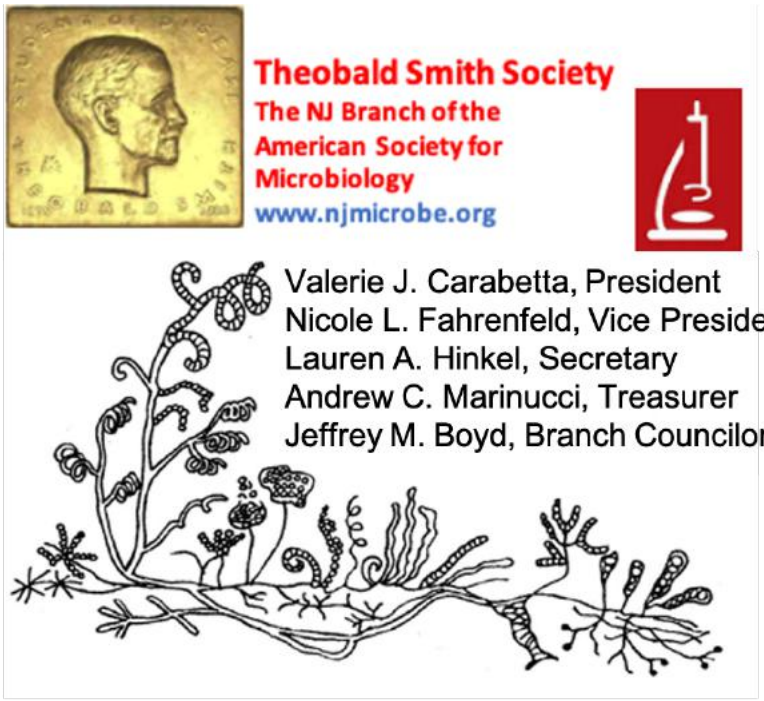

Spring 2022 Symposium Proceedings

25 May 2022

Richard Weeks Hall of Engineering

Rutgers University, Piscataway, NJ

9:30 am

Opening remarks by Valerie Carabetta, President, Theobald Smith Society

Valerie Carabetta serves as the 80th president of the Theobald Smith Society. She is an assistant professor in the Department of Biomedical Sciences at Cooper Medical School of Rowan University. Dr. Carabetta earned her PhD from Princeton University in Molecular Biology and completed her post-doctoral research at the Public Health Research Institute, New Jersey Medical School, Rutgers University.

Dr. Carabetta's primary research interest lies in understanding the physiological consequences of protein acetylation in bacteria, and the underlying mechanisms that control it. Her current work interfaces her experience and knowledge with molecular genetics, microbiology, biochemistry, proteomics and mass spectrometry to tackle difficult biological questions. One long term goal is to determine the feasibility of targeting protein acetylation as a novel therapeutic agent for the treatment of drug resistant bacteria, as has already been successful for treatment of some cancers, and latent viral and fungal infections.

Invited speakers

9:45-9:55 am Dr. Truc Do, Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ. "Phenotype-guided comparative genomics identifies the complete transport pathway of the antimicrobial lasso peptide ubonodin in *Burkholderia."*

9:55-10:05 am Makayla Manfredi, Department of Chemistry and Biochemistry, Seton Hall University, South Orange, NJ. "Inhibiting infectivity of murine hepatitis virus A59 (MHV-A59) by targeting peptide organometallic compound to spike protein."

10:05-10:15 am Dr. Adriana Messyasz, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ. "Genetic and phylogenetic mechanisms that shape *Acidobacteriota* organic matter utilization in polar soils."

10:15-10:25 am Gabrielle Popencuk, Department of Biology, William Paterson University, Wayne, NJ. "Investigation of novel *Candida auris* ERG11 mutations on triazole resistance."

10:25-10:35 am Indira Sawh, Department of Earth and Environmental Sciences, Rutgers University, Newark, NJ. "Tracing the origins of repletism and identifying the honeypot ant gut microbiota."

10:35-10:45 am Gustavo Rios-Delgado, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ. "The role of the small RNA tsr25 in *Staphylococcus aureus* iron homeostasis."

The invited speakers also presented posters. See their talk/poster abstracts below.

10:50-11:00 am

Young Investigator Award Presentation - Dane Parker, Center for Immunity and Inflammation, Department of Pathology and Laboratory Medicine, Rutgers University, New Jersey Medical School, Newark, NJ

Dr. Dane Parker joined the Center for Immunity and Inflammation and the Department of Pathology and Laboratory Medicine in April 2018 as a tenure-track Assistant Professor, where he studies the interaction between bacterial pathogens and the innate immune system. Dr. Parker obtained his PhD from Monash University in Melbourne, Australia where his focus on was the genetics and transcriptional regulation of the pathogen responsible for ovine footrot. In 2007 he moved to the laboratory of Professor Alice Prince at Columbia University where he gained skills in working with several important human bacterial pathogens responsible for respiratory and skin infections, also gaining experience with host innate immune signalling pathways important

for the detection of microorganisms. A major focus of his research is the type I and type III interferon signalling pathways, how bacterial pathogens can activate this pathway and how they influence inflammation and bacterial clearance during infection. He is actively working with the important bacterial species, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Streptococcus pneumoniae*. It is this work that is funded by an R01 grant from the National Heart Lung and Blood Institute. Dr. Parker established his laboratory in 2016 at Columbia University Medical Center and continues this work at Rutgers New Jersey Medical School. Work in the lab uses animal models and in vitro analyses to dissect both the host and bacterial response in infection.

11:00 am-12:00 pm
Keynote Address by Ann Stock, 2022 Waksman Award Winner: Adapting to Life in the Microbial World: Two-Component System Design Principles

Two-component system (TCS) phosphotransfer pathways involving a sensor histidine protein kinase and a phosphorylation-activated response regulator that generates the output response comprise a versatile regulatory scheme that occurs in hundreds of thousands of bacterial regulatory systems. While most TCSs share a few common core features, there are enormous variations in TCS configurations, such as magnitudes of enzyme activities and binding affinities, regulatory strategies, and pathway architectures. Recent research in our lab has focused on how such variations adapt the core phosphotransfer mechanism to the specific needs of individual TCSs. Our studies of design principles in TCS transcriptional regulatory pathways have revealed the importance of signaling protein concentrations and their interface with other system parameters. Perturbations to optimal concentrations of signaling proteins alter response output behavior and compromise cell fitness. Response regulator transcription factors do not exist in great excess to the numbers of their binding sites; they are sensitive to inhibition and potential targets for antimicrobial drug development.

