ASM Washington, D.C. Branch
Spring Meeting 2021

Diseases in the World We Live In

April 16th – 9:30 to 4 pm ET
April 17th – 10 to 4 pm ET

VIRTUAL

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Level: Intermediate

Contact Hours: 2.5

Fee: FREE students/postdocs, $8 professionals

ASM Washington, D.C. Branch Spring Meeting 2021

Diseases in the World We Live In

Friday, April 16, 2021

Zoom link was emailed to all registered attendees

Time		Description	
9:30	9:45	***Welcome***	Mary Brockett President, Uniformed Services University ASM Student Chapter
9:45	10:15	***Early Career Talk*** Identification of Multiple *Staphylococcus aureus* Secreted Factors Sensed by *Pseudomonas aeruginosa*	Tiffany M. Zarrella, Ph.D. National Cancer Institute National Institutes of Health
10:15	11:15	***Invited Speaker*** In Spite of Barriers and Struggles, Early and Later African Americans Microbiologists Have Contributed	Marian Johnson-Thompson, Ph.D. Professor Emerita, University of the District of Columbia Adjunct Professor, University of North Carolina at Chapel Hill
11:15	12:00	***Lunch***	
12:00	1:00	***Invited Speaker*** **Mining for Gold: An Exploration for Antibiotic Discovery**	Danielle Graham, Ph.D. Assistant Professor and Assistant Chair Department of Biological and Forensic Sciences Fayetteville State University
1:00	1:30	***Early Career Talk*** Characterizing Novel Mutations Leading to Antibiotic Resistance in *Staphylococcus aureus*	Kalinga Pavan Thushara Silva, Ph.D. Postdoc Center for Cancer Research National Cancer Institute
1:30	3:30	***Career Panel and Q&A ****	
		Brandon R. Anjuwon-Foster, PhD Associate Research Scientist PPD	Dominique M. Carter, PhD Agricultural Science Advisor U.S. Department of Agriculture	Danielle Graham, Ph.D. Assistant Professor Fayetteville State University
		Jocelyn R. Hauser, Ph.D., D(ABMM) Chief, Molecular Diagnostics Unit DC Public Health Laboratory	Sarah L. Hansen STEM Communications Manager University of Maryland, Baltimore County	Rita Tamayo, PhD Associate Professor University of North Carolina
3:30	3:45	**Concluding Remarks**	Christopher M. Healy Vice President, Uniformed Services University ASM Student Chapter	

**** The Career Panel will be held via Zoom breakout rooms.*** *At the start of the session, attendees will self-select a room to interact with the panelist then every 20 min attendees will be asked to change rooms.*

ASM Washington, D.C. Branch Spring Meeting 2021

Diseases in the World We Live In

Saturday, April 17, 2021

Zoom link was emailed to all registered attendees

Time		Description	
9:45	10:00	***Check-In***	
10:00	10:15	***Welcome***	Kileen Shier, Ph.D., D(ABMM), MLS(ASCP)^{CM} President, DC Branch of ASM
10:20	11:20	***ASM Distinguished Lecturer*** When a clone is not a clone: phenotypic heterogeneity in *C. difficile*	Rita Tamayo, Ph.D. Associate Professor Department of Microbiology and Immunology University of North Carolina at Chapel Hill
11:20	11:40	***Early Career Talk*** Characterization of the role of pstB1 in *Enterococcus faecalis* membrane stress, biofilm formation, and inorganic phosphate uptake	Christopher M. Healy Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences
11:40	12:00	***Early Career Talk*** This way out: Regulation of *Vibrio cholerae* biofilm dispersal	Andrew Bridges, Ph.D. Postdoc, HHMI Fellow Princeton University
12:00	1:00	***Lunch***	
1:00	1:30	***Invited Speaker*** Health Security: Where We've Come From & Where We're Headed	Matthew Watson, M.S. Senior Analyst, Johns Hopkins Center for Health Security Senior Research Associate, Johns Hopkins Bloomberg School of Public Health
1:35	2:05	***Invited Speaker*** Reverse genetic systems for viruses using synthetic genomics	Lauren Oldfield, Ph.D. Assistant Professor Department of Synthetic Biology J. Craig Venter Institute
2:10	2:30	***Lightning Talks***	
2:30	2:45	***Break***	
2:45	3:45	***Poster Session ****	
3:45	4:00	**Concluding Remarks**	Kileen Shier, Ph.D., D(ABMM), MLS(ASCP)^{CM} President, DC Branch of ASM

