5th Recent Advances in Microbial Control-Microbiomes Matter (RAMC)

Saturday, November 3

12:00 PM - 1:00 PM Workshop Registration

Egret, 3rd Level

1:00 PM - 5:00 PM Workshop: Next Generation Sequencing Applications for Industry

Egret, 3rd Level

Sunday, November 4

7:30 AM - 8:30 AM Breakfast-all registered attendees

Pelican/Heron, 3rd Level

7:30 AM - 4:00 PM RAMC Registration

Dunes Foyer, Lobby Level

8:30 AM - 11:30 AM Session: 1: Health Care Microbial Control

Conveners: Jesse Miller, NSF International, Ann Arbor, MI, USA and **Julie Haendiges**, NSF International, Ann Arbor, MI, USA

Dunes Ballroom 1-11, Lobby level

8:30 AM S1: Innovation for Healthcare Prevention Through the Microbial Ecology Lens

A. Halpin[*]*, CDC, Atlanta, GA, USA*
Healthcare-associated infections (HAIs) represent a major public health concern, with over 700,000 HAIs and 75,000 deaths in U.S. acute care hospitals alone, each year. Public health needs novel approaches to prevent and control these infections. Patients, and the healthcare environment they come in contact with, can serve as unrecognized reservoirs of HAI pathogens, including multidrug-resistant organisms. Envision a future where rapid Microbiome Indices are available as tools to aid in prevention of infection and in disruption of transmission. The development of these Microbiome Indices, measures of the microbial communities living in, on, and around us, that assess risk for colonization and transmission of pathogens. These Indices represent the intersection between personalized medicine and precision public health, targeting those populations most at risk for becoming infected with or serving as a source for transmission of pathogens. As with all Advanced Molecular Detection efforts, the development and implementation of Microbiome Indices necessitate laboratorians and epidemiologists work together to improve public health. Only by collaborating to leverage novel technology and analyze the wealth of microbiome and patient data available will we achieve improved population health outcomes. This talk will provide a high-level overview of CDC's perspective on leveraging these microbial communities to improve patient health.

9:00 AM S2: Richard Brooks MD Dept of Health

R. Brooks[*]*, Maryland Department of Health IDE ORB, Baltimore, MD, USA*
Legionella outbreaks in Long-term care facilities and using WGS to analyze isolates.

CRE Colonization and Outbreak investigations in hospitals in Maryland using WGS.

9:30 AM Coffee break/ Exhibits open

10:00 AM S3: Candida auris diagnostics and management in the healthcare environment

J. Sexton, CDC, Atlanta, GA, USA*

Despite being discovered only 10 years ago, the multidrug-resistant pathogenic yeast *Candida auris (C. auris)* has rapidly become a source of global concern. Unlike most fungal pathogens, *C. auris* is highly transmissible and causes persistent healthcare-associated outbreaks. As it is a recently emergent pathogen, gaps in knowledge have created significant challenges for diagnostics, drug susceptibility data interpretation, decolonization efforts and disinfectant guidance that collectively hinder the outbreak response effort. This talk will review recent advances in the *C. auris* outbreak response and will highlight existing needs to better control this emerging MDRO. Although further work remains in all areas of *C. auris* control efforts, this talk will emphasize the urgent need for effective disinfectants in the healthcare setting, which is an essential component of the outbreak response that has seen little progress to date. Hospital surface disinfectants with registered efficacy claims against *C. auris* are greatly needed to successfully control the persistent outbreaks facing many facilities in the US and around the world.

10:30 AM S4: The human gut microbiome in health and disease

J. Libertucci, University of Michigan, Ann Arbor, MI, USA*

The human body is colonized by a diverse community of microorganisms collectively known as the microbiota. This diverse community of microorganisms is essential for the proper function of the host. The human gut microbiota influences the maturation and responses of the immune system, it is required for the breakdown of complex carbohydrates, and is needed for the metabolism of drugs and other xenobiotics. The microbiota can also influence susceptibility to and outcomes of infectious diseases, as it is an essential component for the process of colonization resistance. When the microbial community composition is altered, for example by antibiotics, this reduces colonization resistance and allows for the invasion of non-native microbes like *Clostridium difficile*. Through understanding the dynamics that occur between the host, microbiota, and pathogen, treatment and prevention protocols for *C. difficile* infection can be improved upon. This talk will focus on how the gut microbiota influences *C. difficile* infection and how microbial derived therapies, such as fecal microbiota transplants, can be beneficial for the treatment of infection and diseases that are influenced by the gut microbiota.

11:00 AM S5: Novel nanotherapeutics for the emerging MDRs beyond hospital settings bacteria

A. Limayem, University of South Florida, Tampa, FL, USA*

The multidrug resistant Enterococcus faecium (MEF) strains originating from farm animals are proliferating at a substantial pace to impact downstream food chains and could reach hospitals. This study was conducted to elucidate the drug susceptibility profile of MEF strains collected from poultry products in Ann Arbor, MI area and clinical settings from Michigan State Lab and Moffitt Cancer Center (MCC) in Florida. Presumptive positive Enterococcus isolates at species level were identified by Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) analysis. The antibiotic susceptibility profile for both poultry and clinical strains was determined by the Thermo Scientific's Sensititre conform to the National Committee for Clinical Laboratory Standards (NCCLS) and validated via quantitative real-time PCR (qPCR) methods. Out of 50 poultry samples (Turkey: n = 30; Chicken: n = 20), 36 samples were positive for Enterococcus species from which 20.83% were identified as E. faecium. All the E. faecium isolates were multidrug resistant and displayed resistance to the last alternative drug, quinupristin/dalfopristin (QD) used to treat vancomycin resistant E. faecium (VRE) in hospitals. Results indicate the presence of MEF strains in food animals and clinical settings that are also resistant to QD.

11:30 AM - 1:00 PM Lunch-all registered attendees

Pelican/Heron, 3rd Level

1:00 PM - 4:00 PM Session: 2: Microbiome in agriculture/soil, health productivity and animal health

Conveners: Christine Sansone, Novozymes, Durham, NC, USA and **Varghese Thomas**, Bayer, West Sacramento, CA, USA

Dunes Ballroom 1-11, Lobby level

1:00 PM S6: Using plant-microbe symbiosis to increase plant resilience

S. Doty, A. Sher, Z. Khan, H. Rho, S. Kandel, P. Joubert, M. Aghai, A. Firrincieli, G. Ettl and S.H. Kim, University of Washington, Seattle, WA, USA*

With the relatively long life cycles of plants, symbiosis with microorganisms may allow plants to rapidly overcome environmental challenges. Endophytes are bacteria and fungi that live in intimate association within plants. The plant microbiota provide numerous benefits to the host plant including N-fixation, phytohormone production, reduced stress responses, anti-microbial production, tolerance to heat, salt, and drought, and pollutant degradation. Although some plant species are leguminous or

actinorhizal, associating with rhizobia or Frankia, respectively, in root nodules, many pioneer plant species are non-nodulating and yet thrive in low-nutrient settings. For these plants, N-fixing (diazotrophic) endophytes and other closely associated microorganisms may be the source of this essential macronutrient. We demonstrated N-fixation in poplar and willow, pioneer plant species able to colonize bare rocky substrates. A consortium of the diazotrophic strains was added to hybrid poplar, increasing growth and N-fixation under greenhouse conditions. Not only did the microbes impact this important bioenergy plant species, they also increased growth, health, and yields of an exceptionally broad range of plant species, including rice, tomato, pepper, ryegrasses, and Douglas-fir under nutrient-limited conditions. Inoculation of plants with endophytes also improved water use efficiency and drought tolerance of the inoculated plants including poplar, rice, and conifers. With the increased stress of climate change and continued anthropogenic-caused pollution, the implications of plant-microbe symbioses for agriculture, forestry, and bioenergy production are profound.