Ann Stock is Distinguished Professor of Biochemistry and Molecular Biology at Rutgers-Robert Wood Johnson Medical School and Associate Director of the Center for Advanced Biotechnology and Medicine. She also serves as Co-Director of the Rutgers Graduate Training Program in Biotechnology. Stock obtained an A.B. in Biochemistry (1979) and Ph.D. in Comparative Biochemistry (1986) from the University of California at Berkeley working in the laboratory of Professor Daniel E. Koshland, Jr. and pursued postdoctoral studies in structural biology at Princeton University and Brandeis University with Professor Greg Petsko. She joined the Center for Advanced Biotechnology and Medicine at Rutgers University in 1991. Her research interests focus on bacterial signal transduction and the molecular mechanisms that allow cells to elicit adaptive responses to changes in their environments. She participated in the discovery and characterization of a fundamental two-component phosphotransfer mechanism, now known to mediate the majority of bacterial signaling. Her current research focuses on understanding how specific features of signaling pathway architecture provide regulation of gene expression that is optimized for the specific needs of

individual systems. Stock's research has been supported by NIH and NSF funding, NIH MERIT and MIRA awards and the Howard Hughes Medical Institute (1994-2011). She is an elected fellow of the American Association for the Advancement of Science (2006) and the American Academy of Microbiology (2007). She served as editor for *Journal of Bacteriology* (2011-2021), and currently is a member of the advisory board and editorial board for *PLoS Biology*. Stock is president-elect of the American Society for Biochemistry and Molecular Biology (ASBMB).

12:00 – 1:00 pm
Lunch

1:00 – 2:00 pm
Poster presentations (even numbered posters)

2:00 – 3:00 pm
Poster Presentations (odd numbered posters)

3:00 – 5:00 pm
REMnet Short Course
Undergraduate Concept Labs and Research Terrace
New Jersey Advanced Manufacturing Institute
Richard Weeks Hall of Engineering

The *Research Experience in Microbiomes Network* (REMNet) is a grassroots community of microbiology practitioners who are interested in developing standards for research in microbiomes that can be incorporated into the undergraduate curriculum.

POSTERS

1. Interaction sites of the Nem1-Spo7/Pah1 phosphatase cascade in yeast lipid synthesis

Ruta Jog, Mona Mirheydari, Gil-Soo Han, and George M. Carman

Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

In the yeast *Saccharomyces cerevisiae,* Pah1 phosphatidate (PA) phosphatase catalyzes the dephosphorylation of PA to produce the diacylglycerol used to synthesize triacylglycerol that is stored in lipid droplets. Pah1 is inactive as a phosphorylated form in the cytosol and becomes active as a dephosphorylated form on the nuclear/ER membrane following its recruitment and dephosphorylation by the Nem1-Spo7 protein phosphatase complex. Spo7, the regulatory subunit in this protein phosphatase complex, is required for stability and function of the catalytic subunit Nem1. In this work, we examined regions of Spo7 that are involved with the interaction with Nem1 for Nem1-Spo7 complex formation and interaction with Pah1. By deletion analyses and site-directed mutagenesis, we found that the Spo7 C-terminal residues 240-259 are important for Nem1-Spo7/Pah1 phosphatase function as indicated by a dramatic reduction in triacylglycerol synthesis and lipid droplet formation, along with a temperature-sensitive phenotype. We are currently investigating whether this sequence is required for physical interaction with Nem1 or Pah1. Supported by NIH grant GM136128

2. The yeast glycogen synthase kinase homolog Rim11 phosphorylates the phosphatidic acid phosphatase Pah1 to inhibit its catalytic activity

Shoily Khondker, Gil-Soo Han, and George M. Carman

Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

The yeast phosphatidic acid (PA) phosphatase, Pah1, regulates the synthesis of triacylglycerol (TAG) and phospholipids by controlling the relative amounts of its substrate PA and product diacylglycerol. Pah1 phosphorylation, which is mediated by multiple protein kinases, has been shown to protect the protein against proteasomal degradation and regulates its localization and catalytic activity. Phosphorylated Pah1 is cytoplasmic, but it translocates to the nuclear/endoplasmic reticulum membrane for catalytic function through its dephosphorylation. In this study, we show that Rim11, a yeast homolog of glycogen synthase kinase phosphorylates Pah1. The *rim11*Δ mutant shows alterations in lipid composition with elevated TAG levels during exponential growth, the phase in which Rim11 is the most abundant. Rim11 purified from yeast phosphorylated *Escherichia coli*-expressed Pah1, which lacks endogenous phosphorylation, in a manner that is dependent on reaction time and the amounts of Rim11, Pah1, and ATP. Phosphoamino acid analysis and phosphopeptide mapping showed that Pah1 phosphorylation by Rim11 occurs at multiple serine and threonine residues, which was supported by mass spectrometry analysis which indicated that Rim11 has a major phosphorylation site that is shared with multiple kinases (Ser-602) and two unique minor phosphorylation sites (Thr-163 and Thr-164). Furthermore, in vitro PA phosphatase assays show that Pah1 catalytic activity is inhibited following phosphorylation by Rim11. Supported by NIH grant GM136128

3. Phosphatidic acid mediates the Nem1-Spo7/Pah1 phosphatase cascade in *Saccharomyces cerevisiae*

Joanna M. Kwiatek, Gil-Soo Han, George M. Carman

Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

The Nem1-Spo7/Pah1 phosphatase cascade plays an essential role in the synthesis of the neutral lipid triacylglycerol in the yeast *Saccharomyces cerevisiae*. Pah1 is a phosphatidic acid (PA) phosphatase that is phosphorylated and inactive in the cytoplasm. The Nem1-Spo7 complex is a nuclear/endoplasmic reticulum (ER)-associated protein phosphatase that recruits, dephosphorylates, and activates Pah1. Upon its dephosphorylation, Pah1 hops onto the membrane surface for its interaction with and dephosphorylation of PA to produce the diacylglycerol that is then converted to triacylglycerol. Pah1 then scoots along the membrane to associate with and dephosphorylate another PA molecule. In this work, the Nem1-Spo7 complex was reconstituted into unilamellar phospholipid vesicles (proteoliposomes) composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and PA to mimic the nuclear/ER membrane. The phospholipid composition of the proteoliposomes, which had an average diameter of 60 nm, was confirmed by thin-layer chromatography. The reconstituted Nem1-Spo7 complex supported the recruitment and dephosphorylation of Pah1 phosphorylated by the Pho85-Pho80 protein kinase complex *in vitro* or the Pah1 phosphorylated by multiple protein kinases *in vivo*. The Nem1-Spo7-mediated dephosphorylation of Pah1 was dependent on the presence of PA in the proteoliposomes. Additionally, the dephosphorylated Pah1 catalyzed the dephosphorylation of PA to produce diacylglycerol in the proteoliposomes. This reconstituted system permits a defined membrane environment to examine the regulation of the Nem1-Spo7/Pah1 phosphatase cascade. Supported by NIH grant GM136128

4. Yeast Pah1 PA phosphatase contains a novel domain within its N-terminal intrinsically disordered region.

Geordan J. Stukey, Gil-Soo Han, and George M. Carman

Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

Pah1 PA phosphatase catalyzes the dephosphorylation of PA to produce diacylglycerol. In yeast and higher eukaryotes, the diacylglycerol produced in the reaction is used for the synthesis of triacylglycerol or the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine. Pah1 is inactive as a phosphorylated enzyme in the cytosol and becomes active after its recruitment and dephosphorylation by the ER-associated Nem1-Spo7 protein phosphatase complex. Conserved N-LIP and HAD-like domains are required for PA phosphatase activity and a conserved tryptophan within the WRDPLVDID domain is required for its *in vivo* function in lipid metabolism. Intrinsically disordered regions, which are located between the conserved catalytic domains and at the C terminus, contain multiple sites for phosphorylation and regulation of Pah1 location and function. Prediction of Pah1 structure by AlphaFold identifies a novel domain contained within the N-terminal intrinsically disordered region for which its function is unknown. A truncation mutant that lacks amino acid residues 186-266 have been constructed and expressed in a *pah1*Δ mutant to assess the function of the novel domain for regulating Pah1 phosphorylation, recruitment and dephosphorylation by the Nem1-Spo7 protein phosphatase complex, and function in lipid metabolism. Supported by NIH grant GM136128

5. Examining the stimuli of the SrrAB regulatory system in *Staphylococcus aureus*.

Franklin Román-Rodríguez; Jeffrey M. Boyd.

Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ.

Staphylococcus aureus is one of the leading pathogens in the world employing various metabolic pathways and virulence factors that help it successfully colonize the host and thrive in different environments. The SrrAB Two Component Regulatory System (TCRS) controls the change between fermentative and respiratory growth in *S. aureus* and regulates the expression of many virulence genes including toxic shock toxin. SrrAB has shown response to the buildup of reduced menaquinone and oxygen, but the direct stimuli are unknown. To uncover the stimuli of SrrAB and factors that alter srrA expression we built and screened a transposon mutant library in a strain containing a srrA transcriptional reporter. Preliminary results suggest the SrrA response regulator acts as a repressor of the srr operon as mutants with a srrA mutation show increased promoter activity during aerobic growth. The srrAB promoter activity was also responsive to alterations in media glucose levels which is consistent with it responding to fermentative growth. Furthermore, mutant strains were isolated that had lesions in genes encoding for TCA cycle enzymes including aconitase. The mutations resulted in decreased transcriptional activity independent of SrrAB. Mutants were also isolated containing lesions in the genes pckA and gapB that function in gluconeogenesis. These mutants have increased srrAB transcriptional activity independent of SrrAB. Taken together, these results suggest that an alternate regulatory system detects altered carbon flux through the TCA cycle and gluconeogenesis and responds by altering srrAB transcription. We are currently working on identifying the regulators and metabolites which trigger these responses.

6. Effects of plastic on naproxen degradation via a freshwater consortium.

Zanub Rahman, Abigail W. Porter, Lily Y. Young

Rutgers University, New Brunswick, NJ

Research on naproxen, an anti-inflammatory pain reliever, has established its prevalence in the environment through incomplete degradation via wastewater treatment, along with its primary metabolite, 6-O-desmethylnaproxen (DMN), but there is a lack of work surrounding the interaction of this compound with other aquatic contaminants like plastic. Plastic pollution is extremely prevalent in aquatic environments and it can serve as a surface for biofilm growth. It was hypothesized that these congregations of bacteria may transform chemical compounds, like naproxen, quicker than planktonic bacteria, due to sorption of naproxen to the surface substratum. This project began with cultures from anoxic sediment of the Raritan River which were amended with polystyrene, aluminum, or no surface substratum. These cultures were analyzed via gas chromatography (GC) to measure methane gas production and high-performance liquid chromatography (HPLC) to measure naproxen loss and DMN formation. After the naproxen disappeared, DNA sequencing was conducted to characterize the communities present within the liquid culture and on the surface of the plastic. Analysis revealed that naproxen disappeared within 2 weeks regardless of amendment with surface substratum but the biofilms on plastic were able to metabolize naproxen on their own.

8. Phenotype-guided comparative genomics identifies the complete transport pathway of the antimicrobial lasso peptide ubonodin in *Burkholderia*.

Truc Do[1], Alina Thokkadam[1], Robert Leach[2], A. James Link[1,3,4]

1. Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ
2. Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ
3. Department of Chemistry, Princeton University, Princeton, NJ
4. Department of Molecular Biology, Princeton University, Princeton, NJ

New antibiotics are needed as bacterial infections continue to be a leading cause of death, but efforts to develop compounds with promising antibacterial activity are hindered by a poor understanding of – and limited strategies for elucidating – their modes of action. We recently discovered a novel lasso peptide, ubonodin, that is bioactive against opportunistic human lung pathogens from the *Burkholderia cepacia* complex (Bcc). Ubonodin inhibits RNA polymerase, but only select strains are susceptible, indicating that having a conserved cellular target does not guarantee activity. Given the cytoplasmic target, we hypothesized that cellular uptake of ubonodin determines susceptibility. Although Bcc strains are equipped with numerous nutrient uptake systems, these organisms lack close homologs of the single known lasso peptide membrane receptor FhuA. Thus, a straightforward homology-driven approach failed to uncover the identity of the ubonodin transporter(s). Here, we developed a phenotype-guided comparative genomics approach to identify genes uniquely associated with ubonodin-susceptible Bcc strains, leading to the identification of PupB as the ubonodin outer membrane receptor in *B. cepacia*. Loss of PupB renders *B. cepacia* resistant to ubonodin, whereas expressing PupB sensitizes a resistant strain. We also examine how a widely conserved iron-regulated transcriptional pathway controls PupB to further tune ubonodin susceptibility. PupB is only the second lasso peptide OM receptor to be uncovered and the first outside of enterobacteria. Finally, we elucidate the complete cellular uptake pathway for ubonodin by identifying its inner membrane receptor YddA in *B. cepacia*. Our work provides a complete picture of the mode of action of ubonodin and establishes a general framework for deciphering the transport pathways of other natural products with cytoplasmic targets.

9. Inhibiting infectivity of murine hepatitis virus A59 (MHV-A59) by targeting peptide organometallic compound to spike protein

Makayla Manfredi, Gregory Wiedman, Daniel Nichols, Wyatt Murphy

Department of Chemistry and Biochemistry, Seton Hall University, South Orange, NJ

Murine hepatitis virus A59 (MHV-A59) is a coronavirus that infects mice with diseases similar to human conditions. Pseudoviruses are useful to determine mechanisms of action in viral infectivity because of their versatility and safety. MHV-A59 is a pseudovirus to severe acute respiratory-associated coronavirus 2 (SARS-CoV-2) with common structural features in their viral spike (S) glycoproteins. The S protein on viruses is responsible for membrane fusion and mediating receptor binding. The development of peptides analogous to regions outside the fusion spike protein are the basis for inhibition of viral infectivity. In recent studies, the combination of specified peptides and photosensitizers have demonstrated promising results regarding treatment of infections through photodynamic techniques. The photosensitization process where the transfer of energy excitation from ground-state oxygen to singlet oxygen, is an important aspect in oxidative degradation reactions. This process is the basis of photodynamic therapy that utilizes light, a sensitizer, and oxygen to initiate cell death. This presentation discusses the development of a targeting peptide attached to a photosensitizer organometallic complex in order to inhibit and kill virus particles through its spike protein.

