Theobald Smith Society
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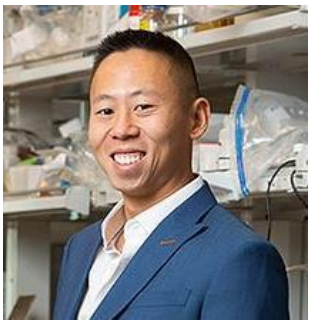
Fall 2021 Symposium Proceedings
held virtually, 8 December 2021

4:55 pm

Opening remarks by Valerie Carabetta, President, Theobald Smith Society

Keynote Speakers

5:00 pm



"Systems biology insights into antimicrobial resistance physiology", Jason H. Yang, Assistant Professor and Chancellor Scholar Microbiology, Biochemistry and Molecular Genetics, Center for Emerging and Re-Emerging Pathogens, Rutgers University, New Jersey Medical School, Newark, NJ

Antimicrobial resistance (AMR) is frequently studied using laboratory evolution, most commonly by serial plating under increasingly stringent antimicrobial concentrations. These approaches have led to the discovery of resistance alleles in drug efflux pumps and drug targets as critical mediators of high-level resistance. However, human usage of antimicrobial treatment differs from laboratory conditions, with circulating drug concentrations typically transient and periodic with drug dose consumption. To better approximate physiologically relevant selection pressures, we performed laboratory evolution experiments on MG1655 *Escherichia coli* cells under transient selection by bactericidal antibiotics. These experiments revealed novel mutations heavily enriched in metabolic genes that conferred low-level resistance. We generated knock-in *E. coli* mutants based on these mutations and validated them as metabolic resistance alleles. We performed bioinformatic analyses on >3,500 publicly available whole-genome sequences from *E. coli* clinical isolates and discovered that specific SNPs selected for by our laboratory evolution protocol were clinically relevant. We performed follow-up experiments to characterize changes in microbial physiology induced by these

resistance alleles and discovered spectrums of changes in cellular respiration and the oxidative stress response to paraquat. We hypothesized that changes in paraquat sensitivity may be driven respiratory activity and validated this hypothesis genetically in oxidative phosphorylation mutants with either high basal respiration (ΔatpA) or low basal respiration (ΔcyoA ΔcydB ΔappB). Finally, we characterized *E. coli* cells harboring resistance alleles from the Antibiotic Resistance Platform and *E. coli* clinical isolates from the Center for Disease Control's Antibiotic Resistance Isolate Bank and discovered these changes in microbial physiology are common features of AMR.

5:30 pm

"Virulence through metabolism in *Staphylococcus aureus*", Dane Parker, Center for Immunity and Inflammation, Department of Pathology and Laboratory Medicine, Rutgers University, New Jersey Medical School, Newark, NJ

Staphylococcus aureus is an important pathogen that leads to high morbidity and mortality. Although *S. aureus* produces many factors important for pathogenesis, few have been validated as playing a role in the pathogenesis of *S. aureus* pneumonia. To gain a better understanding of the genetic elements required for *S. aureus* pathogenesis in the airway, we performed an unbiased genome-wide transposon sequencing (Tn-seq) screen in a model of acute murine pneumonia. We discovered that metabolic genes were overrepresented in those required for lung infection. We demonstrated through phenotyping of deletion mutants in several functional assays that replicative ability and tolerance against host defenses form two key metabolic dimensions of bacterial infection. These dimensions are independent for most pathways but are coupled in central carbon metabolism and highlight the critical role of bacterial metabolism in survival against host defenses during infection. Recent studies have focused on a gene predicted to be involved in the pentose phosphate pathway and we present new data demonstrating its role in several pathogenic traits. Pathogenesis is conventionally understood in terms of the host-pathogen interactions that enable a pathogen to neutralize a host's immune response. We demonstrate with the important bacterial pathogen *S. aureus* that microbial metabolism influences key traits important for *in vivo* infection, independent from host immunomodulation

