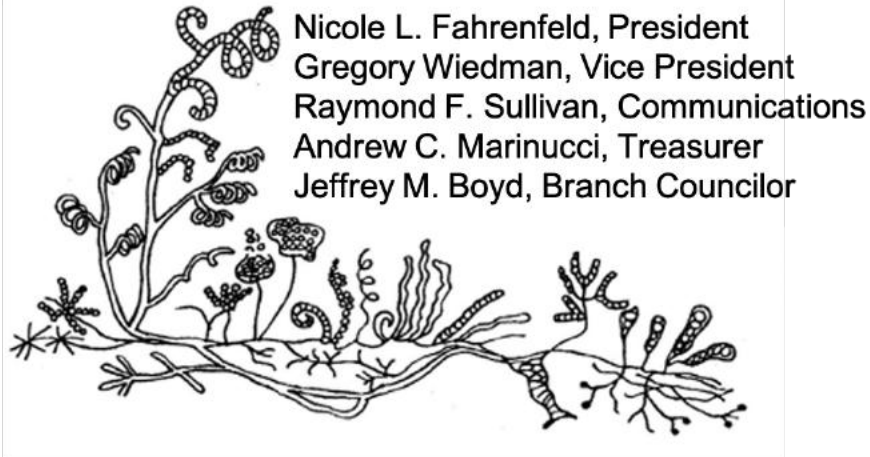




**Theobald Smith Society**  
The NJ Branch of the  
American Society for  
Microbiology  
[www.njmicrobe.org](http://www.njmicrobe.org)



**Fall 2022 Symposium Proceedings**  
**30 November 2022**  
**McNulty Hall**  
**Seton Hall University, South Orange, NJ**

**5:30 pm**

**Opening remarks by Nicole Fahrenfeld, President, Theobald Smith Society**

Nicole Fahrenfeld serves as the 81<sup>th</sup> president of the Theobald Smith Society. She is associate professor of Civil & Environmental Engineering in the School of Engineering, Rutgers University, Piscataway, NJ. Dr. Fahrenfeld earned her PhD and completed her post-doctoral research at Virginia Tech, Blacksburg, VA.

Dr. Fahrenfeld's primary research interest lies in at the interface of environmental chemistry and environmental microbiology to promote water quality and sustainability, with applications in natural, agricultural, and engineered systems. Research interests / projects include microbial source tracking in mixed land use watersheds, end-of-pipe treatment for combined sewer overflows, microbial processes in sewers, microplastic sources and their associated biofilms, antibiotic resistance monitoring in urban waters and bioremediation of oil, solvent, and explosives contaminated aquifer sediments.

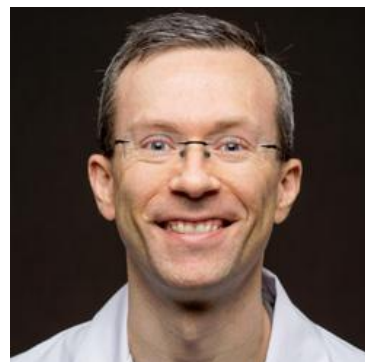
## Invited speakers

**5:45-6:45 pm Esther Babady**, PhD, is an American Society for Microbiology Distinguished Lecturer and Waksman Foundation Lecture for 2021-2023. She is the Director of the Clinical Microbiology Service, the Director of ASM's Subcommittee on Postgraduate Educational Programs (CPEP) Clinical Microbiology Fellowship program, and an Attending Microbiologist and Member (Professor) in the Departments of Laboratory Medicine and Medicine at Memorial Sloan-Kettering Cancer Center in New York City. She received her Ph.D. in Biochemistry and Molecular Biology and completed a postdoctoral CPEP fellowship in clinical microbiology, both at the Mayo Clinic in Rochester, Minn., before joining MSKCC. She is board-certified by the American Board of Medical Microbiology, a fellow of the Infectious Disease Society of America and a fellow of the American Academy of Microbiology. Her research interests include rapid diagnosis of infections in immunocompromised hosts, fungal diagnostics and the development and evaluation of the clinical utility of molecular microbiology assays.