1:30 PM S7: Harnessing soil microbiomes to support plant health

J. Dundore-Arias[*] and L. Kinkel, University of Minnesota, Saint Paul, MN, USA

Plants live in intimate association with diverse microbes, and these associations are essential for plant performance and survival. Our work explores the effects of soil management practices on microbial phenotypic characteristics and species interactions, and the consequences for plant productivity. Using culture-based approaches we have found that soil resource availability plays a critical role in determining the likelihood of antagonistic arms race coevolution vs. niche differentiation among indigenous soil populations, with significant implications for plant disease suppression. Moreover, amplicon sequencing of rhizosphere communities associated with different plant hosts and soil management practices provide insights into non-cultured taxa associated with disease suppression. This presentation will cover what we have learned about the factors that contribute to determining the pathogen-suppressive and plant growth-promoting potential of indigenous soil microbes, and the possibility to create tailored approaches targeting species interactions to enhance plant health and productivity.

2:00 PM Coffee break/ Exhibits open

2:30 PM S8: Diet modulates bacteriophage production by the gut symbiont *Lactobacillus reuteri*

J.P. van Pijkeren[*], University of Wisconsin-Madison, Madison, WI, USA

The mammalian intestinal tract is home to a complex microbial ecosystem that is dominated by lysogens: bacteria that contain in their genomes dormant phages, which are referred to as prophages. About half of the viruses in the intestine are derived from lysogens, which means that these bacteria are exposed to triggers that promote phage production. While it is well-established that our diet influences the composition of the bacteriome and phageome, the molecular underpinnings that drive the interplay between diet, host and microbial viruses in our intestine are mostly unknown. Here, we developed the gut symbiont *Lactobacillus reuteri* as a model to study its prophages, and used a genetic approach to generate a lytic host to quantify bacteriophage production. We show that *L. reuteri* prophages are activated during gastrointestinal transit. We determined that a diet enriched in fructose and exposure to short-chain fatty acids promote phage production. We identified that fructose and short-chain fatty acids activate the Ack pathway, which in-turn promotes phage production in a RecA-dependent manner. Our findings demonstrate that prophages in a bacterial gut symbiont can be induced by both diet and metabolites impacted by diet, which provides a potential mechanistic explanation for the profound effects of diet on the phage community in the intestine.

3:00 PM S9: A Multi-Year White-Nose Syndrome Treatment at Black Diamond Tunnel

K. Gabriel[*] and C.T. Cornelison, Kennesaw State University, Kennesaw, GA, USA; S.A. Crow Jr., Georgia State University, Atlanta, GA, USA

In an effort to mitigate precipitous declines in bat populations due to white-nose syndrome (WNS), a multi-year treatment strategy has been implemented at Black Diamond Tunnel (BDT) in Clayton, Georgia. Once the home to the largest known tri-colored bat population in Georgia, BDT has seen over a 95% decline in tri-colored bats since the detection of *Pseudogymnoascus destructans*, the causative agent of WNS, in 2013. Methodologies are being employed using gaseous antifungal volatile organic compounds (VOCs) that have demonstrated an *in vitro* ability to inhibit *P. destructans*. The compounds being evaluated are associated with a naturally-occurring, plant-associated microbe and are generally recognized as safe (GRAS) by the FDA. Treatment applications occurred during the first week of November, December, and January of the 2016/2017 and 2017/2018 winter seasons. Bat population surveys pre- and post-treatments have been both surprising and positive. This talk provides an update covering the observations of the first two years of a four-year treatment strategy at BDT.

3:30 PM S10: Lactic acid bacteria as probiotics in animal health and nutrition

C. Sansone[*], Novozymes, Durham, NC, USA

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” The ability of these microbes to confer health benefits is dependent on strain specific genetic traits and/or the physiology of the host. Probiotics are most effective during times of stress, including inflammation from subclinical infection, antibiotic use, and diet change. Since stress often results in dysbiosis, beneficial microbes can help reestablish a healthy gut flora mediated

by adherence to gut epithelia. Therefore, the ability to adhere to intestinal epithelial glycoproteins is an essential characteristic of a probiotic. In addition to being associated with the gut mucosa, lactic acid bacteria (LABs) are a diverse clade of facultative anaerobes. These microbes have been shown to be the primary colonizers of the gastrointestinal tract. For these reasons, LABs are commonly used as probiotics. We design *in vitro* screens based on properties that we predict are required for LAB probiotics to perform in the host. These properties can include ability to survive gastrointestinal transit, adhere to the gastrointestinal tract, competitively exclude pathogens, produce antimicrobial peptides, modulate the immune system, increase barrier integrity, and improve nutrient digestion. After characterization of these probiotic candidates, strains are placed in appropriate animal models to bridge the *in vitro* screening with host performance. These LAB probiotics often cause positive shifts in the microbial community and/or accelerate development of a mature microbiome.

5:00 PM - 6:00 PM Keynote Talk: "Opportunities and challenges in industrial and agricultural applications of microbiome profiling"

Conveners: Dan Knights, University of Minnesota, Twin Cities, MN, USA

Dunes Ballroom 1-11, Lobby level

6:00 PM - 7:00 PM Opening Mixer

Dunes Ballroom 1-11, Lobby level

Monday, November 5

7:30 AM - 8:30 AM Breakfast-all registered attendees

Pelican/Heron, 3rd Level

7:30 AM - 4:30 PM RAMC Registration

Dunes Foyer, Lobby Level

8:30 AM - 11:30 AM Session: 3: Ecological and evolutionary factors that drive the microbiology of our food production systems

Conveners: Edan Hosking, Neogen, Lansing, MI, USA and **Andy Benson**, University of Nebraska, Lincoln, NE, USA

Dunes Ballroom 1-11, Lobby level

8:30 AM S11: Setting the stage—why the principles of ecology and evolutionary biology really matter to food microbiology in 2018

A. Benson[*]*, University of Nebraska, Lincoln, NE, USA*

Dramatic changes in diagnostic microbiology are being driven by the use of Next Generation DNA Sequencing (NGS) and other omics technologies as routine diagnostic tools. These changes affect a range of food microbiology applications, including pathogen surveillance, outbreak detection, and attribution of desirable (e.g. flavor) and undesirable (e.g. spoilage) microorganisms. As the applications of omics methods continue to evolve, opportunities to associate some of the most common observations with causal factors will also increase provided we learn to interpret these complex data sets through the lenses of ecology and evolutionary biology. Through such lenses, observations such as increased frequency of a given serotype/subtype of a pathogen or significant changes in diversity or structures of microbial communities are viewed as outcomes of ecological disturbances. This session will feature speakers whose topics emphasize use of different omics methods to study food-related ecological disturbances at different taxonomic scales. The studies they will describe share a common thread of using the fitness advantage of the microbial population as a basis for identifying causes of the disturbances—a paradigm shift that can transform microbiological testing from observational data collection to systematic data collection and mining for actionable information.