Invited Speakers

6:00 pm

"Microbes in the Movies, The Early Years (It All Started in New Jersey!)", John G Warhol, The Warhol Institute, Leonardo, NJ

6:10 pm

"RNA structure-dependent activation of the innate immune response by influenza virus mini-viral RNAs", Emmanuelle Pitré, University of Cambridge, Department of Pathology, Addenbrooke's Hospital, Cambridge, United Kingdom and Lewis Thomas Laboratory, Department of Molecular Biology, Princeton University, NJ

6:20 pm

"Copper inhibits pentose phosphate pathway function in *Staphylococcus aureus",* Javiera Norambuena, Department of Biochemistry and Microbiology, Rutgers, the State University of New Jersey, New Brunswick, NJ

6:30 PM

"DNA uptake in the deep-sea Epsilonproteobacterium, *Caminibacter mediatlanticus*", Avanthika Bharath, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ

6:40 pm

"Development of an in vivo site-specific photo-cross-linking and mass spectrometry-based approach to identify the small protein interactome", Kyle S. Skalenko, Department of Genetics and Waksman Institute, Rutgers University, Piscataway, NJ

6:50 pm

"Reconstitution of the Nem1-Spo7 protein phosphatase complex into unilamellar phospholipid vesicles reveals its dependence on phosphatidic acid for the dephosphorylation of Pah1", Joanna M. Kwiatek, Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ

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

Poster Presentations 7:00-8:30 pm

Marijke Rittmann [1], Guojun Wu [2,3], Dian Sulistyoningrum [4], Tim Green [4], Emily Hanselman [1], Liping Zhao [2,3], Joshua W. Miller [1,2*], Yan Y. Lam [2,5*]

1. Nutritional Sciences, Rutgers University, New Brunswick, NJ

2. Center for Nutrition, Microbiome, and Health, Institute for Food, Nutrition and Health, Rutgers University, New Brunswick, NJ
3. Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ
4. Women and Kids Theme, South Australian Health and Medical Research Institute, Adelaide, SA, Australia
5. Centre for Chinese Herbal Medicine Drug Development, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China
*Co-corresponding authors

Post-doc Presentation Prize Winner:

Graduate Student Presentation Prize Winner:

Microbiome associations with vitamin B12 status in adults.

Marijke Rittmann [1], Guojun Wu [2,3], Dian Sulistyoningrum [4], Tim Green [4], Emily Hanselman [1], Liping Zhao [2,3], Joshua W. Miller [1,2*], Yan Y. Lam [2,5*]

1. Nutritional Sciences, Rutgers University, New Brunswick, NJ

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Introduction: Vitamin B12 is an essential nutrient required for hematological and neurological function. B12 deficiency results in megaloblastic anemia and neurodegenerative disease, and may contribute to cognitive decline and dementia risk in older adults. Some bacteria among human gut microbiota can synthesize B12 while others require B12 from the diet of the host. Consequently, variation in gut microbiota may influence B12 status in humans. The purpose of this study is to assess the association between gut microbiota composition and host B12 status.

Methods: We conducted a cross-sectional cohort study of 92 adults (47 males/45 females; 20-75y) in Sydney, Australia. 16S rRNA gene V3-V4 amplicon sequencing was used to profile gut microbiota at the amplicon sequence variant (ASV) level. Serum total B12 was measured by automated chemiluminescence assay. The Kruskal-Wallis test followed by Dunn's test were employed to assess the association between B12 tertiles and gut microbial diversity. The PERMANOVA test was used to assess associations between B12 tertiles and overall gut microbiota composition. Microbial guilds were identified based on co-abundance clustering. Spearman correlation was used to explore the association between microbial guilds and B12 status.

Results: Mean serum B12 was 389±177 pmol/L. Shannon index (p=0.010), ASV number (p=0.022), and Faith's phylogenetic diversity (p=0.048) were directly correlated with increasing B12 tertiles. No associations were observed between B12 tertiles and overall gut microbiota composition. We identified 30 microbial guilds, among which guilds 3 and 15 were directly and inversely correlated with serum B12, respectively (p=0.079).