**6:45-7:15 pm Dane Parker**, PhD, Center for Immunity and Inflammation, Department of Pathology and Laboratory Medicine, Rutgers University, New Jersey Medical School, Newark, NJ

Dr. Dane Parker is the recipient of the 2022 Theobald Smith Society Young Investigator Award. He 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 signaling pathways important for the detection of microorganisms. A major focus of his research is the type I and type III interferon signaling 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.



**7:15-7:25 pm Lauren Hall**, Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ “*Desulfoluna* is everywhere, the sponge selects: global distribution of anaerobic debrominating bacteria in marine sponges.”

**7:25 -7:35 pm Ty’Celia Young**, Department of Forestry and Environmental Conservation, Clemson University, Clemson, SC. “Determining the suitability of fruit fermentation as a model of succession.”

**7:35-7:45 pm Rachel A. Carr**, Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ. “Characterization of antibiotic susceptibility profiles of extensively- and pan-drug resistant *Acinetobacter baumannii* clinical isolates.”

**7:45-7:55 pm Mellissa Woortman**, Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ. “Breastfeeding mode differences, geographic location, and the milk microbiota.”

**8:00-9:30 pm Poster presentations and dinner**

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

## POSTERS

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

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

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**Ty’Celia Young<sup>1</sup>, Shang Xu<sup>2</sup>, and Lily Khadempour<sup>2</sup>**

1. Department of Forestry and Environmental Conservation, Clemson University, Clemson, SC
2. Department of Earth and Environmental Sciences, Rutgers University, Newark, NJ

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Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ

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**Olaitan Akintunde<sup>1</sup>, Todd Greco<sup>2</sup>, Josiah Hutton<sup>2</sup>, and Valerie J. Carabetta<sup>1</sup>**

1. Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden NJ
2. Department of Molecular Biology, Princeton University, Princeton NJ

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1. Dept. of Cell Biology & Neuroscience, Rutgers University, New Brunswick, NJ
2. Dept. of Entomology, Rutgers University, New Brunswick, NJ
3. Dept. of Civil & Environmental Engineering, Rutgers University, New Brunswick, NJ

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1. Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ
2. Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

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1. Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ,
2. Rowan University School of Osteopathic Medicine, Stratford, NJ 3. Cooper University Hospital, Camden, NJ

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**Taylor Andrews<sup>1</sup>, Siobain Duffy<sup>2</sup>**

1. Microbial Biology Graduate Program, Rutgers University, New Brunswick, NJ
2. Dept. of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ

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**Gyu Lee Kim<sup>1</sup>, Jisun Kim<sup>1</sup>, Javiera Norambuena<sup>2</sup>, Jeffrey Boyd<sup>2</sup> and Dane Parker<sup>1</sup>**

1. Department of Pathology, Immunology and Laboratory Medicine, Center for Immunity and Inflammation, Rutgers New Jersey Medical School, Newark, New Jersey USA
2. Department of Biochemistry and Microbiology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey, USA

# 1. *Desulfoluna* is everywhere, the sponge selects: global distribution of anaerobic debrominating bacteria in marine sponges.

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

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

The marine environment is a rich source of biogenic organohalides. Of particular interest are the marine sponges (*Porifera*) which produce a vast array of bioactive secondary metabolites, including diverse halogenated compounds. Host-specific microbial communities thrive within sponge tissues and this association between sponge and associated microbiota may be driven by the organohalogen chemistry of the sponge animal. Cleavage of the carbon-halogen bond is a critical step in degradation of organohalides and the natural organohalogen cycle. Our overall hypothesis is that dehalogenating bacteria form stable populations within the sponge animal that function in the cycling of organohalide compounds and provide a benefit to the host in their degradation of the secondary metabolite produced by the sponge. A range of sponge species were collected from the Great Barrier Reef, Hawaii and the coast of New Jersey for analysis of their anaerobic debrominating bacterial communities using an enrichment/cultivation and molecular analysis-based approach. Debrominating activity was detected in all sponge animals tested and stable cultures could be enriched from most of the sponges. To determine if dehalogenating strains are host specific, we utilized the long-read capability of the Oxford Nanopore MinION to profile bacterial ribosomal operons of dehalogenating cultures and the sponge holobionts. Divergent *Desulfoluna* spp. strains were detected in the various sponges at all locations, indicating a cosmopolitan association between *Desulfoluna* spp. and marine sponges. Organobromide-rich sponges provide a specialized habitat for organohalide-respiring microbes and *D. spongiiphila* and its close relatives are responsible for reductive dehalogenation in geographically widely distributed sponge species.

