Proceedings

JULY 14-16, 2020

****** All times are Mountain Daylight Time ******

Virtual Meeting, Bozeman, Montana

The Center for Biofilm Engineering and its Industrial Associates gratefully acknowledge Elsevier for sponsoring the 2020 Montana Biofilm Virtual Meeting.

Biofilm focuses on hypothesis- or discovery-driven studies on microbial cells that grow in multicellular communities and demonstrate different gene expression, growth rate, behavior and appearance to those that are in planktonic (free-living) state.

Meet the Editorial Team

Tom Coenye
Ghent University, Gent, Belgium

Ákos T. Kovács
Technical University of Denmark (DTU), Kongens Lyngby, Denmark

Birthe Veno Kjellerup
University of Maryland
College Park, Maryland, USA

Darla Goeres
Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA

journals.elsevier.com/biofilm

Table of Contents

Table of Contents, Agenda

SESSION 4: Field Studies

Day 3 Opening Remarks

Matthew Fields, CBE Director, Professor, Microbiology & Immunology, MSU
Paul Sturman, CBE Industrial Coordinator

SESSION 5: Biofilms and Antimicrobials

SESSION 1: Biofilm Dynamics

Regulatory mechanisms and effectors leading to biofilm dispersion

Presenter: **Karin Sauer**, Professor
Affiliation: Binghamton Biofilm Research Center, Binghamton University, Binghamton, NY, USA.

Dispersion is a mechanism by which bacterial cells actively leave the biofilm in response to various environmental endogenous and exogenous cues including carbon sources and nitric oxide, resulting in a phenotypic switch of dispersing cells returning to the planktonic mode of growth. Compared to biofilms, dispersed cells have been demonstrated to have reduced c-di-GMP levels, increased motility, reduced matrix, and altered virulence and susceptibility. However, beyond dispersion cue sensing and how these cues are translated into the modulation of the c-di-GMP pool, little is known about the down-stream events that enable *P. aeruginosa* to egress from the biofilm or the factors contributing to the virulence-related phenotypes observed in newly dispersed cells. To characterize the genetic requirements for dispersion by *P. aeruginosa* biofilms, we made use of RNA-seq and compared gene expression in planktonic, biofilm, and dispersed cells. The analysis revealed gene expression patterns that are unique to dispersed cells, supporting the notion that newly dispersed cells are physiologically distinct from planktonic and biofilm cells. Genes identified to be induced upon induction of dispersion or found to be dispersion-specific comprised those involved in adaptation and protection, motility/dissemination, drug susceptibility, virulence, matrix components, and matrix degradation. By combining molecular and biochemical analysis with microscopy, we found that genes involved in matrix degradation, such as secreted DNases capable of degrading eDNA present in the biofilm matrix, and glycoside hydrolases, enable dispersion. In contrast, CdrA, a c-di-GMP regulated adhesin that reinforces the biofilm matrix, impedes the dispersion response. Lowering of the intracellular c-di-GMP level, however, was not sufficient to induce dispersion. The finding suggested weakening of the biofilm matrix to be a requisite for biofilm dispersion. Moreover, weakening of the biofilm matrix coincided with cells adopting a hyperadhesive but drug-tolerant phenotype frequently observed in newly dispersed cells. The transcription factor AmrZ regulates the expression of several matrix components and matrix degrading genes. AmrZ was found required for dispersion, by linking dispersion cue sensing to down-stream events that enable *P. aeruginosa* to disperse from the biofilm. Collectively, our findings indicate dispersion to coincide with the activation of nucleases and hydrolases, as well as untethering of polysaccharides as an essential step to the liberation of bacterial cells from the biofilm. Moreover, our findings suggest bacteria to be actively released through specific regulation of degradative factors resulting in the overall reduction of specific components of the biofilm matrix.

Identifying causative relationships and active populations in polymicrobial communities

Presenter: **Matthew Fields**, Director[1], Professor[2]
Co-Authors: Dr. Anna Zelaya[1,2], Dr. Heidi Smith[1,2]
Affiliation: [1]Center for Biofilm Engineering, [2]Microbiology & Immunology, Montana State University, Bozeman, MT, USA.

Microbial communities in most environments can display high spatial diversity, and recent results from a variety of environments have demonstrated high temporal variability as well. While sequencing capabilities have produced substantial insight about potential functionality and population distributions in different environments, DNA-sequencing alone cannot identify active microbial assemblages nor produce informed estimations of causative relationships between particular populations and system constraints. In order to delineate differentially active populations within groundwater samples, BONCAT (biorthogonal non-canonical amino acid tagging) and PMA (propidium monoazide) treatments were compared, and methods were developed to sort BONCAT-active cells via flow cytometry (BONCAT-Seq). Furthermore, vector auto-regressive (VAR) models and Granger causality were used to predict relationships between species richness and key geochemical parameters. Future work includes further refinement of VAR models with active population data applied to different microbial systems.

What we know and don't know about viruses in biofilms

Presenter: **Elinor Pulcini**, Assistant Research Professor
Affiliation: Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilms provide protection to the microbial cells encased within the biofilm matrix. This protection includes increased tolerance to antimicrobials, protection from changes in shear forces and, in the case of a pathogenic biofilm, provide protection from human host immune cells. Viruses, non-living protein encased nucleic acid particles, require host cells in order to replicate. Viruses are often found in the same environment as biofilms; water distribution systems, food processing lines and diseased hosts (human or animal). However, very little is known about the potential role of biofilms in providing protection to viral particles. While viruses cannot replicate within biofilms, biofilms may provide a niche which allows viral particles to survive disinfection and host immune response, thereby providing a potential re-infection source. This presentation will examine the role of biofilms in virus survival with particular emphasis on the health care setting.