10. Identification of small proteins in *Escherichia coli* using translation initiation profiling.

Sangeevan Vellappan[1,2,3], John S. Favate[2], Premal Shah[2,3], Srujana S. Yadavalli[1,2]

1. Waksman Institute of Microbiology, Rutgers University, Piscataway NJ
2. Department of Genetics, Rutgers University, Piscataway NJ
3. Human Genetics Institute of New Jersey, Rutgers University, Piscataway NJ

Many proteins less than 50 amino acids long, derived from short open reading frames, were missed in early genome annotations due to minimum gene length cut-offs. Their true prevalence and function remain unknown due to their small size and hydrophobicity, causing serious limitations on their identification and purification. Recent advances in computational and experimental methods have led to the discovery of more than 150 small proteins in E. coli grown in rich media. Interestingly, a substantial fraction of the known small proteins in *E. coli* is hydrophobic and putative membrane proteins. These proteins may play a role in sensing environmental cues and mediating stress responses by directly interacting with and regulating large transmembrane complexes such as sensor kinases, channel proteins, and drug efflux pumps. It remains unclear which set of small proteins accumulate under specific growth conditions and how they modulate gene expression. To answer this question, we are analyzing how stress conditions alter the expression profiles of small proteins by using an optimized high-throughput translation initiation profiling method – RETapamulin enhanced Ribo-seq (Ribo-RET). Optimization to the original Ribo-RET method increases signal-to-noise ratio and allows us to eliminate potential spurious ribosome binding. Furthermore, by generating Ribo-RET data for *E. coli* cells grown in mildly acidic pH and low Mg^{2+}, we have systematically identified several stress-specific and differentially expressed small proteins. Our current work aims at using deep learning tools to predict unannotated translation start sites of small proteins from the Ribo-RET dataset. Experiments are also on-going to characterize the top hits of both known and unannotated stress-induced small proteins from the Ribo-RET dataset through validation of their in-vivo expression, physiological effects of deletion and overexpression, cellular localization and identification of their potential binding partners.

11. Strategies for sampling and detection of opportunistic pathogens in drinking water distribution systems.

William R. Morales-Medina, Joyce Zhu, Zia Bukhari

American Water, Camden NJ

Waterborne opportunistic pathogens (OPs) like *Legionella pneumophila*, *Pseudomonas aeruginosa* and non-tuberculous mycobacteria (NTM) pose a significant public health impact, causing more than 7 million infection each year in the US. OPs can survive and proliferate in the drinking water distribution system (DWDS) network. Existing drinking water regulations do not directly monitor OPs, and as a result, risk of waterborne disease transmission is unknown. A major impediment has been a lack of standardized protocols for sampling, detecting and quantifying OPs in the distribution network. Monitoring in DWDS is also complicated by the fact that the abundance of OPs may differ at different locations in the distribution system, which makes monitoring site prioritization key for any sampling program. This ongoing research is focused on defining a practical and tractable approach determining the 'what', 'where' and 'how' for sampling and monitoring OPs in DWDS. Initially we built on traditional culture-based analytics and advanced to molecular quantification assays using droplet digital PCR. The latter uses a unique approach to overcome environmental matrix inhibition, improving reliability and robustness of molecular assays in environmental applications. Then a tractable sampling and concentration method for 50L of bulk water was developed using medical dialysis ultrafilters. To identify strategic sampling locations in the DWDS, we developed an interactive visualization application that integrates a modeling based on historic geocoded water quality (WQ) data, which are collected in different location of the DWDS for regulation and compliance purposes by utilities. This web-based application, which provides an innovative approach for prioritizing sites in DWDS for monitoring OPs will be available for other interested utilities. Data will also be discussed to demonstrate how effectively the predicted OPs vulnerable locations from this tool can be corroborated through field sampling and analyses for OPs. Spiking experiments demonstrated effective and reproducible (i.e., 76 - 99%) recovery for all four OPs using molecular end detection. Sampling sites in the distribution system showing medium and high vulnerability, based on data visualization tool free chlorine and temperature, were tested through specific OPs analyses using the sampling and analytical methods. A higher molecular signal and culturable OPs were detected in high vulnerability locations, which had lower free chlorine concentrations. Temperature demonstrated a negative impact on OP concentration (especially with culturable OPs) and lower OPs were detected at colder temperatures. Impact of additional relevant WQ parameter on improving OPs site selection is being explored and utility of machine learning/artificial intelligence is also being assessed. The work discussed in this presentation will provide guidance for utilities interested in quantifying and managing risks of *Legionella* and other opportunistic pathogens in drinking water systems. This is expected to help utilities develop strategic mitigations strategies to better control public health impacts of these important waterborne pathogens.

12. Genetic and phylogenetic mechanisms that shape *Acidobacteriota* organic matter utilization in polar soils.

Adriana Messyasz[1], Lee Kerkhof[1], Dr. Minna Männistö[2], Max Häggblom[1]

1. Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
2. Natural Resources Institute Finland, Rovaniemi, Finland

Climate change is affecting arctic tundra ecosystems severely. Higher temperatures are driving increased microbial respiration of soil organic matter and the release of carbon dioxide and methane. To understand the mechanisms of microbial soil organic matter utilization, we examine the complex diversity of polar soil microbial communities with a focus on the ubiquitous but elusive members of the *Acidobacteriota*. Our aim is to uncover whether genetic and phylogenetic differences in *Acidobacteriota* species or strains link to differences in carbon and nitrogen utilization as well as predation of certain strains/species over others. We utilized the long-read capability of the Oxford Nanopore MinION to profile bacterial ribosomal operons of tundra soil *Acidobacteriota* communities and isolates. The inclusion of the hypervariable ITS region within the rRNA operon allows us conduct strain-level classification of *Acidobacteriota*. These sequences along with metagenomic long-read sequences were then used to assemble full *Acidobacteriota* genomes to annotate genes associated with organic matter utilization and pangenome markers to differentiate *Acidobacteriota* strains. Through this method we have been able to characterize multiple novel *Granulicella*, *Edaphobacter* and *Terriglobus* strains in subdivision 1 of the *Acidobacteriota* and improve the accuracy of the phylogenetic placement of novel and known species and strains. We have been able to separate the *Acidobacteriota* into clades which will be analyzed further to identify functional and genetic differences that match up with differences in organic matter utilization. By understanding the complex diversity of *Acidobacteriota*, we can elucidate the mechanisms which shape microbial communities in polar soils.

13. Antimicrobial and antibiofilm potential of a medicinal plant root extract.

Nuha Sbateen, Imen Elmaraoub, Muizzat Alli, Malika Abakkass, Meriem Bendaoud,

Biology Department, New Jersey City University, Jersey City, NJ

Antibiotic resistance is a growing public health problem. Some bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* are capable of causing severe infectious diseases and are becoming more resistant to many commonly known antibiotics. This public health concern has prompted a worldwide interest in using natural anti-microbial compounds. In this study, we directed our focus on a root extract of a widely used medicinal plant (CR) in the treatment for diabetes and to improve the immune system. However, very little is known about the potential antimicrobial and antibiofilm properties of the CR root extract. In the present study, the antimicrobial effect of CR extract was tested on a wide range of gram-positive and gram-negative bacteria as well as fungi using the broth assay. CR was found to have a strong antibiofilm effect against *S. aureus* and *S. epidermidis* and various antimicrobial effects on other gram-positive and gram-negative bacteria as well as fungi. The active fraction appears to be greater than 100 KDa in size. Heat and Proteinase K treatment of the root extract had no effect on its activity. Further studies will be conducted to characterize and identify the active fraction of the CR medicinal plant.