## 2. Determining the suitability of fruit fermentation as a model of succession.

Ty'Celia Young<sup>1</sup>, Shang Xu<sup>2</sup>, and Lily Khadempour<sup>2</sup>

1. Department of Forestry and Environmental Conservation, Clemson University, Clemson, SC

2. Department of Earth and Environmental Sciences, Rutgers University, Newark, NJ

The environment is always changing, indicating a need for succession models to reflect the changes or stages of a community from primary succession to climax communities. The development of pioneer species to a climax community can be complex and occur over long time periods. This study explores the idea of modeling succession using the growth of microbes and fruit fly (*Drosophila melanogaster*) larvae on fruit. Although fruit flies are a model organism in many fields of study, little is known about their microbial interactions or preference of fruit rot successional stages. Here, we examine the relationship of microbial growth on rotting fruit cubes and the presence of fruit fly larvae to determine whether this is a useful model of succession. First, we developed a protocol for creating fruit cubes using nectarines, pectin, and water and identified the optimal ratio to be 15 g blended fruit, 5 ml water, and 2 g pectin per cube. Then, we examined the succession of fruit cubes at the end of the fourteen-day period in a screen-protected container. Every two days, we removed three cubes from the freezer and placed them in the container where fruit flies were allowed to oviposit. We collected larval abundance, weight difference, color, and pH data at the end of the fourteen-day period. We found significant differences in weight and color in older cubes, likely due to evaporation, however there was no significant difference found in pH. The older fruit cubes had an increased number of both larvae and microbes, indicating the fruit flies preferred to oviposit on rotting fruit and a positive relationship exists between larval presence and microbial abundance. In conclusion, fruit fermentation and microbial growth offers a simple way to study succession. Further studies should examine the effects of successional microbial growth on fruit fly health.



### 3. Uncovering the mechanism by which HBSu acetylation regulates the process of sporulation in *Bacillus subtilis*.

Liya Popova, Olivia Schreiber, Hritisha Pandey, Valerie J. Carabetta

Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ

Under conditions of environmental stress or nutrient starvation, *Bacillus subtilis* produces endospores as a final means of survival. These spores can persist for years even without essential nutrients and will germinate when conditions become favorable for growth. Formation of a mature endospore is a complex process in which many different proteins are involved. The histone-like protein HBSu has been proposed to be important for proper condensation and packaging of DNA into the spore compartment. Previously, we discovered that HBSu is acetylated at seven lysine residues and one physiological function of acetylation is to regulate chromosomal compaction during growth. N $\epsilon$ -lysine acetylation is a common post-translational modification (PTM) in bacteria and can alter a protein's subcellular localization, function, or stability. Recently, we demonstrated that improper acetylation also leads to a severe reduction in sporulation frequency. Moreover, the few spores that were produced were more susceptible to environmental stresses. We proposed that specific acetylation patterns of HBSu are required to ensure proper chromosomal arrangement, packaging, and protection during the process of sporulation. However, the mechanism by which HBSu acetylation influences this process is currently unknown. As HBSu is a functional ortholog of eukaryotic histones, there are two likely possibilities which we will address. First, HBSu may be important for chromosome dynamics during the formation of the axial filament. Second, the HBSu ortholog in *Escherichia coli* was shown to transcriptionally regulate many genes, it may be important for proper gene expression. Using our collection of point mutations that mimic the acetylated form of HBSu, we performed quantitative real-time PCR (qRT-PCR) to examine transcription of important sporulation genes throughout the entire process. We have designed and optimized conditions for qRT-PCR and selected 25 genes that are expressed throughout the sporulation for analysis. Preliminarily, we found that deacetylation of lysine residues at K41 and K75 is important for proper expression of *cotE*, *cotH*, *sigG*, *sigF*, *spoIIQ*, *spoIIE*, *divIC*, *spoIIAB*, *spoIVCA*, *spoIIGA*, *asnO*, *spoVK*, at the late sporulation phase. In addition, we are exploring the use of super-resolution microscopy (dSTORM and PALM) to examine chromosome dynamics during sporulation. So far, our data suggests that acetylation of HBSu is required for proper temporal gene expression during sporulation, but further work is required to confirm and fully assess the role of HBSu acetylation during sporulation.