9:00 AM S12: *Diet driven evolution of an enteric pathogen*

R. Britton[*]*, Baylor College of Medicine, Houston, TX, USA*

As *Clostridium difficile* infections have expanded worldwide in the past 20 years, several reasons for the increase in morbidity

and mortality have been explored. We have identified recent adaptations to the diet as one possible factor that has contributed to the emergence of epidemic, hypervirulent *C. dificile* strains. Ribotype 027 and 078 strains of *C. difficile* have acquired the ability to consume the disaccharide trehalose at much lower concentrations than other *C. difficile* ribotypes. The ability to metabolize trehalose during *C. difficile* infection contributes to the severity of *C. difficile* infection in mice. The mechanisms by which RT027 and RT078 strains metabolize trehalose better than other ribotypes are distinct ; RT027 strains have an altered repressor or trehalose metabolism while RT078 strains have acquired a four-gene operon that enhances trehalose utilization. RT078 strains are also one of the most prevalent ribotypes found in farm animals, which may be a reservoir for human infections. In this presentation I will discuss recent evidence that supports genetic alterations in RT078 strains (and closely related ribotypes also found in farm animals) impacts the abiilty of this ribotype to utilize starches normally found in animal feed. The impact of dietary changes on the evolution of the microbiota and enteric pathogens will be discussed.

9:30 AM Coffee break/ Exhibits open

10:00 AM S13: Ecological and evolutionary factors that drive the microbiology of our food production systems

J.C. Gomes-Neto[*] and A. Benson, Nebraska Food for Health Center, Nebraska Innovation Campus/University of Nebraska, Lincoln, NE, USA

The range of environments encountered by food-borne pathogens include naturally-occurring and non-natural environments that can be created during processing, packaging, and storage. Our understanding of the processes that enable transmission and propagation of foodborne pathogens in these environments is largely descriptive and limited to the species-level, leaving us unable to explain how or why certain subpopulations of pathogens occur repeatedly in production/processing environments versus others that are observed only on rare occasions. Next Generation DNA Sequencing (NGS) and other omics methods provide a powerful opportunity to explain high-frequency subpopulations by systematically defining the signatures of evolutionary and ecological processes in their genomes. These signatures are crucial because the genes and pathways that comprise the signatures affect fitness traits of the organisms, and they can inform a new generation of sanitation strategies that reduce fitness advantages, ultimately reducing occurrence of these pathogens in the production and processing environments. Fundamentally, the concept is simple—identify important genetic markers of fitness in problematic populations of pathogens and develop actionable physical and chemical sanitation strategies that can impair function of genes bearing such markers. In practice, this approach requires implementation of specific designs for microbiological testing, strategic use of NGS technology, and sophisticated methods of genomic analysis. When used strategically, the approach can define candidate genes and pathways bearing hallmarks of evolutionary processes, ultimately highlighting important traits of the organisms to target with sanitation methods. This talk will describe the concept and show examples where the concept and approach have been used successfully.

10:30 AM S14: Metagenomics For: Plant mapping and cleaning validations for probiotic applications in a dry clean facility

M. Sayles[*], Diamond Pet Foods, Topeka, KS, USA

While advances in next generation sequencing continue to push our understanding of isolates, pathogens, and foodborne illness, there are additional NGS applications that can improve aspects of how we manage food safety and food quality. This presentation will provide unique and real-world examples of using next generation sequencing applications to improve the food supply chain. NGS applications include techniques such as metagenomics, targeted amplicon sequencing, antimicrobial resistance, and food authenticity. One of our biggest challenges with NGS is finding practical applications of use. This presentation will include plant applications in which some aspect of NGS is used to understand the novel application of probiotics in the production environment. A central theme of these applications will be metagenomics. Metagenomics is used to identify key changes in a microbiome. This presentation will explore the use of 16s metagenomics to help understand plant mapping and cleaning validations in a dry clean facility. The presentation will briefly review why metagenomics was chosen, the environmental application of probiotics and dive into a case study and share objectives, results and future planned actions.

11:00 AM S15: Examining beef shelf life and storage conditions using metagenomics

M. Bosilevac[*], USDA-ARS, Clay Center, NE, USA

The primary control measure used to maintain shelf life of beef products is refrigeration. However, even small changes in storage temperature can lead to spoilage problems. Shelf life is a subjective measure based on sensory characteristics (odor, visual appearance, color, and texture) but the numbers and types of microorganisms present can be used as a guide to identify the development of these unpalatable qualities. Investigations into the causes of spoilage rely on microbiological techniques that are either too general (counts of generic bacteria groups) to be of much use, or too labor intensive (specific culture enumeration and identification of certain spoilage bacteria) to lead to economical results. The use of 16S metagenomic analysis offers significant advantages in the investigation of spoilage. This talk will present three case studies wherein both microbiological culture and metagenomics were used to investigate problems arising during refrigerated storage of beef.

11:30 AM - 1:00 PM Lunch-all registered attendees

Pelican/Heron, 3rd Level

1:00 PM - 4:00 PM Session: 4: Biofilms and the microbiome

Conveners: Jon J. Calomiris, Sotiria Science, Arnold, MD, USA and **Paul Sturman**, Montana State University, Bozeman, MT, USA

Dunes Ballroom 1-11, Lobby level

1:00 PM S16: Resolving the complexity of natural microbiomes through large-scale single cell genomics

R. Stepanauskas, Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA*

With a recent estimate of over a trillion species, microorganisms constitute the ultimate frontier for discoveries in biology and biotechnology. Single cell genomics and other, cultivation-independent research tools are becoming the predominant sources of information about the composition and function of diverse microbiomes at relevant scales. In my talk I will focus on the following case studies demonstrating recent progress and opportunities in this emerging field: 1) single cell genome- and phenome-based discovery of previously unrecognized microbial metabolisms; 2) in situ studies of phage infections, symbioses and other microbial interactions; 3) generation of genome reference databases for global microbiome studies.

1:30 PM S17: Characterization of ship microbiomes using machine learning

S. Techtmann, Michigan Technological University, Houghton, MI, USA*

Microbe are ubiquitous in the world's oceans and important players in biogeochemical cycling. Distinct environmental conditions in the oceans are known to select for different microbial populations in specific locations. Ships transit the world's oceans on daily basis. Microbes colonize ships and can be carried with them from one port to another. In this project, we are seeking to understand the ability of microbes to colonize vessels and be transported by them on voyages. Samples were collected from 20 ports around the world in order to identify key microbial features that distinguish one location from another. This was accomplished through combining high resolution microbial community analysis using next-generation sequencing with machine learning. Additionally, the microbial community present on ships surfaces and in bilge water was analyzed. Machine learning models were constructed that can classify the location of sample collection from the microbial community composition alone with greater than 97% accuracy. This suggests that there are sufficient differences in the microbial community between locations that can be used differentiate geospatial locations. Our work has also shown that the microbiome of a ship is mostly distinct from the water, but does have some overlap with the microbes found in the water. Recent work has sought to characterize the rate of change in boat microbiomes during voyages to better understand the rates of colonization of ship surfaces during transits. This work will help clarify the potential for ships to serve as conduits for bacterial dispersal in the oceans.