Conclusion: B12 status was directly associated with gut microbial diversity within adults. In addition, two microbial guilds were identified that are associated with B12 status. Further studies are needed to determine the mechanism underlying the association between B12 status and microbial guilds, and if these findings are reproducible in other human cohorts.

The influence of respiration on dispersal of fermentative *Staphylococcus aureus* biofilms.

Franklin Román-Rodríguez, Jeffrey M. Boyd

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

The Staphylococcal biofilm lifecycle is composed of 5 main phases: attachment, multiplication, exodus, maturation, and dispersal. Dispersal is a stress triggered response that releases cells from the biofilm which can then colonize other surfaces of parts of the body. During this step, cell regain their sensitivity to antibiotics making it an ideal moment to target for treatment. This process is influenced by the Two Component Regulatory Systems (TCRS) in *S. aureus*, specifically the Agr quorum sensing and by the SaeRS and SrrAB systems to a lesser extent. Agr is known as the master regulator of dispersal, controlling expression of various proteases that trigger the dispersal process. We have modified a biofilm formation measurement assay to assess the dispersal of fermentative biofilms. Biofilms are grown on 96 well plates for 22 hours under anaerobic conditions and then introduced to a Terminal Electron Acceptor (TEA) (oxygen or nitrate). Biofilm dispersal is then monitored overtime aerobically and anaerobically. Preliminary results indicate that oxygen or nitrate introduction into fermentative biofilms triggers dispersal. Furthermore, our preliminary data shows that an Agr mutant strain undergoes dispersal when oxygen is introduced into the fermentative biofilm. Preliminary results also show fermentative biofilm dispersal of a respiration impaired mutant strain lacking heme. Our preliminary data suggests that Agr is not essential for fermentative biofilm dispersal when a TEA is introduced. This suggests that an underlying mechanism might be triggering the dispersal process after exposure to oxygen. This is further supported by our result where a heme auxotroph showed fermentative biofilm dispersal after oxygen was introduced.

Reconstitution of the Nem1-Spo7 protein phosphatase complex into unilamellar phospholipid vesicles reveals its dependence on phosphatidic acid for the dephosphorylation of Pah1.

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

Rutgers Center for Lipid Research, Rutgers University, 61 Dudley Road, New Brunswick, NJ

Yeast Nem1-Spo7 protein phosphatase complex, conserved in higher eukaryotes, is localized in the nuclear endoplasmic reticulum (ER) membrane. Nem1-Spo7 complex interacts with the Pah1 phosphatidic acid (PA) phosphatase, previously phosphorylated in the cytoplasm by several protein kinases. As the result, Pah1 is dephosphorylated and translocated from the cytoplasm to the nuclear/ER membrane where it hops to the membrane and either binds to a phospholipid molecule or to its substrate PA to catalyze its reaction. Pah1 PA phosphatase then scoots along the ER membrane towards another PA molecule for another round of catalysis. In this work we developed a model system to investigate PA influence on the Nem1-Spo7/Pah1 cascade. The Nem1-Spo7 complex was reconstituted into unilamellar phospholipid vesicles (liposomes) composed of PA and the major ER membrane phospholipids; the complex was examined for its role in regulating PA phosphatase for its membrane interaction and catalytic function. Recombinant Pah1 PA phosphatase was phosphorylated by Pho85-Pho80 and incubated with the proteoliposomes. The reconstituted protein phosphatase catalyzed the dephosphorylation of PA phosphatase and facilitated its membrane interaction and ability to catalyze the dephosphorylation of PA at the membrane surface. We found that Pah1 dephosphorylation in presence of proteoliposomes containing PA was significantly stronger than in proteoliposomes without PA. This finding uncovered a novel regulatory mechanism by which the substrate in the second step of the phosphatase cascade controls the activity of the first enzyme in the cascade.