#### 4. Evaluating the activities and targets of new lysine acetyltransferases from *Bacillus subtilis*.

Olaitan Akintunde<sup>1</sup>, Todd Greco<sup>2</sup>, Josiah Hutton<sup>2</sup>, and Valerie J. Carabetta<sup>1</sup>

1.Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden NJ

2.Department of Molecular Biology, Princeton University, Princeton NJ

Ne-lysine acetylation of proteins can alter enzymatic activity, DNA binding, subcellular localization, or stability. This can have significant consequences and can impact essential processes, such as transcription, DNA replication, central carbon metabolism, etc. The histone-like proteins in bacteria are considered functional homologs of eukaryotic histones, despite no primary or structural conservation. Previously, our lab demonstrated that HBSu is acetylated at seven sites, and that altering the acetylation status of key lysine residues regulates its DNA binding activity, nucleoid compaction and DNA replication. Enzymatic lysine acetylation occurs via the action of the lysine acetyl-transferases (KATs), which catalyze the addition of an acetyl group to the side chain of surface-exposed lysine residues in proteins. This reaction is reversible, by the action of lysine deacetylases (KDACs). The mechanisms of acetylation *Bacillus subtilis* are not completely understood. AcuA is one known *B. subtilis* KAT. AcuA, however, has a limited number of substrates, suggesting that there could be additional enzymes involved, especially since more than 800 proteins are acetylated. Our lab identified a second KAT, YfmK that targets lysine residues in HBSu. Inactivation of YfmK and four other putative KATs (YdgE, Ydhl, YokD, and YjbC) led to a more compacted nucleoid, similar to the phenotype observed for HBSu mutants that could not be acetylated. Here, we used mass spectrometry-based proteomics to determine that HBSu is a bona fide substrate for Ydhl, YjbC, YdgE, and YokD and identify the lysine residues within HBSu that are targeted by these enzymes.



## 5. Investigating the likelihood of influent-associated *Diptera* as reservoirs for the SARS-CoV-2 virus.

Aleena Aziz<sup>1</sup>, Michael A. Monzon<sup>2</sup>, Genevieve Ehasz<sup>3</sup>, Nicole L. Fahrenfeld<sup>3</sup>

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2. Dept. of Entomology, Rutgers University, New Brunswick, NJ

3. Dept. of Civil & Environmental Engineering, Rutgers University, New Brunswick, NJ

"Filth flies" in families like *Muscidae* and *Psychodidae* are associated with human pathogens. A recent study in Iran detected SARS-CoV-2 gene copies in *Musca domestica* near hospitals. Filth flies are common at wastewater treatment plants and wastewater influent can contain SARS-CoV-2. We will study if filth flies harbor SARS-CoV-2 from surrounding influent wastewater. We collected flies from four sites at a municipal wastewater treatment plant in Central NJ pairing 24-hour composite influent samples. Control insects were trapped at a research farm in Rutgers. Composite influent samples were filter concentrated. After RNA extraction from filters and flies, qPCR will detect the SARS-CoV-2 N1 and N2 genes. Results will indicate the potential of filth flies being a vector for SARS-CoV-2.

## 6. Breastfeeding mode differences, geographic location, and the milk microbiota.

Melissa Woortman<sup>1</sup>, Haipeng Sun<sup>2</sup>, Maria Gloria Dominguez-Bello<sup>2</sup>

1. Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ
2. Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