SESSION 2: CBE Paths Forward

Pathways to Innovation: Growing a Regulatory Science Program at the CBE

Presenter: **Darla Goeres**, Research Professor of Regulatory Science[1]; Principal Investigator[2]
Affiliation: [2]Standardized Biofilm Methods Laboratory, [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The Center for Biofilm Engineering is launching a new regulatory science program with the intent to promote innovation by creating a culture that bridges a regulatory science mission and novel technology solutions to real world problems. The CBE is uniquely positioned to host a regulatory science program. Since our inception, the CBE has collaborated with industry to provide unbiased, solutions-based research that encompasses state of the art technology and fundamental biofilm science. This relationship with industry drove the CBE to become a leader in the development of standard test methods for growing, treating, sampling, and analyzing biofilm bacteria, securing our contribution to standard setting organizations, and promoting collaboration with our regulatory colleagues. As an academic institution, the CBE regulatory science program will promote unbiased, reproducible science-based methods that are in the public domain. Under the regulatory science program, the CBE will expand our outreach and engagement with regulatory agencies, standards organizations, and nonprofits at a national and international level. We will foster student engagement, training, and workforce development including offering a graduate program certificate in regulatory science. We will recruit both traditional and non-traditional students and strive to achieve an inclusive learning environment. The regulatory science program has seed funding for five years to establish the program. The final goal is for MSU to receive funding for a Regulatory Science Center.

SESSION 3: Biofilm Methods

Development of a biofilm model on *in vitro* colonized epidermis

Presenter: **Samantha Westgate**, CEO
Co-authors: Louise Suleman, Senior Commercial Scientist; Laura Sellars, Senior Scientist.
Affiliation: Perfectus Biomed, Cheshire, UK.

Human skin provides a formidable barrier to pathogens however, patients with chronic wounds or burn injuries present with impaired barrier function that can lead to severe infection. There is a plethora of academically designed *in vitro* skin and wound models but a shortage of models that incorporate mammalian cells and biofilm encased bacterial cells, within a reproducible assay. Development of commercially focused models will result in more effective screening of topical wound care products *in vitro*. Following incubation *Staphylococcus aureus* was harvested and diluted in Phosphate Buffered Saline (PBS) to 1 x 10^6 (inoculum 1), 1 x 10^7 (inoculum 2) and 1 x 10^8 (inoculum 3) $CFUmL^{-1}$. A commercially available full thickness epidermal tissue model, made from human keratinocytes and fibroblasts formed a model of the human dermis and epidermis. The tissue model was prepared according to manufacturer's instructions and held at 37 ± 2°C with 5% CO_2 prior to treatment. A punch biopsy of the epidermal layer was taken and removed to mimic wounded skin. Aliquots of *S. aureus* were applied to the

wounded area of the epidermis and incubated for 24 and 48 hours at 37 ± 2°C with 5% CO_2. Following incubation, the tissue model was washed to remove non-adherent *S. aureus*. Samples were removed from the culture insert, suspended in recovery medium and sonicated. Following sonication, the suspension was serially diluted, plated onto TSA and incubated at 37 ± 2°C for 24 hours. *S. aureus* colonies were counted and expressed as $Log_{10}CFUmL^{-1}$. N=3. Tissue samples were also taken for Scanning Electron Microscopy (SEM). Skin samples were fixed in glutaraldehyde and dehydrated in increasing concentrations of ethanol for 10 minutes per concentration. Samples were sputter coated prior to SEM imaging. *S. aureus* attached to the tissue model after 24 hours. Variable seed concentrations did not result in significant variations in biofilm development, with 24 hour and 48 hour biofilm development being consistent between replicates at both time points. Scanning electron images revealed microcolony formation of adhered *S. aureus* to the epidermal layer. The results of this study will allow researchers to screen topical antimicrobial products in models that represent low, medium and high-level contamination situations. The model has the potential to be used for the assessment of skin irritation, skin corrosion, UV exposure, permeability, bacterial adhesion and the efficacy of antimicrobial wound dressings and solutions.

Table of Contents, Agenda

Biofilm sensing: An engineering overview

Presenter: **Stephan Warnat**[1,2], Assistant Professor
Co-Authors: Christine Foreman[1,3], Professor. Markus Dieser[1,3], Assistant Research Professor, Matthew McGlennen[1,2], Ph.D. Student
Affiliation: Center for Biofilm Engineering[1], Department of Mechanical and Industrial Engineering[2], Department of Chemical and Biological Engineering[3], Montana State University, Bozeman, MT, USA.