2:00 PM Coffee break/ Exhibits open

2:30 PM S18: Metagenomic analysis of agricultural groundwater wells for risk assessment

A. Paropkari, T. Seher, S. Sindi, A. Hernday and C. Nobile, University of California, Merced, Merced, CA, USA; W. Ludington, Carnegie Institution for Science, Washington, DC, USA; G. Wallace, Pacific Groundwater Group, Seattle, WA, USA*

California uses approximately 15 billion gallons of groundwater per day, and groundwater accounts for about half of California's water supply. Agriculture in the state is highly dependent on groundwater, which can become contaminated from agricultural runoff. How agricultural contamination drives groundwater geochemistry through microbial metabolism is poorly understood. Samples of groundwater were collected from three agriculture wells in addition to an effluent surface water lagoon that fertilizes surrounding corn fields. We analyzed the samples for concentrations of solutes, heavy metals, and USDA coliform bacteria as part of a groundwater monitoring study. Whole metagenome shotgun sequencing revealed taxonomic composition and metabolic potential of the microbial community. Differential levels of solutes and heavy metals were observed at various depths below ground surface. Metagenomic communities in groundwater samples consisted of bacteria that were involved in processes related to breakdown of nitrate. Additionally, several Plantomycetes genomes as well as novel and diverse nano-prokaryotes were also identified in the samples. Secondary metabolites found in the samples were rich in antimicrobial and quorum sensing compounds, which may suggest niche specialization in the well. Overall, we conclude that the groundwater microbiome of these agriculture wells is influenced by nutrient availability, which provides selective pressure on community composition. Groundwater microbial communities have the capacity to naturally remediate effluent ammonium from farm use to mineralized nitrogen. In the long-term, our findings may be useful for risk assessment of microbial contaminants as well as to identify future strategies for maintaining groundwater as a more stable source of water throughout California.

3:00 PM S19: Characterization of premise wastewater plumbing microbial communities in a renovated intensive care unit using next generation sequencing

E. Breaker, CDC/ORAU, Atlanta, GA, USA*
Over 700,000 healthcare-associated infections (HAIs) and 75,000 associated deaths occur in U.S. acute care hospitals annually. The healthcare environment (e.g., premise-plumbing) can serve as a reservoir for HAI pathogens, including antimicrobial-resistant organisms, which have the potential to disperse from P-traps into the sink basin and, ultimately, the patient care environment. To better understand the natural history of premise-plumbing biofilms, this pilot study characterized premise-plumbing bacterial communities in three unoccupied patient rooms of a newly renovated intensive care unit. Serial community profiling using 16S rRNA sequencing of faucet water, and P-trap water and biofilms was completed over five weeks. Number of observed operational taxonomic units (OTUs) per sample ranged from 141 to 191 (Median: 167); 21 OTUs represented >1% of the communities. We identified several shared OTUs between rooms throughout the sampling period, including HAI pathogens: *Acinetobacter, Pseudomonas, Sphingomonas,* and *Stenotrophomonas.* Because frequently sampling P-trap biofilms in occupied rooms has limited feasibility; we compared bacterial communities of one P-trap biofilm to its associated P-trap water, which is more easily obtained. The bacterial communities of the P-trap water and biofilm were similar, demonstrated by beta diversity distance metrics. Here, we demonstrate that even in unoccupied rooms, premise-plumbing biofilms form and evolve over time, incorporating known pathogens. Further work to support whether P-trap water could be used to more easily detect HAI pathogens is needed. Using P-trap water for routine surveillance of premise-plumbing-associated pathogens and for outbreak investigations could provide actionable data to prevent the transmission of HAI pathogens in the healthcare environment.

3:30 PM S20: How biofilm formation impacts antimicrobial resistance, colonization, and interactions ofÂ *Enterococcus faecalis*Â with the host-associated microbiome

G. Dunny, University of Minnesota, Minneapolis, MN, USA*
Enterococcus faecalis has evolved as a ubiquitous gut commensal, and in recent years has received increasing attention because of its high level of acquired and intrinsic antibiotic resistance and its propensity to cause opportunistic infections of immune compromised individuals. These infections are frequently recalcitrant to antimicrobial therapy. Numerous laboratories have characterized enterococcal biofilm formation in vitro, and in some cases have established a correlation between biofilm formation and virulence in models of opportunistic infections. A number of microbiome studies have documented how antibiotic treatments may result in explosive increases in the intestinal populations of enterococci, and how this process may lead to systemic infections of the bloodstream and other sites in the body. However, a major gap in our understanding of enterococcal biology and ecology exists in relation to colonization and persistence of these organisms in healthy hosts, and the role (if any) of biofilm formation. Our laboratory is taking a genetic approach to address these issues. In this presentation I will describe recent work from our laboratory using Tn-Seq based genetic screens for comprehensive analysis of the genetic determinants of persistent intestinal colonization in the absence of antibiotic selection. I present initial results of screens carried out in a mouse model, and correlation between these results and those carried out using in vitro biofilm models.

4:00 PM - 5:00 PM Poster Reception

Dunes Foyer, Lobby Level

5:00 PM - 6:00 PM Poster Presentation

Dunes Ballroom 1-11, Lobby level

5:00 PM - 7:00 PM Poster Session

P1 Novel zinc containing porphyrin (ZnPor) exhibits toxicity in the absence of photoactivation against *Pseudomonas aeruginosa,* an opportunistic pathogenic bacterium, grown under planktonic and biofilm conditions

N. Patel, University of Dayton, Dayton, OH, USA*
One of the greatest threats to human health, and life, is the rise of antibiotic-resistant infections. National summary data from the CDC estimates that at least 2,049,442 million illnesses and 23,000 deaths occur each year as the result of antibiotic resistant bacteria and fungi. We are the co-inventors of two patented, novel technologies for the treatment of antibiotic resistant bacteria. Both treatments make use of a novel porphyrin ZnPor (US Patent # 9,364,537) that does not require using traditional photo-activation i.e., it exhibits unique dark toxicity. In standard tests against planktonic cells ZnPor exhibits broad spectrum activity, for example it is bactericidal towards: *Pseudomonas aeruginosa (*MIC_{90} at 3.125µM), MSSA (MIC_{90} at 1.56µM) and MRSA (MIC_{90} at 1.56µM) strains of *Staphylococcus aureus,* as well as *Listeria monocytogenes* (MIC_{90} at 12.5µM) and *Mycobacterium smegmatis*(MIC_{90} at 12.5µM). We have demonstrated that *P. aeruginosa* (PAO1) cells take up ZnPor rapidly and accumulate it inside the cell. Circular dichroism experiments show that there is a high binding constant of ZnPor and PAO1 DNA. In cells treated with ZnPor there was a substantial loss and destruction of DNA as well. Additionally, we have tested

various uptake/transport systems in PAO1 that aid in uptake of heme, which represents a naturally occurring porphyrin molecule. In biofilm experiments, ZnPor was able to disrupt 16h preformed biofilms on Polyethylene (PE) surface and, more interestingly, enhanced the effect of the antibiotics: Tobramycin and Vancomycin which are resistant to PAO1.