14. Characterization of sponge-associated dehalogenating bacterial strains from Australia, Hawaii, and New Jersey.

Lauren A. Hall[1], Kaitlin A. Decker[1], Katie Scott[1], Max Dvinskikh[1], Kayla Ventura[1], Kate MacKenzie[1], Eric Chiles[1,2], Xiaoyang Su[2], Nicole Webster[3], Lee J. Kerkhof[4], Max M. Häggblom[1]

1. Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
2. Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ
3. Australian Antarctic Division, Tasmania, Australia
4. Dept. of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ

Marine sponges (*Porifera*) naturally produce a diverse range of halogenated secondary metabolites. Host-specificity of sponge-associated microbial communities has been well described and may be driven by the unique organohalogen chemistry of different sponge species. We hypothesize that the presence of these metabolites creates a selective environment enriching for organohalide-respiring bacteria (ORB) and results in stable, host-specific dehalogenating populations that colonize the sponge tissues. To compare the distribution of dehalogenating bacteria, various sponge species were collected in the Great Barrier Reef, Hawaii, and off the New Jersey coast. Debrominating activity was detected in all species and sustained in enrichment cultures from most sponges. To examine host-specificity at the strain level, ribosomal operons from tissues samples, dehalogenating enrichment cultures, and debrominating isolates were sequenced using the Oxford Nanopore MinION. Operons were screened against an NCBI database using BLAST, QA/QC was performed using Geneious, and phylogenetic analysis was conducted using FastTree. Divergent *Desulfoluna* strains were detected in the various sponges from Australia and Hawaii. This suggests considerable strain-level diversity. *D. spongiiphila* and/or close relatives appear to be responsible for reductive dehalogenation in diverse sponge species. Organobromide production in sponge tissues creates a distinct niche for ORB, and sponges harboring ORB strains are widely distributed throughout the global ocean.

15. F-box proteins detect *Mycobacterium tuberculosis* invasion to initiate selective autophagy.

Pamela R. Barnett, Samantha L. Bell

Public Health Research Institute, Rutgers New Jersey Medical School, Newark, NJ

Mycobacterium tuberculosis (Mtb) is the causative agent of one of the deadliest infectious diseases worldwide, tuberculosis, and represents a particular challenge for the global healthcare system. Current treatment options are complex and long in duration, leading to nonadherence and emergence of drug resistant strains of Mtb. As a result, there is an urgent need for novel therapeutics. Host directed therapies are an attractive antitubercular development niche, but one that can only be exploited with further investigation into the mechanisms that underly the immune response to invading Mtb. The first immune cell type typically encountered by Mtb is the macrophage. Mtb is phagocytosed by these cells as part of the host innate immune response. Within the macrophage, Mtb can damage the phagosome and gain access to the cytosol. Importantly, this event also exposes the damaged phagosome and Mtb to mediators of the selective autophagy pathway, which degrades cytosolic cargo including cytosolic pathogens. Approximately 30% of Mtb bacilli are cleared by this antibacterial pathway, but initiation of this process is not well understood. A subset of F-box only (FBXO) proteins, part of the Skp, Cullin, F-box (SCF) ubiquitin-ligase complex, are expressed in macrophages and have been associated with selective autophagy in the context of damaged lysosomes and other intracellular infections. By using a series of genetic manipulations, mainly overexpression, shRNA-based knockdown, and CRISPR-based knockout, we will modulate the expression of the glycan binding FBXO proteins along with the other components of the SCF complex, Skp1 and Cul1. Preliminary results indicate that both Skp1 and Cul1 play a role in restriction of bacterial replication, suggesting the involvement of one or more of the FBXO proteins. We aim to elucidate how each protein functions in selective autophagy, specifically focusing on their roles detecting damaged phagosome, recruiting adaptor proteins, and ultimately controlling Mtb survival and replication.

16. Antimicrobial and anti-biofilm activity of N-acetyl-L-cysteine and L-histidine on different strains of pathogenic bacteria

Muizzat Alli, Nuha Sbateen, Malika Abakkass, Imen Elmaroub, Meriem Bendaoud

Biology Department, New Jersey City University, Jersey City, NJ

Biofilm formation has become one of the leading causes of infections in hospitals due to its resistance to antibiotic treatments. There has been a dire need for new compounds that possess antimicrobial properties that inhibit the growth of biofilms and potentially kill pathogens. As a result, many scientists have turned to natural compounds as a possible solution to this ongoing concern. In this research, our goal was to determine the antimicrobial and antibiofilm activity of two known compounds, N-acetylcysteine, and L-histidine, on pathogenic bacteria and fungi. To achieve this goal, we tested the antibiofilm and antimicrobial effect of the two amino acids against 14 different strains of bacteria and 3 strains of fungi using a biofilm and broth assay. Results showed that N-acetylcysteine had a strong bactericidal effect against all the tested pathogenic bacteria at 50mM. N-acetylcysteine was also capable of biofilm inhibition in some bacteria. L-histidine demonstrated minor inhibition of biofilm formation activity against *Staphylococcus aureus* and *Escherichia coli*, and no bactericidal activity again all tested pathogens. Future studies will focus on studying the cytotoxic effects of N-acetylcysteine on human cells.

17. Investigation of antifungal tolerance genes RTA1 and SLT2 on echinocandin susceptibility and resistance in *Candida glabrata*.

Saira Tahsin, Gabrielle Popencuk, Kelley R. Healey

Department of Biology, William Paterson University, Wayne, NJ

Candida glabrata is a fungal pathogen that can cause invasive infections in immunocompromised patients. These infections are treated with echinocandin antifungal drugs that inhibit fungal cell wall synthesis by targeting beta-1,3-glucan synthase. Mutations in genes (FKS1 or FKS2) that encode for this target enzyme lead to resistance; however, multiple tolerance/adaptation pathways are activated upon drug exposure prior to FKS mutation. We disrupted tolerance genes, including SLT2 (cell wall integrity pathway) and RTA1 (lipid translocator), in FKS mutants to determine their relevance in echinocandin resistance. Competent FKS mutant cells were transformed with a targeted, PCR-amplified deletion marker. Colonies grown up on selective medium were PCR-screened and sequenced to confirm successful gene deletion. Subsequent drug susceptibility assays demonstrated that SLT2 disruption resulted in reversal of echinocandin resistance, while RTA1 disruption resulted in no significant changes. This study has provided additional insight into how targeting specific tolerance factors may influence drug resistance. We plan to further investigate the role of STL2 in antifungal resistance and test more genes implicated in tolerance.

18. Abundance, diversity, and host assignment of total, intracellular, and extracellular antibiotic resistance genes in riverbed sediments.