**** The Poster Session will be held via Zoom breakout rooms.*** *Each virtual poster will be in a single breakout room and at the start of the session, attendees will self-select a room to interact with the presenter and attendees can move between posters freely.*

VIRTUAL POSTER PRESENTATIONS

***Early Career Lightning Talks**

Breakout Room	Lead Author	Title
1 – Atitkar *	Atitkar, Rama Uniformed Services University	Clinical Isolates of Shiga Toxin-producing *Escherichia coli* ith Similar Virulence Gene Profiles Exhibit Differences in Toxin Release and Virulence
2 – Englander *	Englander, Hanna UConn Health, University of Connecticut	Engineered Probiotic to Combat Antibiotic Persistence
6 – Gautam	Gautam, Pratima University of Maryland Baltimore County	Enrichment of Copper Resistant Genes in *Alteromonas macleodii* strain CUKW
7 – Leonard	Leonard, Heidi Pathotrak, Inc.	Rapid Detection of *E. coli* O157:H7, *Salmonella*, and STEC in Leafy Greens and Meats using Pathotrak's Next Generation Enrichment
5 – Magalona	Magalona, Kim George Mason University	The Microbiome Characterization of *Morone saxatilis* as a Tool to Detect Infection with Mycobacteria
8 – Morin	Morin, Katherine George Mason University	Predator vs. Prey: The Lethality of *Vibrio fluvialis* on Highly Pathogenic *Vibrio* Species
3 – Shipley *	Shipley, Alicia U.S. Food and Drug Administration	Detection of *Cyclospora cayetanensis* in Mixed Bagged Pre-Cut Salads by the FDA Bacteriological Analytical Manual (BAM) Chapter 19b Method
9 – Simpson	Simpson, Casey George Mason University	Type Vi Secretion System Facilitates Intraspecies Killing in *V. Vulnificus* Via the Associated Rhs Toxin and Antitoxin Systems
4 – Soare *	Soare, Alexandra Y. University of Maryland, Baltimore	Immune Evasion Mechanisms by Mucormycosis Causing Fungi
10 – Van Nederveen	Van Nederveen, Viktoria Uniformed Services University	Investigating the Role of SepA in Enteroaggregative *Escherichia coli* Biofilm
11 – Wartell	Wartell, Brian A. University of Maryland College Park	Determining SARS-CoV-2 Levels in Wastewater from 5 Treatment Plants in Southern Maryland
12 – Healy	Healy, Christopher Uniformed Services University	Characterization of the role of pstB1 in *Enterococcus faecalis* membrane stress, biofilm formation, and inorganic phosphate uptake

Clinical Isolates of Shiga Toxin-producing *Escherichia coli* With Similar Virulence Gene Profiles Exhibit Differences in Toxin Release and Virulence

Rama Atitkar[1,2], Jocelyn Hauser[1,2], Courtney Petro[1,2], and Angela Melton-Celsa[1]

[1]Uniformed Services University, Bethesda, MD

[2]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) has an estimated global diarrheal burden of about 1 million cases and is the leading infectious cause of hemolytic uremic syndrome (HUS). STEC strains with Stx subtypes 2a+ or 2a+2c+ are associated with more severe disease presentation and HUS in humans. Previous research found that O157:H7 strains with similar virulence gene profiles that make the same types of Stx can show differences in toxin production and mouse virulence. Isolate JH2010 (2a+2c+) was lethal in a streptomycin-treated mouse model, while JH2012 (2a+) was avirulent. JH2010-infected mice had significantly higher fecal cytotoxicity compared to JH2012-infected mice; this difference was not dependent on colonization or production of Stx2c. JH2010 cultures had higher cytotoxicity than JH2012 in the supernatant fraction, while JH2012 had higher cytotoxicity than JH2010 in the cell pellet fraction. Since Stxs are encoded on lysogenic bacteriophage and toxin is released when the bacteriophage enters the lytic cycle, we hypothesized that JH2012 has a lysis defect as compared to JH2010. Initial sequence analyses of the stx_{2a}-phage regions from JH2012 allowed identification of strain PA8 (2a+), with similar stx_{2a}-phage sequences to JH2012; however, PA8 does not exhibit lowered supernatant cytotoxicity like JH2012. Further comparison of the stx_{2a}-phage sequences between JH2012 and PA8 identified a late phage region of dissimilarity containing genes involved in host cell lysis. These results support our hypothesis that host cell lysis impacts Stx release from the bacterium, and that mutations in the phage lytic proteins may contribute to the variable virulence of STEC.