Water supply systems are critical elements of many industrial applications. Microbes in the water can lead to the growth of biofilms, which are 3D accumulations of cells encased in extracellular slime that adhere to surfaces. Biofilms protect microbes from environmental stresses (e.g., changes in temperature, pH, oxygen, and water availability) and concentrate nutrients, thereby promoting their successful colonization. Also, microbial contamination is a significant factor in the degradation of industrial fluids, causing biofouling and corrosion of equipment, the imperilment of product quality, and posing occupational safety risks. Once these fluids are contaminated with microbes, decontamination is a difficult task, particularly in hard to reach locations. Even after meticulous cleaning, residual contamination can quickly repopulate the media as a result of resident biofilms existing in inaccessible regions. Therefore, a proper mechanism to promptly detect biofilm would be beneficial to control industrial processes. However, industrial sites often do not have the resources for wet chemical biofilm analysis. This presentation reviews *in situ* biofilm detection processes that are currently being investigated. The advantages and disadvantages of system integration will be evaluated. Development of a microfabricated impedance microbiology sensor system here at the Center for Biofilm Engineering will be the focus of the second part of this presentation. Impedance microbiology is a powerful technique to monitor electrical changes in various media. The technique is based on applying a single frequency to a device under test (DUT), measuring the complex electrical current. Varying the frequency and calculating the complex resistance/impedance allows modeling of the DUT using electrical equivalent circuits. The recorded spectra indicate *in situ* biofilm formation and an increase of microbial concentrations in the media. The figure below shows the current impedance microbiology test apparatus (a) and schematic that presents the correlation between biofilm formation and the electrical characterization (b).

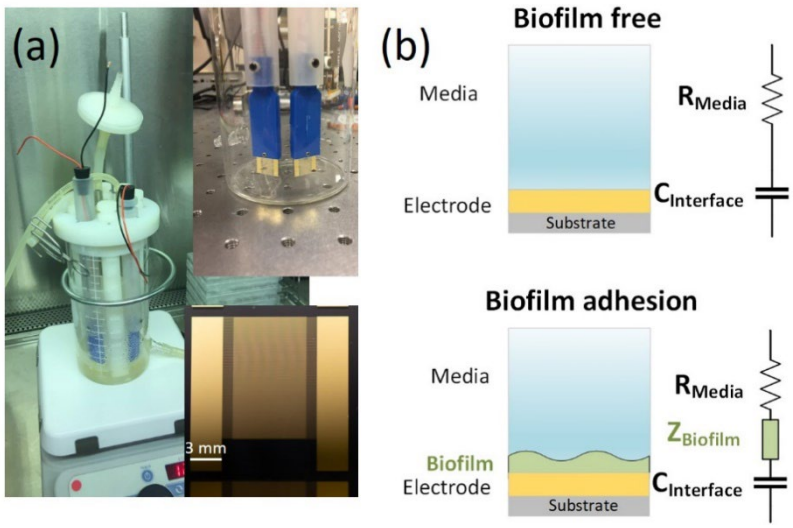

Figure 1. Impedance microbiology test apparatus (a) at the CBE to detect *in situ* biofilm growth in aqueous environments. Biofilm growth is detected through electrical modeling of sensor response (b).

Experimental designs to quantify early aggregation and colonization of biofilms with statistical confidence from confocal images

Presenters: **Brian A. Pettygrove**[1,2], PhD Candidate; **Albert E. Parker**[1,3], Associate Research Professor
Co-authors: Heidi J. Smith[1,2], Jovanka M. Voyich[2], Philip S. Stewart[1,4]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Department of Microbiology and Immunology, Montana State University, Bozeman MT, USA. [3]Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA. [4]Department of Chemical and Biological Engineering, Montana State University, Bozeman MT, USA.

Microscopy is an essential tool for any biofilm researcher as it allows non-invasive, quantitative measurements of various biofilm characteristics over space and time. Little work has been done, however, to assess the requisite experimental parameters, such as how many replicates or fields of view to collect to ensure statistical robustness in biofilm imaging studies. We analyzed existing microscopy data consisting of time-lapse measurements of bacterial biomass during early *Staphylococcus aureus* biofilm colonization to determine the relative sources of variability due to differences among independent experiments, fields of view, and time points. Based on this analysis, recommendations for future experimental designs were generated. Our analysis demonstrated the importance of prioritizing experimental replicates and the ability to decrease the image capture rate for temporal studies. Results suggest that, when monitoring early biofilm formation in the absence of any treatment, at least three experimental replicates should always be performed, and that little statistical benefit is obtained from imaging more than two fields of view per experiment. We also analyzed experiments where adherent *S. aureus* cells were challenged with human neutrophils. Here the variability in the data depended on the log reduction observed, with weakly efficacious treatment conditions resulting in low variation and moderately efficacious treatments producing the most variation. Using this approach, we have generated a tool to help biofilm researchers determine the appropriate design for early colonization experiments based on the expected variation and efficacy of treatments.

SESSION 4: Field Studies

Multi-domain biofilm growing systems and their potential application

Presenter: **Erika J. Espinosa-Ortiz**[1, 2], Assistant Research Professor
Co-authors: Paul Sturman[2], Robin Gerlach[1]
Affiliation: [1]Chemical and Biological Engineering Department, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Research Professor & Industrial Coordinator, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

In many natural and industrial environments, microbes such as bacteria, archaea, fungi and algae cohabit and interact with each other, forming complex multi-domain microbial communities. The interspecies microbial interactions in these complex microbial communities often affect the pathogenicity, survival, stress resistance, growth, nutrition and transport of one or all interacting partners. The unique way microbes communicate and physically associate in multi-domain communities is relevant to various research fields including food production, medicine, agriculture and environmental science. At the CBE, we have developed different bioreactor systems aimed at investigating how microbial interactions influence the development (e.g. attachment and surface colonization), structure and physiology of multi-domain biofilms. This presentation will provide a broad description of the bioreactor systems and some of the characterization tools developed and used at the CBE for studying multi-domain biofilms. Examples of the systems studied include: (i) fungal-bacterial biofilms with relevant pollutant-degrading microbes to develop new biological systems for water and wastewater treatment, (ii) fungal-algal-cyanobacterial communities formed during biodeterioration tests of different building materials, and (iii) bacterial-yeast (fungal) communities formed in laboratory draught beer lines.