Breastfeeding has a protective effect on infant health and gut microbiome development, but unknowns about its components remain, including the breast milk microbiome. Differences in milk microbiome composition are seen with maternal diet, age, health status, and lifestyle. Other factors, such as geographic location and expressing breast milk to feed infants via bottle are not as well studied. We hypothesized that human milk microbiota composition is different between breastfeeding modes and that milk microbiota differs by geographic location. To investigate breastfeeding mode, breast milk samples from 26 mothers from the UPSIDE cohort, 14 who only fed directly at the breast and 12 that additionally provided some expressed breast milk, were analyzed to compare differences in breast milk microbiomes. Differences in alpha or beta diversity were not observed, but taxa were discordant between the feeding modes. Linear discriminate analysis of effect size (LEfSe) comparing the two infant feeding modes showed that Xanthomonadales were enriched in the direct group at 1 month of age, and Aeromonadales, and *Schlegelella* in the expressed group and *Stenotrophomonas* in the direct group were enriched at 6 months of age. To address geographical differences, breast milk samples from 502 lactating mothers from 14 populations across 4 continents were compared for microbiota diversity. Breast milk microbiota from the Americas (North and South), Europe, and Africa displayed differences in alpha diversity ( $p = 0.01$ ; Kruskal-Wallis for Faith's Phylogenetic Diversity) and beta diversity ( $p = 0.02$ ; PERMANOVA for Jaccard Distance), confirming geographic differences in alpha and beta diversity. These results shed light on the role that geographic location and breastfeeding mode have on the breast milk microbiome and highlight the need of further studies to determine potential impact of these factors on infant development.

## 7. Characterization of antibiotic susceptibility profiles of extensively- and pan-drug resistant *Acinetobacter baumannii* clinical isolates.

Rachel A. Carr<sup>1</sup>, Justin Halim<sup>1</sup>, Rebecca Florent<sup>2</sup>, Henry Fraimow<sup>3</sup>, Dejan Nikolic<sup>3</sup>, Valerie Carabetta<sup>1</sup>

1. Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ, 2. Rowan University School of Osteopathic Medicine, Stratford, NJ 3. Cooper University Hospital, Camden, NJ

*Acinetobacter baumannii* is an opportunistic pathogen common in intensive care units (ICUs), particularly among immunocompromised individuals. Nosocomial *A. baumannii* infections have become increasingly problematic in recent years, as these bacteria rapidly acquire antibiotic resistance, leading to the emergence of multidrug, extensively drug and pan drug-resistant (MDR, XDR, and PDR, respectively) isolates. Recently, Cooper University Hospital (CUH) experienced a large increase in highly drug-resistant *A. baumannii* infections, which had a mortality rate of 60%. Oftentimes, physicians had to turn to combinations of drugs with no experimental verification or historically shelved antibiotics, such as the polymyxins, in a desperate attempt to save lives. This highlights the critical need for more research to identify new, effective treatment options for these difficult-to-treat infections. Here, we determined the susceptibility of 22 patient isolates from CUH against 22 standard-of-care drugs and three newly released antibiotics (eravacycline, omadacycline and plazomicin) by the standard broth microdilution technique. We found that the isolates in this collection were 70% XDR and 30% PDR, meaning there were little to no treatment options available. Overall, the collection was most susceptible to minocycline (77.3%), followed by rifampin (55%) and amikacin (40.9%). While official breakpoint data is not available from the Clinical Laboratory Standards Institute for the new tetracycline-class drugs, a number of strains had low minimum inhibitory concentrations (MICs) to eravacycline and omadacycline, suggesting that these new drugs may be effective in treatment of highly drug-resistant strains. The drug plazomicin was largely ineffective against these strains, with high MICs. We plan to explore novel combinations of eravacycline and omadacycline with the standard-of-care drugs and to search for synergistic combinatorial effects using checkerboard assays. This information can ultimately be used to design new treatment regimens against drug-resistant *A. baumannii* infections.

## 8. Does coinfection help or hinder viral emergence on novel hosts?

Taylor Andrews<sup>1</sup>, Siobain Duffy<sup>2</sup>

1. Microbial Biology Graduate Program, Rutgers University, New Brunswick, NJ

2. Dept. of Ecology, Evolution, and Natural Resources, Rutgers University, New Brunswick, NJ

Viral coinfection, when multiple viruses simultaneously infect a single host cell, occurs in environments with high viral density and can accelerate viral evolution by facilitating genetic exchange. Coinfection occurs commonly in nature, and thus contributes significantly to the evolution of viral populations. Progeny with hybrid genomes created via reassortment during coinfection can bring together beneficial mutations on different segments much more rapidly than is expected with spontaneous mutation. However, when viruses frequently coinfect, complementation and cheating can occur, which may prevent viruses from adapting as rapidly to novel environments. A major gap in our understanding of viral evolution is how coinfection affects host range expansion, when viruses evolve to be able to infect a new host species and then subsequently adapt to improve their fitness on the novel host. In this project, I seek to determine whether coinfection helps or hinders viral emergence on novel hosts.