Engineered Probiotic to Combat Antibiotic Persistence

Hanna Englander[1,2] and Dr. Wendy W.K. Mok[1]

[1]Department of Molecular Biology & Biophysics, UConn Health

[2]Department of Physiology & Neurobiology, University of Connecticut

Antibiotic persistence poses a threat to infectious disease treatment. Persistence refers to a non-inherited phenotypic state that enables bacteria to transiently tolerate an antibiotic and survive treatment. After the course of antibiotic treatment is over, persisters can regrow and contribute to infection relapse. We previously discovered that persisters from stationary phase cultures that survive fluoroquinolone treatment need to engage in DNA repair as they recover after the course of treatment is over. An effective therapy to combat the rise of antibiotic treatment failure could be one that prevents persister cell recovery. In past experiments, we found that the probiotic strain *E. coli* Nissle (EcN) can kill *E. coli* persisters as they recover from treatment. Now, we want to assess whether EcN will be able to produce these same results when they are administered to a pathogenic *E. coli* strain at the onset of fluoroquinolone treatment. To ensure survival of EcN, we created model persisters of EcN with an inducible toxin-antitoxin system in which accumulation of the toxin has been linked to increased persistence. When we treated the engineered EcN strain with two different classes of antibiotics, we found that the toxin-accumulating strain were uniformly tolerant to two classes of antibiotics, fluoroquinolones and β-lactams. This data indicates that we produced model probiotic persister populations with our engineered EcN strain. We envision that these EcN model persisters can potentially be a promising new adjuvant to combat antibiotic treatment failure and eradicate persisters as they reawaken from antibiotic therapy.

Enrichment of Copper Resistant Genes in *Alteromonas macleodii* strain CUKW

Pratima Gautam, Ivan Erill, and Kathleen Cusick

University of Maryland Baltimore County, Baltimore, MD 21227

Copper-based antimicrobial paints are frequently used to inhibit the biofouling of marine vessels. However, some bacterial species are able to overcome this copper challenge and form the initial biofilm. *Alteromonas* species are one of the early colonizers of copper-based antifouling surfaces; but little is known about the mechanism(s) they use to overcome the copper challenge. Our lab isolated a strain of *A. macleodii* (CUKW) from copper test coupons, used for testing the effects of the coating process on substrate material, by suspending them in seawater at the US Naval Research Lab Test Facility in Key West. CUKW grows at copper concentration lethal to most microbes. To understand their mechanism of copper homeostasis, PacBio whole genome sequencing of CUKW was performed, and comparative genomic study was carried out. The main model of copper homeostasis includes *Escherichia coli* chromosome-based Cue and Cus systems and the plasmid-based Pco system, and the *Pseudomonas syringae* plasmid-based Cop system. These were first studied in strains isolated from copper rich environments. Our study suggests that CUKW harbors genetic elements from all these systems, often as multiple copies. Expression profiling of copper-associated genes showed induction of multiple plasmid-borne genes when grown in presence of copper. Two copies of plasmid-borne copA which is known to be the key player in cytoplasmic copper detoxification was highly induced. Genomic analysis showed presence of a nearly identical gene cluster surrounding the two copA copies with a putative regulator immediately upstream of copA. One of the copA variants occurred in a region identified as genomic island, signifying a horizontal transfer. Overall, genomic analysis showed that CUKW possesses an enhanced genetic repertoire of homologs of copper resistance systems, and plasmid-based genes from the multiple systems were induced when grown in the presence of elevated copper levels at static liquid cultures.