Optimization of waste-produced polyhydroxyalkanoates (PHAs) bioplastic production from wastewater activated sludge.

Bo Deng, Lucia Rodríguez-Freire

Department of Civil and Environmental Engineering, New Jersey Institute of Technology, Newark, NJ

Nowadays, over 6,300 million tons of fossil-fuel plastic has been generated, with only 9% of it being recycled and 79% ending in landfills. Plastic persistence in the environment is causing serious environmental problem such as white pollution, entering the food chain and threatening the life of animals and plants. As the demand of plastic production keeps increasing, expected to be 12,000 million tons by 2050, there is a need to promote alternative materials for biodegradable plastic production. Polyhydroxyalkanoate (PHA) is a biopolymer which has similar properties as fossil-fuel based plastic while being completely biodegradable under environmental conditions. This project aims to develop a new process to produce bioplastic PHA using activated sludge from wastewater. In this project, PHA is synthesized by endogenous microorganisms found in wastewater activated sludge. 13 different sludge samples were collected from 6 wastewater treatment plants (WWTP) from the New York City metropolitan area. The sludge samples are characterized for PHA synthesis capacity (microbial community diversity), total suspended solid (TSS), volatile fatty acids (VFAs) and PHA content. Our preliminary results indicate that all activated sludge samples have the capacity of PHA generation. Microbial community analysis showed that PHA-production affiliated microorganisms account for up to 29.2% and important PHA-accumulating organisms are up to 4.8%. Current efforts are focused on developing an enrichment process to optimize the microbial community and PHA accumulation process through feast-famine cycles. In this process, an alternating relatively short periods of external substrate accessibility (feast) and unavailability (famine) is applied as environmental pressure to selectively increase the PHA-accumulating organisms. Preliminary results have shown that the famine-feast cycles successfully drive the microbial community change over cycles/time. GC/MS analysis showed the PHA peak area increased over 700% between cycle 1 and 2; then increased nearly another 200% between cycle 2 and 3. Additionally, the efficiency of a hydrolytic enzyme (α-amylase) was evaluated as a function of VFAs and TOC change during the process. The use of the enzyme enhanced the fermentation and improve the VFAs generation. In conclusion, PHA production can be optimized through microbial community engineering in order to apply this technology in real WWTP systems. This study is expected to enhance the PHA production by using abundant activated sludge from WWTP.

Sphingolipid metabolism in gram-negative bacteria.

Lauren A. Hinkel[1], Eric Klein[1], and Matthew J. Wargo[2]

1. Department of Biology, Rutgers University-Camden, Camden, NJ
2. Department of Microbiology and Molecular Genetics, The University of Vermont Larner College of Medicine, Burlington, VT

Sphingolipids are amphipathic antimicrobial lipids that have many biological functions. While most extensively studied in eukaryotes, sphingolipids are found in many organisms including bacteria. Sphingolipids are frequently encountered by bacteria as they are present, to varying degrees, in many bacterial niches. Although genes putatively involved in sphingolipid metabolism have been identified in a wide array of bacteria few have been thoroughly characterized. Sphingosine-responsive genes were identified through RNA-seq analysis in the gram-negative bacterium *Pseudomonas aeruginosa* PAO1. The protein sequences of *Pseudomonas* sphingosine-responsive genes were used to identify homologs in *Caulobacter crescentus* NA1000. Homologous recombination and microbial culturing techniques were used to determine phenotypic differences between sphingosine-gene deletion and wild type strains grown in the presence or absence of antimicrobial sphingosine. Using these methods, we observed that the putative sphingosine metabolic genes identified in *P. aeruginosa*, sphBCD, are essential for maximal *Pseudomonas* growth in the presence of sphingosine. Similarly, the sphC homolog CCNA_01277 of *C. crescentus* contributes to *Caulobacter's* growth in the presence of sphingosine.

PFAS distribution in contaminated soils and impact on rhizosphere and plant microbiota.