Figure 1

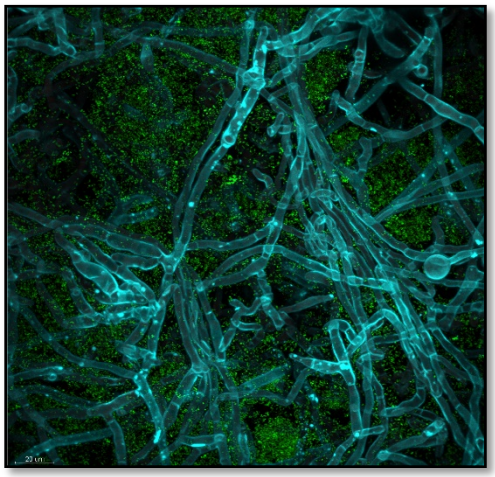

Figure 1. Fungal-bacterial biofilm.
Blue→ fungal hyphae
Green→ bacteria

Figure 2

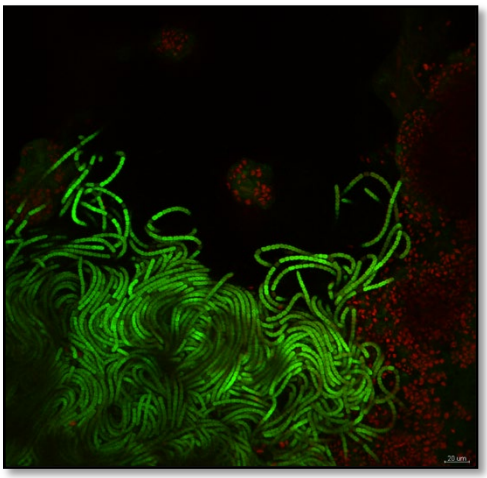

Figure 2: Algal-cyanobacterial biofilm.
Green→ cyanobacteria
Red→ algae

Microbially induced calcium carbonate precipitation (MICP): bio-cement's journey from the laboratory to the field.

Presenters: **Adrienne Phillips**[1], Associate Professor, Civil Engineering
Catherine Kirkland[1], Assistant Professor, Civil Engineering
Co-authors: Robin Gerlach[1], Al Cunningham[1], Randy Hiebert[2] Lee Spangler[3], Jim Kirksey[4]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Montana Emergent Technologies, Butte, MT, USA. [3]Energy Research Institute, Montana State University, Bozeman, MT, USA. [4]Loudon Technical Services, Charleston, WV, USA.

Microbially-induced calcite precipitation (MICP) is being widely researched as an emerging technology for subsurface engineering applications including sealing defects in wellbore cement to mitigate the escape of fugitive greenhouse gases to the atmosphere. MICP relies on enzyme catalysis to promote hydrolysis of urea and subsequent precipitation of calcium carbonate ($CaCO_3$) in undesired flow paths. The process is particularly effective in engineering applications where a biofilm comprised of the ureolytic bacterium, *Sporosarcina pasteurii*, provides the source of the urease enzyme to initiate the process. The resulting $CaCO_3$ mineral forms in association with the biofilm and 'grows' as a bio-cement with repeated injections of microbes and nutrients to seal the subsurface leakage pathway. For more than a decade, our research team has worked in the laboratory to develop injection strategies to control the spatial distribution of biofilm and subsequent bio-cement formation under various temperature, pressure, and flow conditions. Experiments have spanned a range of scales, from bench-scale column studies, to meso-scale experiments at high pressure, to five field scale demonstrations of the biotechnology in two wells with identified leakage pathways in Alabama and Indiana. The field work required the design and construction of a custom mobile laboratory equipped with bioreactors to grow and inject large volumes of microbes into the wells. This presentation will summarize our efforts to take advantage of biofilm to enhance wellbore integrity and seal leakage pathways in oil and gas wells. In addition, the commercial potential of the technology developed in the Center for Biofilm Engineering laboratories will be highlighted with updates from our small business partner, Montana Emergent Technologies.

Large-scale water and facility decontamination testing capabilities

Presenter: **Steve Reese**, Research Engineer
Affiliation: Idaho National Laboratory, Idaho Falls, ID, USA.

The Idaho National Laboratory has established large scale testing facilities for evaluating decontamination methods of potable water distribution systems and building-scale airborne and surface decontamination methods. The Water Security Test Bed (WSTB)and the bio-containment facility, better known as the BOTE (Bio-Response Operational Testing and Evaluation) building, offer the ability to test and train on chemical and biological decontamination methods at large scale. The facilities offer the ability to evaluate performance after scaling up

processes or treatments that show promise at the bench-scale. The presentation will detail the infrastructure and capabilities offered by the facilities and provide an overview of past research conducted at the facilities, as well as discuss how the facilities could be used going forward to investigate pertinent biocontamination-related issues. The WSTB offers a ~450', 8" diameter pipe main and a premise plumbing facility connected to the pipe main via a 1" service connection. The BOTE building is a two-story office building, 4,000 ft.^{2} per floor. The BOTE building has been used in the past to test decontamination of a biological contaminant dispersed by the building's HVAC facilities. It offers potential as a testing platform for validating models of how contaminants are transported in a commercial or residential dwelling, as well as for evaluating the efficacy of surface decontamination methods. The WSTB offers the ability to test water treatment and decontamination methods at large-scale. Currently, the facility is being upgraded to enable testing of cyber-physical intrusions. Both facilities offer flexibility to augment or modify existing capabilities to suit future research directions. The facilities are located in an isolated, secure location, enabling testing and training that would not be practical in a populated area.