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Human health risk assessment for environmental antibiotic resistant microbes requires not only quantifying the abundance of antibiotic resistance genes (ARGs) in environmental matrices, but also understanding their hosts and genetic context. Further, differentiating ARGs in intracellular and extracellular DNA (iDNA and eDNA) fractions may help refine our understanding of ARG transferability. The objectives of this study were to understand the (O1) abundance and diversity of extracellular, intracellular, and total ARGs along a land use gradient and (O2) impact of bioinformatics pipeline on the assignment of putative hosts for the ARGs observed in the different DNA fractions. Sediment samples were collected along a land use gradient in the Raritan River, New Jersey, USA. DNA was extracted to separate eDNA and iDNA and qPCR was performed for select ARGs and the 16S rRNA gene. Shotgun metagenomic sequencing was performed on DNA extracts for the different DNA fractions. ARG hosts were assigned via two different bioinformatic pipelines: network analysis of raw reads versus assembly. Results of the two pipelines were compared to evaluate their performance in terms of number and diversity of linkages and accuracy of in silico matrix spike host assignments. No differences were observed in the 16S rRNA gene normalized sul1 concentrations between the DNA fractions. The overall microbial community structure was more similar for iDNA and total DNA compared to eDNA and generally clustered by sampling site. ARGs associated with mobile genetic elements increased in iDNA for the downstream sites. Regarding host assignment, the raw reads pipeline via network analysis identified 247 ARG hosts as compared to 53 hosts identified by assembly pipeline. Other comparisons between the pipelines were made including ARG assignment to taxa containing waterborne pathogens and practical considerations regarding processing time.

19. Investigation of novel *Candida auris* ERG11 mutations on triazole resistance.

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Candida auris is an emerging fungal pathogen that can cause invasive infections in immunocompromised patients and demonstrates high rates of drug resistance, including to triazole antifungals. Triazoles inhibit an enzyme (Erg11) in the ergosterol biosynthesis pathway. Specific Erg11 amino acid substitutions identified in isolates of *C. auris* have been shown to directly contribute to triazole resistance. Two new amino acid substitutions (E102K and G459S) were recently identified in clinical isolates of clade IV. To determine the role of these novel mutations on triazole susceptibility, we expressed each Erg11 allele in the model organism *Saccharomyces cerevisiae* using gap-repair cloning. Plasmid pRS416 was linearized with SmaI endonuclease and then treated with shrimp alkaline phosphatase (SAP) to help prevent recirculation. The Erg11-E102K and Erg11-G459S alleles were PCR amplified from clinical isolate DNA with primers that included overhang regions homologous to each side of the SmaI site on pRS416. S. cerevisiae cells were then co-transformed with purified PCR and digested plasmid. Colonies were PCR screened to confirm plasmid presence. Following whole cell DNA isolation, the entire *C. auris* ERG11 gene insert from two transformants was PCR amplified, purified, and sequenced to confirm the expected gene sequences. Drug susceptibility assays were performed with short-tailed triazole antifungals. Results demonstrate little (≤ 2-fold) differences in triazole susceptibilities upon expression of pErg11-E102K in *S. cerevisiae* compared to the wild type Erg11 and moderate decreases in susceptibilities (2- to 8-fold) with pErg11-G459S expression.

20. Dual functions of a biosynthetic enzyme (QueE) in bacterial stress response and translation.

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Queuosine (Q), a hypermodified guanosine, is a universally conserved tRNA wobble modification, which occurs in the anticodon loop(G34UN) of specific tRNAs. QueE is one of the enzymes in the biosynthetic pathway essential for Q formation. Previous work from our lab illustrated a new role for QueE during stress response in *E. coli*. When *E. coli* cells are grown in sub-inhibitory concentrations of cationic antimicrobial peptides, a condition known to activate the broadly conserved two-component signaling system, PhoQ/PhoP, there is an upregulation of QueE expression. High levels of QueE block septation in *E. coli* cells resulting in filamentous growth. An intriguing question is whether the role of QueE as an inhibitor of cell division is functionally distinct from its role in tRNA modification. Additionally, the mechanism by which QueE causes cell division inhibition remains unclear. In this work, using alanine scanning and APB-gel tRNA northern blot analyses, we show that the role of QueE in tRNA modification is not functionally linked to its secondary role in blocking division by identifying amino acid residues that specifically affect each of the two functions. Using site-specific in vivo crosslinking technique coupled with mass spectrometry, we have identified proteins in the divisome machinery that interact with QueE. Finally, we seek to validate the hits identified from our crosslinking study to elucidate the interactions of QueE with the division proteins, using bacterial two-hybrid assays and genetic analysis. Deciphering the mechanism of QueE's dual activities in the cell will provide insights into how RNA metabolism is interconnected to critical cellular processes such as cell division and stress responses.

21. Tracing the origins of repletism and identifying the honeypot ant gut microbiota.

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Ants with a replete caste have acquired an efficient means of food storage for colony survival. Repletism is a behavior where a specific group of workers store liquid food in their crop, resulting in the expansion of their abdomen and are often referred to as honeypot ants. The stored food is regurgitated to other colony members, when food availability is scarce. Repletism is found across distantly related ant genera, but the temporal history and phylogenetic distribution of this phenotypic syndrome remains unknown. Here, we reconstruct the evolutionary history of this adaptive behavior, and examine the role of microbes in repletism. We expect that this dietary specialization would be consistent with a conserved microbiota. We describe the first two known instances of repletism in the fossil record; replete workers of the genera *Leptomyrmex* from Dominican amber and *Prenolepis* from Baltic amber, dating to approximately 16 mya and 34 mya, respectively. We mapped the history of this trait onto a time-calibrated phylogeny of extant ants; results suggest that certain lineages are more prone to repletism, and that most origins likely occurred in the Eocene and Miocene. To determine if honeypot ants have a resident gut microbiome, we isolated bacteria from the crop and midgut of *Myrmecosystus mexicanus* honeypot ants. In our preliminary work, we were able to grow 112 total isolates of microbes, belonging to the classes *Bacilli*, *Actinomycetia*, and Gammaproteobacteria. We found the greatest genus richness from replete honey, consisting primarily of *Bacilli*. Importantly, our early data suggest that honeypot ants have a resident gut microbiome with *Pseudomonas* sp. and *Curtobacterium* sp. consistent in the crop and *Peribacillus* sp. and *Acinetobacter* sp. in the midgut. The gut microbiome may play an important role in the ability of repletes to retain liquid for long periods of time, toxin remediation, or nutrient supplementation for the ants. Our study of the evolutionary history of this behavior in combination with analysis of the gut microbiome provides the starting point to further studies of the evolution and natural history of this convergently-evolved trait.

22. Continued investigations into a Cryptococcal antifungal peptide.

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To expand on the work presented in the latest publication on the topic of Cryptococcal targeting antifungal peptides, the author sought to rationalize the activity of the Cryptococcal antifungal peptide dubbed AW9-Ma. This peptide inhibited the growth of *C. neoformans* on its own at 64μg/mL. This peptide potentiated the effects of caspofungin when mixed in a checkerboard assay leading to an FIC index of 0.5. Annexin assays revealed this peptide was a potent phosphatidylserine (PS) flippase inhibitor. The peptide was also somewhat hemolytic. Protein databank structures of the principal PS flippase in red blood cells (RBCs), ATP11C-Cdc50a was analyzed using Pymol software. A 14Å distance between the binding pocket of PS and the drug analog in Cdc50a was found and correlates well with the length of the myristic acid peptide fatty acid tail. AW9-Ma was found to potentiate the effects of a variety of antifungal drugs in-vitro. Subsequent peptide modifications led to dramatic improvement of the antifungal activity of the peptide.