Boran Wang[1], James F. White[2], Lucia Rodriguez-Freire[1]

1. Department of Civil and Environmental Engineering, New Jersey Institute of Technology, Newark, NJ

2. Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ

Rhizosphere horizons are particularly susceptive to heavy metals and organic contamination, including per- and polyfluoroalkyl substances (PFAS), due to their specific biogeochemical processes. The main objective of this project is to evaluate the distribution of PFAS in plants exposed to mixed contaminants, and their effect in the rhizosphere and in planta microbial community, to provide a comprehensive understanding of the mechanisms controlling PFAS effect on plant growth and microbial community changes. Field measurements are combined with soil sorption experiment, hydroponic experiments using the model plant Arabidopsis thaliana and in vitro experiments with *Poa reptans* to assess the mechanisms of PFAS plant translocation, microbiome changes, and toxicity. This work focuses on the Ringwood/Ford Superfund, NJ site where lead, arsenic, chloroethane, benzene and 1,4- dioxane are the main contaminants of concern. However, PFAS have been measured in surface water downstream a paint sludge disposal area (PFOS 445 ng/L, PFOA 23.78 ng/L, PFNA 25.69 ng/L, sediments (PFOS 2.18 ng/g), rhizosphere (PFOS 2.45 ng/g) and plants (PFOS 0.811 ng/g roots). The experiment of PFAS adsorption on soils shows organic matter plays more important role compare to clay, the partition of PFOS in soil is higher compare to PFOA. Hydroponic investigation on PFAS uptake by *Arabidopsis thaliana* shows PFOA preferred accumulation in shoots and PFOS preferred accumulation in roots, and a direct relationship with PFAS solution concentration. The changes in microbial communities are being evaluated using next-generation DNA Illumina sequencing. In vitro laboratory experiments showed that endophytic microbial community decreased PFAS toxicity to *Poa reptans* seedlings. PFAS exposure resulted in damage to the root hairs and lack of internal cyclosis only in the absence of bacteria seedlings. Further, the presence of microorganisms seems to prevent PFAS uptake by exposed seedlings. This work is expected to provide a holistic understanding of the fate and transformation of PFAS within the various environmental compartments, it will inform future remediation strategies and exposure prevention alternatives.

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; New Jersey Institute for Food, Nutrition, and Health, 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

Microbes in the Movies, The Early Years (It All Started in New Jersey!).

John G Warhol

The Warhol Institute, Leonardo, NJ

Why do little kids say “I want to be an astronaut!” but never say “I want to be a microbiologist!”? To unravel this enigma, I examined some of the factors that influence a person’s worldview relative to science interest and career choice. Media exposure is a strong influence, so I began with the first multimedia mass communication vehicle, The Movies. There are almost 78,000 movies that deal with space travel, 26,000 that deal with outer space, and more than 2,600 that feature astronauts. Most of these are hopeful in a futuristic kind of way. In stark contrast, there are 26 movies that key on microbiology, and 4,500 that mention viruses. Few of these are hopeful. This may, in part, explain why astronauts are popular and microbes are feared, but it neglects the obvious – that all life on Earth begins and ends with microbes, they are found everywhere, and they’re really hard to see. Understanding this, I began to re-analyze every movie ever made for signs of microbial life. The evolving results are that microbes have vital and obvious roles in moving pictures, but their prevalence has been underestimated. They abound, and are visible with the proper scientific and social lens. When viewed this way, it’s apparent that microbes truly are everywhere, and both influence and reflect human civilization as seen in the movies. Microbes feature prominently in the first American movie, Blacksmith Scene (1893) produced by Thomas Edison in East Orange, New Jersey. You might say that microbes are the heroes of the film. Blacksmith Scene was so impactful that it was re-shot in France (1895) with a French accent by Auguste and Louis Lumiere as Les Forgerons (The Blacksmiths). Also in 1895, the first science fiction movie, La Charcuterie Mecanique (The Mechanical Butcher) depicts a quintessential microbially processed food as the first product made by a “robotic” butcher. Equally important, the first commercial advertisement using moving film was produced in 1897 for a fermented beverage. The doom and gloom genre of microbe movies may be traced to another Edison Film, Fred Ott Sneezing, in 1894. It’s debatable whether Fred Ott had an allergy or an infection, so the bona fide progenitor of all pathogen movies is House of the Devil, first shown in 1896. Here, everyone’s favorite vector, the bat, makes a brief but crucial appearance. This presentation is part of a larger ongoing project that traces the role of microbes in the movies up to the present day.