SESSION 5: Biofilms and Antimicrobials

Continued metabolism of bacteria in antimicrobial-treated biofilms

Presenter: **Philip S. Stewart**, Regents Professor
Affiliation: Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Bacterial biofilms that have been treated with an antimicrobial agent often continue to respire and consume metabolic substrates following the treatment. The reduction in utilization of metabolic substrates or electron acceptors can be much smaller than the measured reduction in viable cells (Figure 1), leading to the question of whether the non-culturable cells in the biofilm are actually dead. Several examples of this phenomenon are examined spanning six species of bacteria and antimicrobial chemistries from hydrogen peroxide and monochloramine to antibiotics such as gentamicin, ciprofloxacin, and tigecycline. A binary species biofilm treated with monochloramine exhibited a 3.1 log reduction in viable plate counts with only approximately 30% reduction (corresponding to a 0.15 log reduction) in overall glucose uptake. A *Pseudomonas aeruginosa* biofilm treated with ciprofloxacin continued to consume oxygen and produce carbon dioxide as quantified by respirometry. In this example, the log reduction in viable cells was 3.4 whereas the log reduction in carbon dioxide production rate was 0.24. A *Staphylococcus aureus* biofilm treated with daptomycin for 24 h under continuous flow conditions experienced a 1.36 log reduction in viable cell numbers while the combined consumption of amino acids, glucose, and lactate dropped by 66% (a 0.47 log reduction). Other examples of the discrepancy between reduction in viable cells and catabolic activity have been demonstrated with oxygen microelectrode measurements and by ^{19}F oximetry. Three mechanisms that could be behind these effects are discussed.

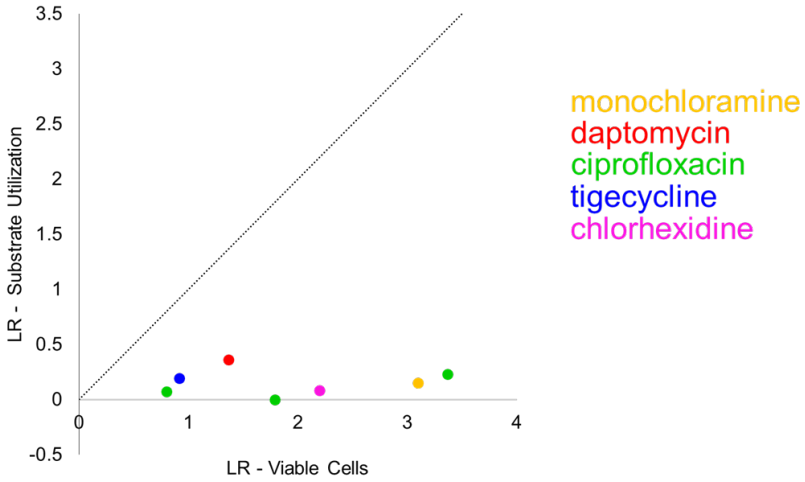

Figure 1. The log reduction (LR) in the rate of substrate utilization is much smaller than the LR in viable cells for bacterial biofilms treated with antimicrobial agents.

The *Pseudomonas aeruginosa* biofilm matrix and antimicrobial tolerance

Presenter: **Matt Parsek**, Professor
Affiliation: Microbiology, University of Washington, Seattle, WA, USA.

Biofilm cells are more tolerant to antimicrobials than their planktonic counterparts. While mechanisms underpinning this phenomenon are multifactorial, the ability of the matrix to reduce antibiotic penetration into the biofilm is thought to be one explanation for some antibiotics. We monitored the transport of two clinically relevant antibiotics, tobramycin and ciprofloxacin, into non-mucoid *P. aeruginosa* biofilms. We found that the positively charged antibiotic tobramycin is sequestered to the biofilm periphery, while the neutral antibiotic ciprofloxacin readily penetrated. We provide evidence that tobramycin in the biofilm periphery both stimulated a localized stress response and killed bacteria in these regions but not in the underlying biofilm. These data suggested that ionic interactions of tobramycin with the biofilm matrix limit its penetration. We propose that tobramycin sequestration at the biofilm periphery is an important mechanism in protecting metabolically active cells that lie just below the zone of sequestration. Another potential factor that contributes to antimicrobial tolerance is the activity of biofilm matrix proteins. *Pseudomonas aeruginosa* produces an extracellular biofilm matrix that consists of nucleic acids, exopolysaccharides, lipid vesicles, and proteins. In general, the protein component of the biofilm matrix is poorly defined and understudied relative to the other major matrix constituents. While matrix proteins have been suggested to provide many functions to the biofilm, only proteins that play a structural role have been characterized thus far. We identified the serine protease inhibitor ecotin (PA2755) as a key matrix protein. This protein is able to inhibit neutrophil elastase, a bactericidal enzyme produced by the host immune system during *P. aeruginosa* biofilm infections. We show that ecotin binds to the key biofilm matrix exopolysaccharide Psl and that it can inhibit neutrophil elastase when associated with Psl. Finally, we show that ecotin protects both planktonic and biofilm *P. aeruginosa* cells from neutrophil elastase-mediated killing. This may represent a novel mechanism of protection for biofilms to increase their tolerance against the innate immune response.