P2 Bacterial communities in ethanol biorefineries

F. Firmino[*] and J.L. Steele, University of Wisconsin-Madison, Madison, WI, USA

Microbial contamination in the US bioethanol industry results in an estimated yearly loss of more than $200 million USD. These losses are due to microbial contaminants, primarily lactic acid bacteria, “stealing” carbohydrates from the yeast and thus reducing ethanol yields. To minimize these losses, the industry has become heavily reliant on antibiotics. This practice promotes the emergence of antibiotic-resistant strains in the ethanol biorefineries and more importantly in animal agriculture, as these compounds find their way into the food supply through a major co-product of ethanol production, dried distiller grains with solubles. To obtain a better understanding of the organisms present and how population dynamics change in ethanol biorefineries we employed both traditional culture-dependent and 16S rRNA sequencing approaches to examine this microbiota. A total of 401,262 sequences across 134 samples were obtained from samples obtained from four Midwestern ethanol plants and the reads were clustered into 42,518 Operation Taxonomic Units (OTUs). The majority of OTUs belonged to two main phylum, Firmicutes (90%) and Proteobacteria (9%). Among the Firmicutes, the genus *Lactobacillus* dominated making up 84% of all OTUs with this phylum. On a per plant basis, the abundance of *Lactobacillus* ranged from 37 to 99% of the reads. Results from traditional plating with speciation by full length 16S rRNA sequencing differ significantly from the community sequencing results, highlighting the importance of culture-independent methods for the characterization of microbial communities. These results will assist in the development biological control methods for controlling microbial contaminants in biofuel fermentations.

P3 Antifungal properties ofÂ *Rhodococcus rhodochrousÂ* DAP 96253 following pre-pilot fed-batch fermentation

M. de la Croix[*], N. Wijewantha, K. Cannon and G.E. Pierce, Georgia State University, Atlanta, GA, USA

Rhodococcus rhodochrous is a Gram-positive, aerobic, non-pathogenic bacterium ubiquitous in soil. The bacterium has a broad metabolic and physiological diversity that allows for numerous practical applications. Environmentally derived *Rhodococcus* strains have been used for over 70 years in industrial fermentation to produce pharmaceutical grade products. Current research, in our laboratory, has shown that under specific inducing conditions, *R. rhodochrous* DAP 96252, when grown in fed-batch fermentation, produces volatiles exhibit antifungal properties against select fungi during contact-independent applications.

Induced cells of *R. rhodochrous* DAP 96253 demonstrated the ability to act as a contact-independent antifungal catalyst for stored refrigerated strawberries. Cardboard inserts, holding the strawberries in clamshells, were sprayed with an immobilized whole-cell edible fruit wax and showed to inhibit the growth and germination of phytopathogenic *Aspergillus spp*. These fungal control methods play an important role in the agricultural post-harvest production and prevent massive loss of inventory due to spoilage. Pre-pilot fed-batch fermentation allows to produce induced cells of *R. rhodochrous* DAP 96253 in large quantities for future large-scale commercialization.

Volatile analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS). Headspace volatiles were sampled using microextraction fibers. Analysis has shown *R. rhodochrous* DAP 96253 to initially produce benzaldehyde, dimethyltrisulfide, and decanal which dissipated rapidly, followed by the production of oxalic acid and dimethyl ether at seven to nine days of storage.

P4 *Bacillus* isolates as seed treatments for control of the important soil-borne plant pathogen *Sclerotinia sclerotiorum* on oilseed rape

D. Roberts[*] and X. Hu, USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD, USA

Treatments containing microbial biological control agents must provide enhanced levels of plant disease suppression and consistency of control before they are widely used in agriculture. Treatments containing *Bacillus megaterium* A6 and two genetically distinct *B. subtilis* isolates, BY-2 and Tu-100, were applied individually and in combinations as seed treatments and tested in field trials conducted at four locations with different soils for control of *Sclerotinia sclerotiorum* on oilseed rape to determine if combining the isolates enhanced performance. Seed treatment with A-6 or Tu-100 resulted in a significant decrease in disease at two locations while seed treatment with BY-2 resulted in significant reductions in disease at all four locations. There was an incremental, but not significant, reduction in disease with increasing number of strains in the treatments at three locations. In plant growth promotion studies conducted in pots, the treatment containing all three isolates resulted in oilseed rape seed yield significantly greater than the non-treated control in four of the five soils tested. The treatment containing isolates A-6 and Tu-100 significantly increased seed yield in one soil. No other treatment containing a single isolate, or two isolates, significantly increased yield in these pot studies. These field experiments indicate that isolate BY-2 provided consistent reduction of disease over the four locations with different soil types. Pot studies suggest that combining other *Bacillus* isolates with BY-2 can provide the added benefit of plant growth promotion.

P5 Next generation sequencing of biofilm samples from industrial paper and packaging process

environment

V. Kukkurainen, K. Partti-Pellinen and K.J. Riihinen, Stora Enso Oyj Research Centre, Imatra, Finland*

In the industrial processes the screening of microbial diversity has been traditionally dependent on the conventional cultivation-driven methods. Also, molecular methods such as quantitative polymerase chain reaction (qPCR) with the ability to detect viable and nonviable microbes is used. While these microbial techniques are still respectively usable methods and gives insights into the microbial diversity, with next generation sequencing (NGS) characterization of bacterial and fungal diversity and phylogenetic classification is possible without any a priori knowledge of the sample. In this research, the phylogenetic characterization of biofilms from the paper and packaging board process was studied, using Illumina MiSeq whole genome sequencing (WGS). Results show the versatile nature of microbial diversity in the biofilms within the studied environment. Results indicate also that even with good possibility to identify microbial diversity with whole genome sequencing, the amplicon sequencing approach for fungal community analysis should be studied more to gain better sequencing depth.

P6 A metagenomics study of the corn kernel and silk microbiome following infection with aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* strains

G. Moore, K. Rajasekaran, J. Cary, M. Gilbert and B. Mack, USDA-ARS, New Orleans, LA, USA; S. Chalivendra and A. Nguyen, Louisiana State University, Baton Rouge, LA, USA; A. Rivers, USDA-ARS, Gainsville, FL, USA*

Metagenomic sequencing of microbiomes affiliated with agricultural commodities is an important avenue of research. In light of the increasing use of non-aflatoxigenic *A. flavus* strains as pre-harvest biocontrol, we are investigating the effect of introducing a biocontrol agent (K49) to the inherent microbiomes of two corn varieties (B73 = susceptible and CML322 = resistant). Concomitant with the introduction of the biocontrol agent, we are also interested in evaluating the impact of exposure of the inherent microbiome to a highly toxigenic *A. flavus* strain (TOX4). To more easily discern between the introduced *A. flavus* strains, K49 has been tagged with green fluorescent protein (GFP), and TOX4 with a red fluorescent protein (RFP, mCherry). Thus, we have four treatments for each corn variety as follows: Control (no spores), K49-GFP alone, TOX4-RFP alone, and mixture of K49-GFP+TOX4-RFP. Samples from corn ears will be collected throughout the growing season and prepared for metagenomics sequencing. Any changes in microbial community structure (bacterial and fungal) in response to the introduced strains, compared to the inherent microbiomes of ears without spore treatments, will be documented for evaluation. This analysis will enable us to identify any organism(s) that either help or inhibit the biocontrol strain as it seeks to prevent infection by the toxigenic strain. Additionally, microbes may be identified that are unique or present at significantly different levels in resistant versus susceptible corn kernels/silks that can be correlated with resistance to *A. flavus* infection. Progress of the research study and preliminary metagenomics data will be presented.