Copper inhibits pentose phosphate pathway function in *Staphylococcus aureus.*

Javiera Norambuena[1], Hassan Al-Tameemi[1], William Beavers[2], Dane Parker[3], Eric Skaar[2] and Jeffrey M. Boyd[1]

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2. Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN
3. Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ

Copper (Cu) has been successfully used as an antimicrobial by humans for millennia; however, questions remain about how Cu toxifies bacteria and how Cu is detoxified. *Staphylococcus aureus* is a human bacterial pathogen of worldwide public concern. We and others have discovered two Cu detoxification pathways in *S. aureus*. The copAZ operon is present in all *S. aureus* encodes for a Cu(I) exporter (CopA) and cytosolic buffer (CopZ). The copBL operon, which is found on the ACME transposable element, encodes for a second Cu(I) exporter (CopB) and a Cu-binding lipoprotein. We studied Cu toxicity in a *S. aureus* USA300 ΔcopBL ΔcopAZ mutant strain that lacks genes involved in Cu resistance (cop-). This strain is very sensitive to Cu due to its inability to effectively efflux Cu. A transposon (Tn) mutant library in the cop- background was screened to determine other gene products involved in Cu homeostasis. Some of the Tn insertions that increased Cu resistance mapped to apt (Adenine phosphoribosyltransferase). Apt catalyzes the conversion of PRPP and adenine to AMP. A metabolomic analysis found that cells exposed to Cu had increased PPP intermediates upstream of the metabolite PRPP. The enzyme Prs catalyzes the conversion of ATP and ribose 5-phosphate to PRPP and AMP. Overexpression of prs resulted in increased resistance to Cu. PRPP is a precursor for pyrimidine, purine, histidine, tryptophan, and nicotinamide synthesis. Metabolites found in these pathways were decreased after treatment with Cu. Moreover, the cop- strain was more resistant to Cu if supplemented with these metabolites. These data support the hypothesis that Cu inhibits Prs and thereby decreases the titers of the essential metabolite PRPP.

Triclosan activation of SaeRS-dependent virulence factor expression in *Staphylococcus aureus.*

Erin E. Price and Jeffrey M. Boyd

Department of Biochemistry & Microbiology, Rutgers University, New Brunswick, NJ

In the human pathogen *Staphylococcus aureus*, the two-component system SaeRS is responsible for the regulation of virulence factors essential for successful pathogenesis. SaeRS can be stimulated by neutrophil-derived signals and cellular respiratory status, but has also recently been shown to be inactivated by the presence of fatty acids. In this study, we demonstrate that triclosan, an anti-bacterial drug that targets fatty acid synthesis, functions as an activator of SaeRS. These findings present implications for the widespread use of triclosan as an anti-microbial agent in household products, as well as a persistent environmental pollutant.

Antimicrobial and Antibiofilm Potential of a Medicinal Plant Root Extract.

Nuha Sbateen, Muizzat Alli, Malika Abakkass, and Meriem Bendaoud

Biology Department, The 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 *Staphylococcus aureus* and *Staphylococcus 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.

The antimicrobial and antibiofilm activity of N-acetyl-L-cysteine and L-histidine on different strains of pathogenic bacteria and fungi.