Rapid Detection of *E. coli* O157:H7, *Salmonella*, and STEC in Leafy Greens and Meats using Pathotrak's Next Generation Enrichment

Heidi Leonard and Javier Atencia

Pathotrak, Inc, College Park, MD 20742

With increasingly stringent microbial testing requirements comes the need for a pathogen detection method that can be completed during a single shift of a microbiology lab. Thus, we have tackled the time-consuming bottleneck of food safety testing, namely enrichment or incubation time. We developed a Next Generation Enrichment technology to reduce enrichment time from 8-24 hours down to 4 hours for the detection of single CFU's of *Salmonella*, *E. coli* O157:H7, and non-O157 STEC. By streamlining Pathotrak's Next Generation Enrichment with qPCR, we have demonstrated that microbial contamination can be identified in 375 g of various leafy greens and meat products within 6 hours of sample reception.

The Microbiome Characterization of *Morone saxatilis* as a Tool to Detect Infection with Mycobacteria

Kim Magalona and Jennifer Salerno

Environmental Science and Policy, George Mason University, Fairfax, VA

Striped bass, *Morone saxatilis*, is an important species for commercial and recreational fishing in the Chesapeake Bay region. However, the population has been in decline since 2004.Since 1997, *M. saxatalis* in the Chesapeake Bay have been impacted by an outbreak of mycobacteriosis, a bacterial disease that infects the spleen and kidneys, leading to emaciation, lower growth rates, increased natural mortality, decreased reproductive output, and the development of skin ulcers in a small percentage of infected fish. The underlying cause of this outbreak is unknown; however, it has been proposed that it may be due, in part, to stress associated with a decrease in the main food source of striped bass, Atlantic menhaden, *Brevoortia tyrannus* (Jacobs, 2007). Menhaden are also capable of being infected by mycobacteriosis, which could potentially be serving as a disease vector to other species (Kane et al., 2007). Further complicating disease investigations are outstanding questions regarding the identity and nature of the possible pathogens responsible for causing mycobacteriosis. The disease can be polymicrobial in nature, meaning that it may involve more than one species or phylotype of mycobacteria, or could be the result of dysbiosis, a departure from the typical microbiome composition during healthy, non-stressed or non-diseased states (Rhodes et al., 2004). Despite the increasing decline of the striped bass population, relatively few studies have investigated the role of the microbiome, or the whole community of associated microbes, in disease mediation and/or detection. Bacteria respond and adapt quickly to environmental changes, making them potential indicators of organism and ecosystem health. Shifts in striped bass microbiome composition may reflect a change in health state prior to the external presentation of disease signs. My proposed study aims to characterize the microbiome composition of different tissues from fish not infected with mycobacteria and mycobacterium-infected striped bass to determine if there is a relationship between whole microbiome composition and mycobacterial infection. Samples will be taken from the skin, spleen, kidney, and gut tissues of *Morone saxatilis* in Chesapeake Bay. My sampling scheme will be based on documented observations of 75% mycobacteriosis disease prevalence in the Bay. It is currently estimated that <30% of infected fish exhibit exterior symptoms such as skin ulcers, but most have spleen lesions which can only be assessed by euthanizing fish and performing necropsies (Overton et al., 2003). Our objective is to determine if mycobacteriosis infection is correlated with the microbiome composition of skin swab samples taken prior to, or in the absence of, lesion presentation, which could lead to the development of a less invasive tool for confirming infection. Such a diagnostic test will allow for easier detection of infected fish and a better understanding of the number of infected individuals in the striped bass population. More effective management and policies can also be informed by this knowledge to start the recovery of the species in the Atlantic.