P7 Impact of the genomics revolution on the taxonomy of bacterial biocontrol agents

C. Dunlap, United States Department of Agriculture, Peoria, IL, USA*

The revolution in DNA sequencing technology has led to an improved understanding of genetics and taxonomy of biocontrol agents. Our lab recently reported the genomes of some important *Bacillus* bacterial biocontrol agents, which in turn resulted in a change of taxonomy for these commercially important strains. Our study showed that four species of *Bacillus* (*Bacillus amyloliquefaciens* subsp. *plantarum, Bacillus methylotrophicus, Bacillus oryzicola* and *Bacillus velezensis*) are the same species. Under the rules of prokaryote taxonomy, the name of the first described species takes precedent over the other names. In this case, *Bacillus velezensis*, is the correct nomenclature for these strains. This class of *Bacillus* strains is the second most popular commercial prokaryotic biocontrol agents after *Bacillus thuringiensis*. In addition, our knowledge of what defines a given “species” is has greatly changed in the last five years. Now correctly identifying your strains can directly provide more information about the physiology and phenotype of your strain.

P8 Inhibitory and fungicidal effects of select essential oils on *Candida auris*

R. Parker and C.T. Cornelison, Kennesaw State University, Kennesaw, GA, USA*

Essential oils represent an underutilized source of therapeutic antimicrobials. Many of these oils have demonstrated antiviral, antibacterial and antifungal properties. Previous research has also displayed an ability of some oils to enhance the efficacy of other antimicrobial drugs, a phenomenon called synergism. *Candida auris* is an emerging opportunistic fungal pathogen that is frequently drug-resistant, including an alarming number of strains that are resistant to all three classes of antifungal drugs commonly used. This study uses the classic microbiological method of broth microdilutions to test the antifungal efficacy of select essential oils on *Candida auris.* The results thus far have displayed efficacy for several oils, some of which have minimum inhibitory concentrations far below the previously published values for safe dermal use. The future aim of this line of inquiry is screen the most efficacious oils for synergism with commonly used antifungals in an attempt to restore their usefulness against drug-resistant *Candida auris* strains.

P9 A twelve-week study of performic acid (PFA) batch efficacy tests on two municipal waste waters

I. Porat and C. Snyder, Kemira Chemicals, Inc, Atlanta, GA, USA; C. Brosseau, Kemira Water Solutions Canada Inc.,*

Varennes, QC, Canada; M. Polverari, Kemira Chemicals Inc., Saint Leonard, QC, Canada
Performic acid (PFA) is an oxidizing agent used for disinfection in municipal wastewater treatment plants (WWTP) which produces no by-products and does not contribute to biological or chemical oxygen demand (BOD, COD). PFA reacts quickly and degrades to carbon dioxide and water. We previously developed a fully automated PFA delivery system which can be easily delivered and installed at customer site. Publications reported PFA's high efficacy against bacteria and coliphage at European WWTPs using the PFA delivery system. To test the efficacy of PFA in North American WWTPs, batch experiments were performed over twelve weeks, at two locations, twice per week using three PFA concentrations (1, 2 and 10 mg/L) at two contacts times (5 and 30 minutes). The surviving microorganisms were plated in Petrifilms for the detection of total bacteria, Enterobacteria, E. coli/coliform and Yeast. PFA efficacy results from WWTP-1 showed an average of 1.8, 3.3 and 1 log reduction for total bacteria; Enterobacteria, E. coli and Coliform; and yeast, respectively; in all conditions tested. In comparison, the results from WWTP-2 showed an average of 0.6, 1.3 and 4.5 log reduction for dosages of 1, 2 and 10 mg/L, respectively, combining all types of microorganisms and contacts times. WWTP-1 and WWTP-2 differ by the treatment plant steps and by their type of influents. In conclusion, tests in North America water show that PFA is a highly effective disinfectant for wastewater, in agreement with European application data. Not yet approved for sale in North America.

P10 Whole genome analysis and lysine degradation genes related to osmotic stress resistance in *Lysinibacillus capsisi* PB300

J. Cadena, L. Sastoque and M. Burkett-Cadena, Pathway Biologic, Plant City, FL, USA; C. Dunlap, United States Department of Agriculture, Peoria, IL, USA*
Lysinibacillus capsisi PB300 is a growth promoting rhizobacteria of cultivated plants that has recently been described as a new species within the *Lysinibacillus group.* PB300 is a gram-positive, strictly aerobic, motile, rod-shaped, endospore forming bacterium originally isolated from the rhizosphere of a pepper plant in Arizona, USA. Genome sequencing and annotation revealed that PB300 has 4657 protein coding sequences (CDS) divided in 463 subsystems, 11 transfer RNA (tRNA) genes, and 4 ribosomal genes. The annotation included 1354 hypothetical proteins and 3293 proteins with functional assignments. Genomic comparison to closest species showed that PB300 possess a saccharopine dehydrogenase gene (SDH) that has been found in *Lysinibacillus sphaericus* and other bacterial species. This SDH gene has an essential role in the lysine catabolism pathway in higher eukaryotes and has been related to osmotic stress resistance in prokaryotes. To verify whether SDH genes are induced by osmotic stress, PB300 was grown with and without lysine at different salt concentrations, showing that presence of lysine does not have a significant effect on the bacterial concentration. However, the bacterial growth rate was extended in the lysine treatments, suggesting expression of SDH gene and the use of this pathway may be an adaptation to high salt environments. This work contributes to our knowledge of the *Lysinibacillus* group and this particular finding is of special interest because PB300 behavior suggests a possible connection between the lysine catabolism pathway and stress adaptation that are induced in response to osmotic stresses.

P11 Evaluating the microbiota of a mushroom farm in Nigeria for lignocellulolytic enzyme production

T. Nwagu, C.G. Chukwu, O.C. Amadi, A.N. Moneke and B.N. Okolo, University of Nigeria, Nsukka, Nigeria; R. Agu, The Scotch Whiskey Research Institute, Scotland, United Kingdom*
Lignocellulose abounds in nature and its conversion to fermentable sugars holds enormous prospects for the biofuel industry. To optimally generate these sugars, enzymes capable of hydrolysing cellulose, lignin and hemicellulose are required. In this work, the microbial diversity of soil collected from a mushroom farm was explored and the number of multifunctional lignocellulolytic enzyme secreting organisms were determined. A total of 438 microorganisms were isolated using the culture dependent technique. The organisms were predominately bacteria; highest number was from nutrient agar (133), followed by MacConkey agar (66), and various selective and differential media. Sixty-six isolates were fungi from potato dextrose agar (PDA), sabouraud dextrose agar and peptone yeast agar. Of the 33 isolates from PDA, 31 secreted considerable amounts of cellulase and endo-1,4-β-glucanase, most produced xylanase and ligninase while 14 were incapable of secreting laccase. Most isolates from nutrient agar secreted poor levels of cellulase and endo-1,4-β-glucanase but no ligninase, xylanase or laccase. Most isolates obtained on MacConkey agar were unable to synthesize cellulase, xylanase, ligninase, and laccase but produced endo-1,4-β-glucanase. Seventeen isolates possessed the multi-enzyme system for the synthesis of all the enzymes investigated. Amongst the best were a bacterium (*Bacillus amyloliquefaciens* subsp. plantarum) and two fungi (*Yarrowia lipolytica strains*). The highest amount of cellulase, ligninase, laccase, manganese peroxide and endo-1,4-β-glucanase were produced by the fungal strains. This indicates that the microbiota obtained from the snail farm soil include potential sources of the multi-enzyme complex required for the conversion of lignocellulose to simple sugars and other value added products.