The immediate and urgent need for products with broad antimicrobial activity and how to get there

Presenter: **Alicia Tetlak**, Director
Co-authors: Judith Mitchell, Chief Executive Officer; Matthew F. Myntti, Chief Technology Officer
Affiliation: Biological Sciences, Next Science, Jacksonville, FL, USA.

The emergence of COVID-19 and the lack of effective treatments for this pathogen has revealed that there is a gap in the healthcare community's arsenal of medicines to treat non-specific organisms. The lack of broad-spectrum antimicrobials in the market contributed to the establishment of quarantine and social distancing mandates as the only viable option of reducing viral spread during this pandemic. It has become apparent that better treatments for broad-spectrum microbial infections must be developed. Viruses must use host cells to replicate, making the development of antiviral treatments extremely difficult. At present, the most effective way to prevent the spread of viral pathogens is through the vaccination of the host population. If there are no vaccines available, the patient's own immune system must independently identify the invading pathogen and develop antibodies to prevent viral proliferation. Even with an appropriate vaccine, the body may fail to recognize the virus or infected cells. If this occurs, protein inhibitors may be used to reduce the duration of illness however data demonstrating effectiveness have been limited. Because treatments are often strain specific and viral mutation occurs rapidly, current regimens are ineffective at treating emerging viruses. Due to these constraints, the most responsible and most commercially viable outcome is to develop treatments that destroy viral groups rather than specific strains, treatments that target broad species of bacteria that often complicate the viral infections, and treatments that protect the body from the viral infection. Since many emerging viruses attack the body through the respiratory tract, treatments should be focused there. The first proposed modality is a nebulized broad-spectrum antimicrobial treatment to reduce the bio-load in the patient's lungs. This treatment must be safe for long-term exposure to the respiratory tissues, especially the alveoli which are already stressed by the presence of the infection, while simultaneously attacking the bacteria and viruses that infect the lung. Pneumonia is the most common cause for mortality in these patients with these types of viral infection. The thickening of the alveoli tissue will decrease the amount of oxygen available to the rest of the body thereby resulting in patient death by asphyxiation. As such, treating this condition can vastly improve patient outcomes. The current treatments for viral pneumonia are ventilation to increase the available oxygen for the patient and steroids to reduce inflammation which have exhibited minimal efficacy and side-effects, including a decrease in the patients' ability to fight off the very infection that is killing them. As such, a

second possible modality for the development of treatments is the development of anti-inflammatories that can be applied systemically or preferentially topically to the lungs to prevent alveolar thickening.

Evaluation of ND-7901 (HT-01): A fast-acting bactericidal antibacterial agent with broad activity

Presenter: **Garrett C Moraski**[1], Research Scientist
Co-authors: Jane A Thanassi[2], Steven D Podos[2], Michael J Pucci[2,3], William D Claypool[4], and Marvin J Miller[4,5]
Affiliation: [1]Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA. [2]Achillion Pharmaceuticals, New Haven, CT, USA. [3]Spero Therapeutics, Cambridge, MA, USA. [4]Hsiri Therapeutics, Media, PA, USA. [5]Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA.

ND-7901 (HT-01) is a small molecule antibacterial agent (molecular weight = 245.2 amu) with pan activity against a host of bacterial strains. It is bactericidal and inhibits multiple targets (RNA, DNA and protein synthesis). It is endowed with fast time kill kinetics and an extremely low frequency of spontaneous mutant formation (MRSA < $6\text{-}10$). It has excellent potency against Gram-positive bacteria (>20 strains tested with an MIC range of 0.5 to 4 mg/mL) including drug resistant and MRSA biofilms. It has excellent potency against anaerobic bacteria (>50 strains tested with an MIC range of 0.015 to 4 mg/mL). It has variable potency against Gram-negative strains (>20 stains tested with an MIC range of 2 to >64 mg/mL) and Mycobacteria (5 strains tested with an MIC range of 4 to >50 mg/mL). Finally, ND-7901 (HT-01) is a robust chemical structure easily detectable by UV light, stable to strong acid (HCl) and has a long shelf life even (>1 year) even as a solution.

D7: A highly effective, broad-spectrum disinfectant for use against pathogenic organisms and biofilms in food safety applications

Presenter: **Mark Tucker**, Chief Scientific Officer
Affiliation: Decon7 Systems, Scottsdale, AZ, USA.

D7 is an aqueous-based disinfectant that can rapidly neutralize highly toxic chemical and biological materials. The formulation:

- is effective for killing pathogenic organisms including vegetative and spore-forming bacteria, viruses, biofilms, and fungi and for neutralizing highly toxic chemicals;
- utilizes very mild ingredients that gives it very low toxicity and corrosivity properties;
- can be deployed as a foam, liquid spray, or fog on a wide variety of materials and surfaces.

