CsgB. Among others, our algorithm produces a simplified amino acid alphabet for amyloid proteins. It groups amino acid together into six groups based on their physicochemical features. We combined sequences of around 500 triplets CsgA-CsgB-CsgC from closely related gram-negative bacteria and used EVmutation software (Hopf et al., 2017) to find out which residues are especially susceptible to simultaneous mutations. **Results:** Both CsgA and CsgB are characterized by a regional structure of five repeated motifs. We found out that the general motif (S-X5-Q-X-G-X2-N-X-A-X3-Q) (the serin in absent in the case of CsgB) is faithfully preserved among different variants of CsgA and CsgB. The residual variability in motifs of both proteins does not affect the sequence of other protein. The opposite situation happens in CsgC, where mutations are not limited to specific motifs. On the other hand, CsgA and CsgC keep a similar level of the sequence divergence aside from species of Enterobacter and Salmonella genera, where CsgC accumulates more mutations. **Conclusions:** The interplay of CsgA, CsgB and CsgC suggests that if a mutation occurs in the region responsible for protein interaction, it should be compensated by mutations in other two proteins. For example, the presence of mutations that decrease the aggregation rate of CsgA, allows CsgC to become a less effective inhibitor without affecting the wellbeing of bacteria. Our preliminary study partially confirms this hypothesis by finding out that genera with less efficient CsgA, as Salmonella, can accumulate more mutations in the sequence of CsgC. We have not identified any simultaneous mutations between CsgA and CsgB. This may be due that single mutation in one region is not enough to change the protein function and to cause mutations in another protein. Probably, the compensation of single mutations by the regional structure of mentioned proteins is sufficient.