Tuesday, November 6

7:30 AM - 8:30 AM Breakfast-all registered attendees

Pelican/Heron, 3rd Level

7:30 AM - 4:30 PM RAMC Registration

Dunes Foyer, Lobby Level

8:30 AM - 11:30 AM Session: 5: Microbiomes and built environments

Conveners: George Garrity, Michigan State University, East Lansing, MI, USA and **William Schwingel**, Masco, Taylor, MI, USA

Dunes Ballroom 1-11, Lobby level

8:30 AM S22: Considerations in assessing new microbiological measurement technologies from a standard perspective

J. Morrow, NIST, Rockville, MD, USA*

As our ability to measure microbial systems advances, technology evaluation processes face challenges in understanding the impact of both biological and technical variability on decision-making. Technology evaluation, including test and evaluation for regulatory purposes, is a critical step in technology emergence and commercialization. Accurate microbial identification and quantitation is becoming increasingly important for assessing the efficacy of new and emerging microbial community-based treatments and defining the market potential of novel technologies. Specifically, accurate microbial identification and quantification is critical to effective validation and evaluation of microorganism characterization technologies and microbiome treatment strategies. This talk will cover measurement assurance principles and standards development efforts focused on improving the quality of microbiological measurement and in turn confidence in measurement results. Highlights on the role of standards and measurement assurance processes in underpinning decision-making essential to begin to account for biological variability and opportunities to improve measurement accuracy will be presented. Opportunities to engage in standards development activities currently underway to better assess and understand biological system health, disease states and clinical outcomes will be discussed.

9:00 AM S23: Challenges implementing rapid detection technologies in the characterization the pharmaceutical microbiome

A. Bragdon, Eli Lilly and Company, Indianapolis, IN, USA*

Quantification and characterization of microbiological contamination in pharmaceutical manufacturing environments is both critical to patient safety and a regulatory expectation. The evaluation of microbiomes, associated with pharmaceutical clean rooms, continues to rely primarily on decades-old recovery methodologies that are deeply rooted in traditional microbiology. Use of rapid detection technologies would be highly advantageous to a pharmaceutical sterility assurance program, facilitating a quicker response to atypical and / or adverse results, particularly associated with critical aseptic product processing areas. However, significant regulatory hurdles exist that hinder the adoption of such technologies, and as such, rapid detection and characterization of bioburden within the pharma industry continues to remain in its infancy.

9:30 AM Coffee break/ Exhibits open

10:00 AM S24: Fungal microbiomes associated with oceanic plastic debris: environmental sources, biodegradation, and fragmentation of plasticized marine polyvinyl chloride.

D. Price, Interface Inc., Lagrange, GA, USA and D.G. Ahearn, Georgia State University, Marietta, GA, USA*

A central theme for Interface, Inc. is “environmental sustainability” accordingly we have been monitoring over several years peer reviewed literature on the fate of plastics in the oceans. Our oral presentation for RAMC 2018 includes review of the source, fate, and effects of microplastics (MP) in the world’s oceans. The review includes both metagenomics and classical procedures detailing microbiomes associated with plastics with emphasis on plasticized polyvinyl chloride (pPVC) and associated fungi. There are few publications on this topic. Most related literature on microbial biodegradation of plastics in aquatic environments center on bacteria and polyethylene (PE) and polypropylene (PP). Microbiome functions on plastics may vary with substrate and environment and time. Recognition and roles for Cryptomycota and other non-culturable anaerobic and microaerophiic fungi from the depths of the oceans remain speculative because of insufficient operational taxonomic units (OUT’s). Unfortunately, the current status of metagenomic identifications of environmental fungi mostly recognizes only the species complex.
Our data, obtained with classic procedures, identifies fungal consortia involved in the uncommon, relatively-rapid biodegradations of marine vinyl formulated for recalcitrance to harsh marine exposures. The Discussion in the recent review by Alimi et al 2018 *full reference* indicate the need for such baseline data. The classic approach appears necessary for establishing baseline data to answer questions on the colonization and transport of contaminants associated with microplastics and nanoplastics.

10:30 AM S25: Observations on the impact of rapid changes in prokaryotic taxonomies and nomenclature

G. Garrity, Michigan State University, East Lansing, MI, USA*
East Lansing, MI, USA

The concept of precise taxonomies of bacteria and archaea has been the subject of a number of recent publications. The goal of these efforts is improved identification of taxa in microbiomes, improved reliability of predictions about the function of each identified taxon and the identification of novel taxa. Typically these studies rely on various public databases of reference sequences and taxonomic metadata. However, the taxonomy and nomenclature of prokaryotes is not static, and these databases may be significantly out of date and may not accurately reflect the current consensus view. Addition of newly named or reclassified taxa are presently accumulating at a rate >1,000/year. Each change results in either an implied or explicit alteration to the taxonomic hierarchy at higher and lower levels. Failure to take these changes into consideration leads to classification errors that cannot be corrected using probabilistic methods. Here, we report on ongoing studies to determine the effects that monthly/yearly/decadal changes in prokaryotic taxonomy/nomenclature have on taxon identification using common pipelines for metagenomic analysis. Using input taxonomies based on their known state at different times, ranging from 1980 – 2018, we find statistically significant differences among the taxonomic assignments for the same sequences at the 99.5% confidence limits. These results suggest that comparisons of data generated at different times or by different methods should be re-annotated using the most recent taxonomy and nomenclature to ensure that inferences or predictions reflect the current state of knowledge.

11:00 AM S25 A : The Microbiome of Sturgeon Egg: Relevance to Hatchery Rearing

T. Marsh, R. Angoshtari and D. Ye, Michigan State University, East Lansing, East Lansing, MI, USA; J. Bauman, F. Trail and K. Scribner, Michigan State University, East Lansing, MI, USA; M. Fujimoto, University of Florida, Gainesville, FL, USA*
During the past three decades aquaculture has increased from 5 million to 63 million tons of fish representing ~16% of animal protein consumed. The hatcheries, ponds and suspended cages of aquaculture are “built” environments with a unique microbiome. Our investigations have focused on the microbiome of sturgeon eggs and how manipulation of this microbiome might provide greater egg survival within the hatchery. The egg, essentially sterile when extruded into the river during spawning, becomes coated within 15 minutes with a diverse community dominated by *Pseudomonas*, *Aeromonas*, *Geobacillus* and *Bacillariophyta.* After 24 hours exposure, the community shifted significantly and was dominated by *Comamonadaceae*, *Rheinheimera*, *Undibacterium*, *Bacillariophyta*, *Rhodobacteraceae* and *Methylophilus*. Community analysis with 18S revealed ciliates, algae and *Saprolegnia* at low concentrations while analysis targeting the ITS region identified 30 genera in 20 sampled eggs. *Aureobasidium*, *Cryptococcus*, *Neobulgaria*, *Pythium* and two unidentified groups dominated.