Predator vs. Prey: The Lethality of *Vibrio fluvialis* on Highly Pathogenic *Vibrio* Species

Katherine Morin[1], Rachel Canty[2], and Brett Froelich[1,3]

[1]George Mason University, School of Systems Biology, Fairfax, VA

[2]University of North Carolina, NC

[3]George Mason University, Department of Biology, Fairfax, VA

Vibrio parahaemolyticus and *Vibrio vulnificus* are two species of bacteria that are responsible for a large percentage of gastroenteritis and necrotizing fasciitis cases as a result of consuming pathogen-containing raw oysters. *Vibrio fluvialis* is a gram-negative bacterium with bent-rod morphology, capable of killing these disease-causing species of *Vibrio* via type VI secretion contact-dependent killing. If *V. fluvialis* were to solely kill the pathogenic *Vibrio* species, but not non-pathogenic bacteria, it could potentially serve as a probiotic in oysters prior to human consumption. The purpose of this research is to test the extent of lethality of *V. fluvialis*, including how many strains of *Vibrio* species it kills as well as strains non-pathogenic bacteria of different classification. This process involves growing bacteria in co-culture competition assays to test how deadly *V. fluvialis* is against other bacteria. Preliminary data using a competition assay indicated cessation of growth in *V. vulnificus* after five hours of competition with *V. fluvialis* ($P<0.0001$). This study will also investigate the lethality of *V. fluvialis* against *V. parahaemolyticus* using competition assays at different bacterial concentrations. In addition, metagenomic analysis of a lab-grown oyster colony before and after inoculation of varying concentrations of *V. fluvialis* will be investigated to determine probiotic potential.

Detection of *Cyclospora cayetanensis* in Mixed Bagged Pre-Cut Salads by the FDA Bacteriological Analytical Manual (BAM) Chapter 19b Method

Alicia Shipley[1,2] and Sonia Almeria[1]

[1]U.S. Food and Drug Administration, Joint Institute for Food Safety and Applied Nutrition

[2]University of Maryland, College Park, MD

Recent *Cyclospora cayetanensis* outbreaks were linked to the consumption of salads containing romaine lettuce and carrots in 2018, and bagged salad mixes containing iceberg lettuce, carrots, and red cabbage in 2020. The FDA Bacteriological Analytical Manual (BAM) Chapter 19b method was validated in carrots and romaine lettuce by matrix extension studies but has not been evaluated in mixed pre-cut salads containing these ingredients. In this study, the BAM Chapter 19b method was evaluated in two ready-to-eat (RTE) mixed salads. Twenty-five-gram samples of pre-cut mixed salad 1 (containing romaine and iceberg lettuce, carrots and red cabbage) and mixed salad 2 (containing romaine and iceberg lettuce, carrots, red cabbage, radish and pea pods) were seeded with 5 and 200 *C. cayetanensis* oocysts. Unseeded produce was used as negative control. The method included washing of the produce, extraction of *C. cayetanensis* DNA, and molecular detection using a Taqman assay targeting the 18S rRNA gene with an internal amplification control (IAC). As few as five oocysts were detected in both mixed salads (n=10 in each type) with positive detection rates of 30% and 60%, respectively for mixed salad 1 and mixed salad 2. All unseeded salad samples were negative, and all salad samples seeded with 200 oocysts (n=7 in each type) were positive. Statistically significant differences were observed in 18S rRNA *C. cayetanensis* CT values in samples seeded with 200 oocysts between both salads ($p<0.05$). The results showed that the method was robust, reproducible and able to detect as few as 5 oocysts in RTE salads.

Type Vi Secretion System Facilitates Intraspecies Killing in *V. Vulnificus* Via the Associated Rhs Toxin and Antitoxin Systems

Casey Simpson and Brett Froelich

George Mason University, Department of Biology, Fairfax, VA

Vibrio vulnificus is an emerging and lethal human pathogen that causes a wide array of symptoms, including gastrointestinal symptoms and blistering skin lesions. Most of these infections are transmitted by coming into contact with raw shellfish or contaminated water that is colonized with these pathogenic bacteria. There are many secretion systems present in *V. vulnificus* which function in a variety of different ways including aiding in virulence. Most of these secretion systems are well characterized in *V. vulnificus* but the Type VI secretion system (T6SS) remains largely ambiguous. The T6SS works in a contact-dependent manner with a syringe-like apparatus injecting toxins into neighboring cells. We found that some strains of *V. vulnificus* significantly out-competed other strains in vitro by calculation the competitive index. *V. vulnificus* JY1306 outcompeted *V. vulnificus* MO6, with a competitive index of 2 ($p < 0.05$). most likely due to the effector proteins or toxins that are being secreted through the T6SS. Here we propose that the T6SS works alongside the Rhs toxins and antitoxins to facilitate intraspecies killing in *V. vulnificus*.