23. Isolation and identification of anaerobic debrominating bacteria from marine sponges.

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Halogenated organic compounds (organohalides) are toxic, often resistant of biotic and abiotic breakdown compounds of natural and anthropogenic origin, and various industrial processes result in production of toxic amount of the compounds. The breakdown of naturally produced organohalides is a process that is associated with activity of anaerobic dehalogenating bacteria that are found in microbiome of ocean sponges. The sponges produce organohalides as part of their natural defense against predators, which introduces the niche for organisms that can break down the toxic compounds by breaking the carbon-halogen bond. The goal of this study was to isolate bacteria capable of dehalogenation. They were isolated from samples of ocean sponges that were collected at the Great Barrier reef (Australia). The original cultures were enriched with 2,6-dibromophenol (2,6-DBP) to maintain debromination activity, after which serial dilution to extinction with 2,6-DBP as sole electron acceptor was performed to isolate dehalogenating bacteria. The hypothesis was that different sponge species harbor distinct dehalogenating bacterial strains. Debromination activity was observed in some of the samples, and the isolation and identification of the bacteria proved the hypothesis.

24. Protective effect of a fiber formula on human gut microbiota against microaerobic conditions in ex vivo fermentation system.

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Hypothesis/Central Question: The gut microbiome of Inflammatory Bowel Diseases (IBDs) patients has been characterized by a decrease in obligate anaerobes and an increase in facultative bacteria, which suggests that oxygen play a role in disruption of anaerobiosis (Lionel et al, 2013). A fiber formula which contains wheat bran, oat bran, corn bran, sorghum bran, inulin and Fibersol-2 has been developed. We hypothesize that this fiber formula that contains diverse fibers, might reverse the disruption of gut bacteria community induced by oxygen through 1) scavenging oxygen and its derivatives via polyphenols and 2) promoting growth of anaerobic beneficial bacteria as a preferred energy source.

Summary/Results and Conclusion(s): Fresh fecal slurry from a healthy donor were inoculated in 4 conditions: 1) (-)O_2/(-)Fibers; 2) (+)O_2/(-)Fibers; 3) (-)O_2/(+)Fibers; 4) (+)O_2/(+)Fibers. During 48 hours, fermented samples were collected to determine the microbiota composition. 552 amplicon sequence variant (ASVs) were identified. Alpha diversity showed that when no fibers added, Shannon index of oxygen group was significantly lower than non-oxygen group while no significant differences at 24 hours with fibers presented. PCoA based on Bray-Curtis distance showed no significant difference between +/- O_2 groups when fibers presented at the first 24 hours while significant separation was shown between +/- O_2 groups without fibers (Fig 1.) After grouping 552 ASVs into 15 co-abundant groups (CAGs), GAG2 was significantly promoted in the (+)O_2/(-)Fibers while CAG10 was significantly promoted in (+)O_2/(+)Fibers group. CAG2 included *Eisenbergiella*, *Intestinimonas* and *Lachnospiraceae*, while CAG10 included *Bifidobacterium*, *Lactobacillus* and *Sutterella*, which are conceived as beneficial bacteria. This primary ex vivo results indicates that fiber formula can promote beneficial bacteria and reverse the disruptive effect induced by oxygen in the gut microbiota.

25. Rapid mutation during geminivirus infection of cassava, tomato, and *Arabidopsis thaliana*.

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Geminiviruses are circular ssDNA viruses with high nucleotide-substitution rates that may enable them to adapt rapidly to plants. We and others are using deep sequencing to analyze mutation in geminivirus populations initiated from infectious clones. Because the starting sequence of the plasmid inoculum is known, any sequence variants detected almost certainly emerged via spontaneous mutation. We recently showed (https://doi.org/HKXQ) that a geminivirus reproducibly mutates during 28-day infection of *Arabidopsis thaliana* at a codon that may affect coat protein phosphorylation and a candidate Rep binding site (iteron) in the second genome component (DNA-B). We are using similar experiments to analyze changes over the course serial whitefly transmission between tomato plants and serial vegetative propagation of cassava.

26. Strand-specific codon usage patterns across Cressdnaviricota corroborate atypical gene orientations of unclassified CRESS DNA viruses.

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The rapidly expanding phylum Cressdnaviricota contains circular, Rep-encoding single-stranded (CRESS) DNA viruses that are organized within seven established families, but many CRESS DNA virus sequences are not taxonomically defined. We hypothesized that genes in Cressdnaviricota ambisense genomes exhibit strand-specific signatures due to a cytosine to thymine transition bias that can help determine the orientation of the genome: which strand is packaged and is in the "virion sense". We developed a statistical test to identify relative codon overrepresentation between ambisense sequence pairs of the two major opposite sense open reading frames for 712 classified CRESS DNA virus exemplars and an additional 137 unclassified CRESS DNA viruses. Codons grouped by the identity of their third-position nucleotide display both strand- and genus-specific patterns across Cressdnaviricota. Roughly 70% of virion-sense sequences have a relative overrepresentation of thymine-ending codons while ~80% of anti-sense sequences display a relative overrepresentation of adenine-ending codons (corresponding to a relative overrepresentation of thymine in these genes as packaged). Thirteen of the 137 unclassified viruses show strong evidence of having the rarer circovirus-like genome orientation, and likely represent novel genera or families within Cressdnaviricota. Given the strong strand-specific patterns of relative codon overrepresentation, the results suggest that the relative codon overrepresentation test can serve as a tool to help corroborate the genome orientation of unclassified CRESS DNA viruses.

27. Role of a biosynthetic enzyme, QueE in oxidative stress response in *E. coli*

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The concept of antimicrobial resistance has taken on different roles due to the emergence of different types of lethal bacteria. Bacterial resistance is well studied, and it's essential to understand the pathway in which the bacterial cell responds to other stressors. Our lab studied the PhoQ/PhoP signaling system and the role of an enzyme called QueE in response to antimicrobial peptides. A high level of activation of PhoQ in response to sub-lethal levels of cationic antimicrobial peptides causes cell division to halt, resulting in the filamentous growth of *E. coli*. QueE, an enzyme in the Queuosine (Q) tRNA modification pathway, is expressed at high levels during this response and it is shown to co-localize with the cell division complex. Therefore, QueE plays dual roles in the cell (i) blocking division during AMP stress response and (ii) formation of Q-tRNAs important for translation. My research will investigate the pleiotropic effects QueE has on the cell. Oxidative stress reagents and antimicrobial agents can affect cell growth and enzyme function in *E. coli*. Previous findings show that filamentation is caused due to the presence of high levels of QueE in the cell, and this filamentation is likely an adaptive mechanism. The research question then is, what advantages does the presence of QueE confer to a cell? Our initial data indicates growth defects when the cell has a loss of queE. Understanding the molecular response of E. coli to these stressors will indicate how QueE links distinct cellular processes such as RNA metabolism and translation with cell division and stress response.