Disinfection and deadhesion treatments are common practices in hatcheries. Disinfection causes increases in *Flavobacterium* and losses to *Sphaerotilus* and *Rheinheimera* populations on the egg. Treatments to reduce egg aggregation shift the community to *Flavobacterium* while losing *Comamonadaceae, Oxalobacteraceae Cryomorphaceae and Sphingomonadaceae*. Substantially reducing the bacterial load in water reduces egg mortality while coincubating the eggs during fertilization with *Acidovorax* reduces mortality by 30%.
Continued identification of probiotics and judicious intervention during the assembly of egg-associated microbial communities can reduce mortality and the use of harmful chemicals in the hatchery. Additional studies on hatchery architecture will help to identify pathogen reservoirs and improve mortality rates and larval robustness.

11:30 AM - 1:00 PM Lunch-all registered attendees

Pelican/Heron, 3rd Level

1:00 PM - 4:00 PM Session: 6: Microbiomes in energy production

Conveners: Jana S. Rajan, Dow Microbial Control, Collegeville, PA, USA

Dunes Ballroom 1-11, Lobby level

1:00 PM S26: Overview of microbiome in energy production

J.S. Rajan, Dow Microbial Control, Collegeville, PA, USA*

1:30 PM S27: Implications of *Halanaerobium* Growth and Persistence in Hydraulically-Fractured Shale Ecosystems

A. Booker and T. Meulia, Ohio State University, Columbus, OH, USA; M. Wilkins, The Ohio State University, Columbus, OH, USA; D. Hoyt and M. Lipton, Pacific Northwest National Laboratory, Richland, WA, USA*
Bacterial *Halanaerobium* strains become the dominant microbial community member in produced fluids across hydraulically fractured (HF) shales. *Halanaerobium* is not native to the subsurface, but is introduced during the drilling and fracturing process.

The accumulation of biomass in pipelines and shale formations is detrimental due to corrosion and bio-clogging that could negatively impact oil and gas recovery. Here, we used *Halanaerobium congolense* strain WG8 isolated from a HF well to identify metabolic and physiological responses to growth under high-pressure subsurface conditions. Laboratory incubations confirmed the capability of strain WG8 to grow under pressures representative of the subsurface (21-48 MPa). Shotgun proteomic measurements identified higher abundances of proteins associated with extracellular polymeric substances (EPS) production, and utilization of 1,2 propanediol when WG8 was grown under pressure. Hydrogenase proteins were less abundant under the same growth conditions. Confocal laser scanning microscopy and scanning electron microscopy indicated EPS production was associated with greater cell aggregation and attachment to shale surfaces under high pressure conditions. NMR and gas chromatography measurements of fermentation products revealed changes in strain WG8 central carbon metabolism under high pressure growth. Twice as much ethanol, acetate and propanol were generated per cell under high pressure conditions, while hydrogen production almost completely ceased. These metabolic shifts were associated with carbon flux through 1,2 propanediol in response to slower fluxes of carbon through stage 3 of glycolysis. Overall, these results revealed the potential for bio-clogging and corrosion (via organic acid fermentation products) associated with persistent *Halanaerobium* growth in deep, hydraulically-fractured shale ecosystems.

2:00 PM S28: Use of metagenomic data to identify phage and bacteriocin biocontrol targets in industrial systems, including oil and gas, biofuel fermentation, wastewater, and cooling towers

E. Summer[*] and G. Hamblen, Ecolyse, Inc, College Station, TX, USA

We have been applying 16S amplicon metagenomics in order to evaluate microbial populations in oil and gas production, biofuel ethanol fermentation, wastewater treatment facilities, and water-cooling towers. Microbial growth impacts each of these important processes through some combination of fouling, corrosion, sulfidogenisis, inhibition, and competition. Chemical methods, including biocides and antibiotics, are frequently used for microbial control. There has been speculation about using natural antimicrobials, such as bacteriophage (phage) or bacteriocins for industrial control applications. Phage are the natural viral predators of bacteria while bacteriocins are diverse antimicrobial compounds that are produced by bacteria to kill other bacteria. While phage and bacteriocins have traits that make them ideal anti-microbial agents, however, both exhibit sometimes extremely restricted target specificity. Therefore, their application requires specific information on problem-causing microbial populations. The feasibility of targeted bacterial population control was evaluated in each of the indicated industrial systems. Analysis of data from thousands of samples emphasizes overall high microbial diversity, complex population structures, and unexpected target identities. Diversity is correlated to the type of system, system openness, physiological and chemical complexity, and process time. The industrial systems were ranked in order of feasibility for use of targeted control agents. Due to the short process time, closed system, and defined conditions, biofuel ethanol fermentation exhibited the most promising characteristics for application of phage and bacteriocin products. In contrast, the diversity of target organisms in oil and gas systems introduces challenges for targeted applications, such as phage and bacteriocin.

2:30 PM Coffee break/ Exhibits open

3:00 PM S29: Roundtable Discussion

J.S. Rajan[*], Dow Microbial Control, Collegeville, PA, USA

This session will be an interactive session where the organizing committee in a roundtable format will summarize the meeting and facilitate a discussion on what challenges and opportunities exist in this area. Topics for discussion will also include gaps in knowledge/technology to address the needs of microbial control in all fields of applied microbiology, including the control of microbial populations in agricultural, food/food safety, built environments and in “man-made” materials.

5:00 PM - 6:00 PM Banquet Reception

Outside reception space, Lobby Level

6:00 PM - 8:00 PM Banquet and Guest Speaker "The Aquarium Microbiome Project: exploring the "built aquatic environment" Chrissy Cabay, Shedd Aquarium, Chicago, IL

Dunes Ballroom 1-11, Lobby level

Banquet and Guest Speaker Chrissy Cabay, Shedd Aquarium, Chicago, IL

C. Cabay[*], John G. Shedd Aquarium, Chicago, IL, USA

The John G. Shedd Aquarium is one of the most biodiverse aquariums in the nation, housing over 1,200 species and welcoming 2 million visitors annually. Its inland location means that Shedd must use municipal water sources to re-create environments

ranging from the North Pacific Ocean to the Amazon Basin. To preserve clarity, remove waste products and control pathogens, many of these water systems are subject to filtration, disinfection and rapid turnover. Yet the impact of these activities on entire microbial communities – and the potential health consequences for the resident animals that live alongside them – are not well-understood. In 2015 Shedd Aquarium joined with partners including USDA, University of Chicago and Argonne National Labs to establish the Aquarium Microbiome Project. Its goal: to understand how microbiomes within aquatic habitats can be manipulated to reduce the incidence of opportunistic infection, support immune health and otherwise create optimal environments for animals in human care. A generous gift enabled the Aquarium to construct an onsite microbial ecology laboratory and build a research team. The Project's results have since had wide-ranging impacts, from informing policy on cetacean welfare to changing how vulnerable endangered species are reared and re-introduced to the wild.