D7 contains surfactants, mild solvents, inorganic salts, a low concentration of hydrogen peroxide (~3.5%), a hydrogen peroxide activator, and water. The surfactants soften the cell walls of pathogens which allows the activated peroxide to penetrate to the interior for complete kill. This unique combination of mild ingredients works synergistically to kill persistent biological pathogens which has been demonstrated in testing at numerous government and private facilities and in many field applications – outperforming formulations that contain much harsher chemicals. The D7 chemistry is licensed by Decon7 Systems from Sandia National Laboratories where it was originally developed to decontaminate chemical and biological warfare agents for military and homeland security applications. Decon7 Systems has greatly expanded its use to many other applications. Recent laboratory testing and field applications have demonstrated that D7 has high efficacy against pathogens of concern in agriculture and food processing such as *Listeria*, *E. coli*, *Salmonella*, the African Swine Fever virus, the Porcine Epidemic Diarrhea Virus, *Salmonella* and *E. coli* biofilms, Avian Influenza, and *Bacillus* spores. These results clearly demonstrate that the use of D7 can significantly improve food safety. D7 is also highly effective against biofilms which has been demonstrated in both laboratory testing and field applications. The surfactants and mild solvents in D7 enable it to penetrate and lift biofilms off of surfaces allowing the reactive species in the formulation to attack and kill the pathogenic organisms. This penetrating and lifting action allows D7 to effectively disinfect surfaces containing biofilms and other organic material where other disinfectants fail. The results of laboratory studies and field application for D7 against biofilms will be the primary topic of this presentation.

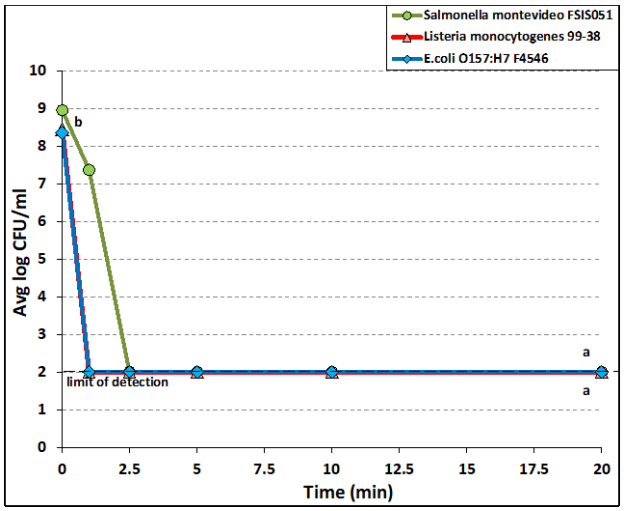

Figure 1. Removal of biofilms and kill of embedded bacteria by the D7 Disinfectant.

Influence of material type and coating on biofilm accumulation by an ISS isolate

Presenter: **Madelyn Mettler**[1], Graduate student
Co-authors: Ceth Parker[2], Post-doc researcher
Brent M. Peyton[1], Professor of Chemical & Biological Engineering
Kasthuri Venkateswaran[2], Senior Research Scientist
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA.

Studies of biofilm growth and prevention are extensive in medical and industrial systems on Earth. It is known that biofilms can exist in almost every microenvironment on Earth, but they also extend to human habitable systems such as International Space Station (ISS) and crew vehicles used in space travel. Biofilms found on the ISS pose increased threats to human health and equipment integrity compared to those found on Earth due to the difficulty of medical care, equipment replacement, and regular cleaning in space. A better understanding of how biofilms differ on earth and in space and how they can be treated or prevented is necessary for prolonged space travel to places such as Mars or for a flight base on the Moon. This project aims to evaluate the effect of material type on biofilm growth of microbial isolates from the ISS. Additionally, the project includes the evaluation of a novel antimicrobial coating intended to reduce biofilm growth. The materials evaluated include uncoated controls and antimicrobial-coated stainless steel, Teflon, Inconel (nickel-chromium alloy), and titanium. The tests were completed using slight modifications to the ASTM E3161-18 Standard Practice for Preparing a *Pseudomonas aeruginosa* or *Staphylococcus aureus* biofilm using the CDC Biofilm Reactor. Results from *P. aeruginosa* using the system will be presented as well as initial investigation of *Rhodotorula mucilaginosa*, a yeast commonly found on the ISS. The data collected to date indicate the novel antimicrobial coating significantly reduced biofilm growth and planktonic cell growth for at least two days. Longer term tests are ongoing.

Simulated microgravity experimentation and molecular mechanism behind biofilm formation in ISS isolates

Presenter: **Ceth Parker**[1], Postdoctoral Researcher
Co-authors: Robert Daudu[1], Madelyn Mettler[2], Brent M. Peyton[2], Kasthuri Venkateswaran[1]
Affiliation: [1]Jet Propulsion Laboratory, NASA, Pasadena, CA, USA. [2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

As NASA seeks to send humans back to the moon and deeper into space, expanding our understanding of microgravity's (µGravity) effects on the adaptation of microorganisms to µGravity environments will be essential to protect crew health and space-habitat longevity. Microorganisms that form biofilms can pose a unique risk to both currently crewed (International Space Station [ISS]) and future (Lunar Gateway) space habitats as these organisms can clog and incapacitate water systems required for waste water management, human consumption, and environmental control (humidity etc.). Numerous sampling campaigns have isolated a variety of bacteria,

fungus, and yeast from the ISS; with *Rhodotorula mucilaginosa* as the most common yeast cultured. *R. mucilaginosa,* a biofilm forming reddish-pink pigmented yeast has been implicated in hospital associated opportunistic infections. Here we show evidence of increased biofilm production when *R. mucilaginosa* and other ISS fungal isolates are grown under simulated μGravity in a three-dimensional clinostat, as compared to standard gravity controls. Scanning Electron Microscopy (SEM) analysis has demonstrated that *R. mucilaginosa* can produce a variety of morphologies that may aid in biofilm formation, including high production levels of exopolysaccharide and a switch from a smooth yeast cell body to a "hairy" morphology with yeast cells covered in fine filaments. Additionally, carotenoid pigmentation has previously been identified to aid in radiation protection and biofilm formation, and despite two distinctive coloration morphologies (light red/low viscosity and dark red/higher viscosity), genetic analysis of conserved marker genes (ITS, CytB, and D1-D2) indicates that all isolates of *R. mucilaginosa* are within the same clade. Further multi-omics analyses are planned to better understand the molecular mechanisms and how their differential expression increases the biofilm formation ability of the yeast under simulated μGravity conditions.