28. Rwandan mosquito bacterial microbiome communities are both host and habitat specific.

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Mosquitoes are a complex nuisance around the world, and tropical countries bear the greatest brunt of the burden of mosquito-borne diseases. Rwanda has had success in reducing malaria and some arboviral diseases over the last few years, but still faces challenges to elimination. By building our understanding of in situ mosquito communities in Rwanda at a disturbed, human-occupied site and at a natural, preserved site, we can build our understanding of natural mosquito microbiomes toward the goal of implementing novel microbial control methods. Here, we examined the composition of collected mosquitoes and their microbiomes at two diverse sites using Cytochrome c Oxidase I sequencing and 16S V4 barcode sequencing. The majority of mosquitoes captured and characterized in this study are the first-known record of their species for Rwanda but have been characterized in other nations in East Africa. Beta diversity metrics were significantly different between sampling sites, mosquito genera, and mosquito species, but not between mosquito sexes, catch method, or presumed bloodfed status. Bacteria of interest for arbovirus control, *Asaia*, *Serratia*, and *Wolbachia*, were found in abundance at both sites, but were more prevalent at the disturbed site and varied greatly by species. Additional studies to build our understanding of naturally-formed microbial communities are essential to safely employing microbial control methods and further reducing the burden of mosquito-borne diseases.

30. Isolation of microorganisms from ten years old southern pine beetle galleries.

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Southern Pine Beetle (SPB, *Dendroctonus frontalis*) is a pest of pine trees. These beetles lay eggs along galleries in the phloem of the pine trees, causing both ecological and economic problems due to the damage they inflict on pine forests. Therefore, it is essential to understand the mechanisms of SPB attack. There is a symbiotic relationship between the SBP and different microorganisms. An organ called the mycangium on SPBs serves as a vector to store and transport fungal symbionts such as *Entomocorticium* sp. A, *Ceratocystiopsis ranaculosus* and *Ophiostoma minus*. *Ophiostoma minus* is a microbe that helps the beetle during invasion, while *Entomocorticium* sp. A and *C. ranaculosus* are a food sources for larvae. These microbes grow at different temperature ranges, which is important for determining how they affect the host and invasion. Our study focuses on the difference in the components of microbiota in each invading time period of SPB. We collected the bark from pine trees which were invaded by SBP from the early, middle, and late stages of attack from the New Jersey Pinelands in 2012. We grew these samples onto fungal and bacterial media, and isolated pure colonies for Sanger sequencing of ribosomal RNA to identify which microbes were present. Recent results show that most of the samples are fungi, and some species in genus *Ophiostoma* were present in the early invasion. Two other species, which are *Umbelopsis ovata* and *Mortierella kuhlmanii,* present most in the middle attack stage. From the late attack stage samples, we did not find a particular species that dominated. Ultimately, our goal is to compare these isolates and their temperature tolerances to isolates that we will obtain from a similar field site in 2022, in order to determine if there are changes in the traits of the microbes over the 10 years' time frame. This study will contribute to our understanding of the mechanisms of SPB attack and help us to forecast how future changes in climate may affect the spread of the SPB.

31. Characterization of small proteins induced under low magnesium stress in *Escherichia coli*.

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Small proteins are usually less than 50 amino acids (aa) in length and are encoded by small open reading frames (sORFs). In the past, they have been largely overlooked by genome annotation studies due to their small size. However, advancements in bioinformatics, deep sequencing methods and gene expression studies have expanded our knowledge on small proteins. Approximately 150 small proteins have been documented in E. coli. Barring some in-silico data, many of these small proteins remain uncharacterized in terms of their structure, function and regulation. Previous work has indicated that small proteins may play important roles in bacterial stress response by aiding the cell sense, adapt and survive in unfavorable environments. They are speculated to do so by interacting with larger protein complexes on the membrane and regulating them. My project focuses on characterizing small proteins that accumulate under low magnesium stress in *E. coli*. Using a high-throughput translation-initiation profiling method, we have identified a list of 16 small proteins expressed three-fold or higher in response to low magnesium. Most of these proteins have no known function. Our initial efforts in characterizing these hits involve cellular localization by tagging them with green fluorescence protein (GFP) and characterizing their regulation at the transcriptional level by using fluorescent promoter reporter assays. Functional characterization of the small proteins using genetic and biochemical assays will provide insights into the potential role of small proteins as stress-induced regulators.

32. The role of the small RNA tsr25 in *Staphylococcus aureus* iron homeostasis.

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Host tissues sequester iron to protect against infections in a process known as nutritional immunity. In response, pathogenic bacteria alter gene expression to adapt to iron starvation. This change in gene expression is mediated, in part, by the ferric uptake regulator (Fur), which acts as an iron-dependent transcriptional repressor. Under iron starvation, Fur repression is alleviated, allowing for increased expression of iron uptake genes. In addition, in its apo-form, Fur also decreases the expression of iron-requiring processes such as the tricarboxylic acid cycle and oxygen respiration. Similar regulation has been described in *Escherichia coli,* which uses the small RNA RyhB to mediate mRNA degradation of non-essential genes encoding iron-utilizing enzymes. *S. aureus* does not have a RyhB. We hypothesized that Fur conducts positive regulation of gene expression in *S. aureus* using a small non-coding RNA. Using a suppressor screen, we selected strains with a second site mutation that suppressed the growth defect of a Δfur mutant and had increased TCA cycle activity. Whole-genome sequencing of the suppressors revealed that all selected strains contained a mutation in the sRNA tsr25. We created a Δtsr25 mutant and determined that it could suppress the varied phenotypes of the Δfur mutant. We also genetically complemented the Δtsr25 mutants, showing that the altered function of tsr25 mutant alleles functioned to conduct regulation in the Δfur mutant. We propose that tsr25 is an important mediator of the fur-driven response to iron starvation in *S. aureus*.

33. Enrichment and isolation of organobromine respiring bacteria from New Jersey estuarine sediment.

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Brominated Flame Retardants (BFR's) are highly brominated organic compounds added to commercial products to increase their thermal stability. Through their life cycles, they enter the environment through direct spills, industrial waste, and leaching from improperly disposed products in landfills. Due to their hydrophobic nature, BFRs partition into sediments where they bioaccumulate and persist. Concerns of human and environmental toxicity, and biomagnification, have placed these chemicals in the forefront for remediation efforts. Metabolism of organobromine compounds by microbial reductive dehalogenation, is a critical step enabling further breakdown. Some key players in this process are organohalide-respiring bacteria (OHRB) which use halogenated compounds as terminal electron acceptors for energy generation, making them important for bioremediation efforts. This study aims to expand the knowledge on anaerobic bacterial communities capable of reductive debromination in estuarine sediments, as well as the factors influencing their activity. Anaerobic microcosms amended with organobromine compounds were established using sediment from contaminated and pristine estuarine sites, to investigate their native debromination potential and isolate novel OHRB species and strains. This information can further expand our ability to screen for native debromination potential of contaminated sites, that are active in natural attenuation.

34. Convergent evolution of bacterial ceramide synthesis.

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The bacterial domain produces numerous types of sphingolipids with various physiological functions. In the human microbiome, commensal and pathogenic bacteria use these lipids to modulate the host inflammatory system. Despite their growing importance, their biosynthetic pathway remains undefined since several key eukaryotic ceramide synthesis enzymes have no bacterial homolog. Here we used genomic and biochemical approaches to identify six proteins comprising the complete pathway for bacterial ceramide synthesis. Bioinformatic analyses revealed the widespread potential for bacterial ceramide synthesis leading to our discovery of a Gram-positive species that produces ceramides. Biochemical evidence demonstrated that the bacterial pathway operates in a different order from that in eukaryotes. Furthermore, phylogenetic analyses support the hypothesis that the bacterial and eukaryotic ceramide pathways evolved independently.