Immune Evasion Mechanisms by Mucormycosis Causing Fungi

Alexandra Y. Soare and Vincent M. Bruno

University of Maryland, Baltimore

Mucormycosis is a NIAID-classified emerging infectious disease caused by fungi belonging to the Order Mucorales. Hallmarks of disease progression include angioinvasion and tissue necrosis that often results in significant, irreversible tissue damage or death. While most cases are found in immunosuppressed individuals, mucormycosis is an increasingly common invasive fungal infection. Limited antifungal medications often leave surgical debridement as the only treatment option. Coupled with the unacceptably high mortality rate (70-100% depending on dissemination), there is a clear urgency to understand the host-pathogen interactions in the context of mucormycosis. Mucorales spores can resist killing by macrophages, immune cells that play a crucial role in host defense against microbes. Due to the paucity of knowledge on interactions between macrophages and Mucorales, we sought to characterize the macrophage response to Mucorales. Inducible nitric oxide synthase (iNos) is an enzyme expressed by macrophages in response to pathogens. Activation of iNos leads to the production of nitric oxide (NO), a free radical molecule which is toxic to many pathogens. Transcriptomic data from Mucorales-infected alveolar macrophages showed increased expression of iNos mRNA compared to uninfected macrophages. However, despite the increased expression of iNos mRNA, these macrophages were unable to produce NO, even when co-incubated with NO-producing stimuli. Our experiments demonstrate that this complete reduction of NO is not due to a lack of iNos protein accumulation or detoxification of NO by the fungi. Ongoing experiments are directed towards understanding the mechanism by which Mucorales mold inhibit NO production and how this immunosuppressive activity contributes to disease progression.

Investigating the Role of SepA in Enteroaggregative *Escherichia coli* Biofilm

Viktoria Van Nederveen[1,2] and Angela Melton-Celsa[1]

[1]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, MD

[2]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD

Enteroaggregative *E. coli* (EAEC) is associated with acute and chronic diarrhea. EAEC adheres in an aggregative pattern and creates thick biofilms on the intestinal mucosa, a process thought to contribute to diarrhea. Epidemiological data suggests that SepA, a serine protease autotransporter of Enterobacteriaceae (SPATE), is important for disease caused by EAEC. All SPATEs have a secreted protease domain. The genes for SepA and many of the proteins important for EAEC biofilm formation and aggregative adherence are encoded on the pAA plasmid. We deleted sepA in six clinical EAEC strains and assessed biofilm formation. We observed that four of the ΔsepA strains demonstrated increased biofilm staining compared to the wild-type control. However, all of the strains secreted similar amounts of SepA. We therefore hypothesized that SepA modulates biofilm formation via cleavage of a target present in some EAEC strains but not others. To determine if the pAA encodes the SepA target, we transferred the pAA from EAEC strain K261 or K261ΔsepA (shows enhanced biofilm staining) into commensal strain HS. We found that the pAA alone is sufficient to confer the biofilm staining phenotype found for the wt or ΔsepA mutant EAEC onto HS. We concluded that the target of SepA is encoded on the pAA. By understanding the function of SepA in biofilm formation, we hope to better understand the role of SepA in EAEC pathogenesis and uncover therapeutic targets or vaccine candidates.