MONTANA BIOFILM VIRTUAL MEETING

Montana Biofilm Science & Technology *Virtual Meeting*

CENTER FOR BIOFILM ENGINEERING 30th ANNIVERSARY 1990 - 2020

July 14-16, 2020

MONTANA STATE UNIVERSITY

7/1/2020 3:46 PM

Draft AGENDA

****All times are Mountain Daylight Time (MDT)**

Tuesday July 14

9:15–9:25
Opening Remarks
Matthew Fields, CBE Director, Professor, Microbiology & Immunology, MSU
Paul Sturman, CBE Industrial Coordinator

SESSION 1: Biofilm Dynamics

9:25–9:30
Session Introduction
Matthew Fields

9:30–10:00
Regulatory mechanisms and effectors leading to biofilm dispersion
Karin Sauer, Chair, Dept. of Biological Sciences; Co-Director, Binghamton Biofilm Research Center, Binghamton University

10:00–10:30
Identifying causative relationships and active populations in polymicrobial communities
Matthew Fields

10:30–11:00
What we know and don't know about viruses in biofilms
Elinor Pulcini, Assistant Research Professor, Chemical & Biological Engineering, MSU, CBE

11:00–11:30 Break

SESSION 2: CBE Paths Forward

11:30–12:00
State of the CBE
Matthew Fields

12:00–12:30
Pathways to Innovation: Growing a Regulatory Science Program at the CBE
Darla Goeres, Research Professor of Regulatory Science, MSU; PI Standardized Biofilm Methods Laboratory, CBE

12:30–1:00 Discussion

Wednesday July 15

9:15–9:25
Opening Remarks
Matthew Fields
Paul Sturman

SESSION 3: Biofilm Methods

9:25–9:30
Session Introduction
Darla Goeres

9:30–10:00
Development of a biofilm model on in vitro colonized epidermis
Samantha Westgate, CEO, Perfectus Biomed

10:00–10:30
Biofilm sensing: An engineering overview
Stephan Warnat, Assistant Professor, Mechanical & Industrial Eng., MSU, CBE

10:30–11:00
Experimental designs to quantify early aggregation and colonization of biofilms with statistical confidence from confocal images
Brian Pettygrove, PhD Candidate, Microbiology & Immunology, MSU, CBE
Al Parker, Biostatistician, CBE; Assoc. Research Professor, Mathematical Sciences, MSU

11:00–11:30 Break

SESSION 4: Field Studies

11:30–11:35
Session Introduction
Paul Sturman

11:35–12:05
Multi-domain biofilm growing systems and their potential application
Erika Espinosa-Ortiz, Asst. Research Professor, Chemical & Biological Eng., MSU, CBE

12:05–12:35
Microbially induced calcium carbonate precipitation (MICP): Bio-cement's journey from the laboratory to the field
Catherine Kirkland, Assistant Professor, Civil Engineering, MSU, CBE
Adie Phillips, Assoc. Professor, Civil Engineering, MSU, CBE

12:35–1:05
Large scale water and facility decontamination testing capabilities
Steve Reese, Research Engineer, Idaho National Laboratory

1:05–1:30 Discussion

Thursday
July 16

9:15–9:25
Opening Remarks
Matthew Fields
Paul Sturman

SESSION 5: Biofilms and Antimicrobials

9:25–9:30
Session Introduction
Phil Stewart, Regents Professor, Chemical & Biological Engineering, MSU, CBE

9:30–10:00
Continued metabolism of bacteria in antimicrobial-treated biofilms
Phil Stewart

10:00–10:30
The *Pseudomonas aeruginosa* biofilm matrix and antimicrobial tolerance
Matt Parsek, Professor, Microbiology, University of Washington

10:30–11:00
The immediate and urgent need for products with broad antimicrobial activity and how to get there
Alicia Tetlak, Director, Biological Sciences, Next Science

11:00–11:30 Break

11:30–12:00
Evaluation of ND-7901 (HT-01): A fast acting bactericidal antibacterial agent with broad activity
Garrett Moraski, Research Scientist, Chemistry & Biochemistry, MSU

12:00–12:30
A highly effective, broad spectrum disinfectant for use against pathogenic organisms and biofilms in food safety applications
Mark Tucker, Chief Scientific Officer Decon7 Systems

12:30–12:50
Influence of material type and coating on biofilm accumulation by an ISS isolate
Madelyn Mettler, Research Assistant, Chemical & Bio. Eng., MSU, CBE

12:50–1:10
Simulated microgravity experimentation and molecular mechanism behind biofilm formation in ISS isolates
Ceth Parker, Postdoctoral Researcher NASA Jet Propulsion Laboratory