## 9. Synthetic biochemical reconstitution of *S. aureus* agr quorum sensing reveals a direct role for the integral membrane protease MroQ in pheromone biosynthesis

Steven Bodine, Tom Muir

Department of Molecular Biology, Princeton University

In the opportunistic pathogen *Staphylococcus aureus*, a master regulator of pathogenicity is the accessory gene regulator (*agr*) quorum sensing circuit. Via the production and recognition of a secreted autoinducing peptide (AIP) signaling molecule, *S. aureus* communicates within its local environment to coordinate gene expression and behavior. Disruption of *agr* quorum sensing is sufficient to ablate *S. aureus* cytotoxin expression, making this quorum sensing system an attractive therapeutic target. One proposed mechanism to disrupt *agr* quorum sensing is the inhibition of AIP biosynthesis. The AIP is a ribosomally synthesized and post translationally modified peptide (RiPP) derived from the cleavage of a precursor peptide AgrD in a multistep peptidolytic cascade. The first cleavage event in this biosynthetic pathway is well studied; the integral membrane protease AgrB cleaves the C-terminal domain of AgrD and cyclizes the C-terminus of the cleavage product with an internal cysteine thiol to generate a macrocyclic biosynthetic intermediate. However, despite decades of research, the identity of the protease(s) responsible for the second step(s) of AIP maturation has not been confirmed. Recently, the integral membrane protease MroQ was discovered in *S. aureus* and shown to exhibit regulatory behavior over *agr*. We demonstrate that MroQ serves as the protease catalyzing the second proteolytic cleavage event of AIP biosynthesis. Genetic complementation reveals that MroQ proteolytic activity is necessary for AIP biosynthesis in multiple *S. aureus agr* variants, while *in vitro* biochemical experiments demonstrate that AgrD, AgrB, and MroQ are sufficient for AIP biosynthesis in *agr* specificity groups -I and -II. Furthermore, we elucidate the molecular determinants of MroQ cleavage-site recognition, which are critical to MroQ's differential activity between distinct *agr* specificity groups. Altogether, this study deepens the collective understanding of *agr* quorum sensing and identifies a novel target for anti-virulence pharmaceutical development.

## 10. Impact of the pentose phosphate pathway on *Staphylococcus aureus* metabolism and pathogenesis

Gyu Lee Kim<sup>1</sup>, Jisun Kim<sup>1</sup>, Javiera Norambuena<sup>2</sup>, Jeffrey Boyd<sup>2</sup> and Dane Parker<sup>1</sup>

1. Department of Pathology, Immunology and Laboratory Medicine, Center for Immunity and Inflammation, Rutgers New Jersey Medical School, Newark, New Jersey USA
2. Department of Biochemistry and Microbiology, Rutgers, The State University of New Jersey, New Brunswick, New Jersey, USA

*Staphylococcus aureus* is an important pathogen that leads to significant disease through multiple routes of infection. We recently published a Tn-seq screen in a mouse acute pneumonia model and identified a hypothetical gene (SAUSA300\_1902) with similarity to a lactonase of *Escherichia coli* involved in the pentose phosphate pathway (PPP) that was conditionally essential. Transposon mutant libraries of *S. aureus* as well as targeted attempts have been able to generate mutations in several genes involved in the PPP. We show here that mutation of 1902 has significant impacts on ATP output and respiration. RNA-seq analysis identified compensatory changes in gene expression for glucose and gluconate as well as reductions in the pyrimidine biosynthesis locus. These differences were also evident through unbiased metabolomics studies and <sup>13</sup>C labeling experiments that showed mutation of 1902 led to increases in glucose and 6-phosphogluconate and reductions in ribose-5P, UMP, GMP and pyruvate. These nucleotide reductions also impacted the amount of extracellular DNA in biofilms and reduced biofilm formation. Mutation also limited the capacity of the strain to resist oxidant damage induced by hydrogen peroxide and paraquat. We demonstrated the importance of these changes on virulence in three different models of infection, covering respiratory, skin and septicemia, demonstrating significant attenuation in all of these models. This work demonstrates the multifaceted role metabolism can play in multiple aspects of *S. aureus* pathogenesis.