Determining SARS-CoV-2 Levels in Wastewater from 5 Treatment Plants in Southern Maryland

Brian A. Wartell[1,3], Devrim Kaya[1,2], Debra Niemeier[1,3,] Birthe V. Kjellerup[1,3]

[1]University of Maryland College Park, Department of Civil and Environmental Engineering, College Park, MD

[2]Oregon State University, School of Chemical, Biological, and Environmental Engineering, Corvallis, OR

[3]Maryland Transportation Institute, College Park, MD

Wastewater surveillance of viral RNA, also known as Waste Based Epidemiology (WBE) provides unique insights not afforded by clinical testing alone. In this study, evaluation of analytical methods for SARS-CoV-2 detection in wastewater was performed with influent samples from five wastewater treatment plant (WWTP) locations in southern Maryland. The main purpose of this study was to analyze trends over time with how they corresponded to observed trends measured in the regions those WWTPs served. Wastewater samples were collected during the period of September 15, 2020 to March 15, 2021 by operators at the WWTPs. Populations served by the WWTPs ranged from 5,000-250,000, with an average of 136,000. The samples were 24 h flow-dependent composite samples collected in volumes of either 500 or 1000 mL. Samples were processed via Centrifugation at 3400g for 20 min to remove large solids, followed by ultrafiltration via Amicon Ultra-15 Centrifugal Filter Devices with a cut-off of 100 kDa. Duplicates were then combined and concentrated to a final volume of 1 mL. RNA was extracted via Quick-RNA^{TM} Miniprep kits. RNA was converted to cDNA and analyzed via RT-PCR. Prior to processing, all samples were spiked with 1 µL/1 mL of surrogate bovine respiratory syncytial virus (BRSV) as a process control. The specific plasmids targeted were N1 and N2. Results indicated predicted trends corresponding to SARS-CoV-2 levels observed in Maryland and calculated RNA levels were measured to range from 10^3-10^6 gc/L for N1 and $5x10^2$ - $4x10^5$ gc/L for N2, with average detected values of $1.27x10^5$ gc/L and $3.12x10^4$ gc/L, respectively. N2 values were lower than N1 values by one order of magnitude but is consistent within similar findings in the literature. The highest SARS-CoV-2 values reported tended to occur during early- to mid-January, when the state and much of the country was also experiencing a surge in the virus, thereby indicating this method and wastewater surveillance overall, to be a useful method to track viral RNA over specific areas through time.

Characterization of the Role of pstB1 in *Enterococcus faecalis* Membrane Stress, Biofilm Formation, and Inorganic Phosphate Uptake

Christopher M. Healy, Biko McMillan, Candace N. Rouchon, Scott D. Schaffer, Zahra Zubair-Nizami, and Kristi L. Frank

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences

Enterococcus faecalis is a Gram-positive gastrointestinal commensal that has emerged as a leading healthcare-associated opportunistic bacterium. Clinical isolates are often resistant to antibacterial agents and readily form biofilms, leading to infections that can require surgery to effectively treat. Understanding the genetic mechanisms responsible for *E. faecalis* biofilm formation and regulation is critical in finding novel methods to treat infections. We used recombination-based in vivo expression technology (RIVET) screens to identify *E. faecalis* promoters that are up-regulated in three biofilm conditions: rabbit foreign-body abscess and endocarditis, and in vitro-grown biofilms. A putative promoter upstream of pstB1 was identified in all three conditions, suggesting that pstB1 and the pst-phoU operon where it is found play an important role in enterococcal biofilms. The pst-phoU operon encodes a well-conserved ATP-powered inorganic phosphate (Pi) importer. Pi homeostasis and the pst-phoU operon are both linked to virulence in a large number of bacterial species. However, the roles of Pi and the pst-phoU operon in *E. faecalis* biofilms have not been determined. We have generated an in-frame ΔpstB1 deletion strain and have initiated phenotypic characterization studies to evaluate the role of pstB1 in membrane stress, biofilm formation, and Pi uptake. When compared to wild-type OG1RF, ΔpstB1 exhibited increased susceptibility to bile salts and detergent on solid medium. We were able to rescue the phenotype via complementation in trans with pstB1. When grown in a reduced-phosphate chemically defined medium (CDM), we observed an early biofilm-formation defect in ΔpstB1 compared to OG1RF, which was partially rescued with complementation. Finally we have identified that ΔpstB1 displays a diminished capacity to take up Pi when compared to OG1RF and complemented ΔpstB1. These findings demonstrate that Pi and pstB1 plays an important role in the ability of *E. faecalis* to form biofilms, and that ΔpstB1 displays membrane instability and reduced Pi uptake.