Muizzat Alli, Nuha Sbateen, Malika Abakass , and Meriem Bendaoud

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

Over the years biofilm formation has become one of the leading causes of infections in hospitals due to its resistance to antibiotic treatments. There has been dire need for new compounds that possess antimicrobial properties that not only inhibit the growth of biofilms, but 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 antimicrobial and antibiofilm activity of two amino acids, 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 8mg/ml. 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 against all tested pathogens. Future studies will focus on studying the cytotoxic effects of the amino acids in human cells.

Antimicrobial and anti-biofilm activity of natural compounds produced by unknown marine bacteria or the human body.

Malika Abakkass, Muizzat Alli, Nuha Sbateen, and Meriem Bendaoud

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

The identification of new antimicrobial and antibiofilm compounds is becoming a research priority around the world to solve the growing concern of antibiotic resistance. Biofilms form when microorganisms produce an extracellular polymeric matrix that enables attachment and growth on various surfaces. The aim of this research was to find new antimicrobial or antibiofilm compounds naturally produced by marine bacteria or the human body to fight an increasing number of infectious diseases. The disc diffusion, biofilm, and broth assay were used to test unknown marine bacteria extracts against different pathogens. We also tested the antimicrobial properties of taurine, beta alanine, and taurocyamine (GES), which are compounds naturally produced by the human body. Results indicated that the bacterial extract from unknown 1 (U1) affected biofilm formation of strains of *Staphylococcus aureus, Escherichia coli*, and *Staphylococcus epidermidis,* while other unknown extracts showed a moderate inhibition zone against pathogenic bacteria. Taurine, beta alanine, and GES displayed a significant inhibition against gram-positive pathogenic bacteria and no significant effect against gram-negative bacteria. In future studies, the focus will be on identifying the active compound in the unknown bacterial extracts and to test taurine, beta alanine, and GES against more pathogens using different concentrations.

Extracellular, intracellular and total antibiotic resistance genes: abundance in river water, biofilm, and sediment and diversity and host assignment in bed sediments.

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Human health risk assessment for environmental antibiotic resistant microbes requires not only an understanding of the abundance of antibiotic resistance genes (ARGs) in environmental matrices, but also understanding of their hosts and genetic context. The abundance of ARGs is well studied for select genes via qPCR, but the hosts of environmental ARGs are comparatively poorly characterized aside from low throughput isolate studies. Linking ARG to hosts provides key data on the risk associated with and factors driving the proliferation of ARGs. Further, differentiating ARGs in intracellular and extracellular DNA (iDNA and eDNA) fractions is needed to understand ARG persistence and transferability. The objectives of this study were to compare (O1) the concentration of select extracellular and intracellular ARGs in water, biofilm, and sediment samples and (O2) the diversity and host assignments for ARGs in the different DNA fractions from sediment samples. For O1, water, biofilm and sediment samples were collected from various sites along the Raritan River. DNA was extracted to separate eDNA, iDNA and total DNA, and qPCR analysis was performed for select ARGs and the 16S rRNA gene. For O2, shotgun metagenomic sequencing was performed on sediment samples and ARG hosts assigned via two different pipelines and compared to evaluate their performance in terms of number and diversity of linkages and accuracy of in silico matrix spike host assignments. sul1 ARG was quantifiable in iDNA and total DNA from all samples and in eDNA only from sediment and biofilm samples. The overall microbial community structure was similar for iDNA and total DNA, compared to eDNA and generally clustered by sampling site. ARG hosts and association with mobile genetic elements will be compared across DNA fractions and sites. Finally, one-to-one comparison of the two pipelines tested will be explained with respect to ARG-host linkages.

Synergistic effect of the combination of diverse dietary fibers on modulating human gut microbiota.

Yongjia Gong, Guojin Wu, Cuiping Zhao, Yan Y. Lam, Liping Zhao

Department of Biochemistry & Microbiology, Rutgers University, New Brunswick, NJ

Dietary fibers are shown to provide energy source to gut bacteria and support production of beneficial microbial products such as short chain fatty acids. However, most studies on the use of dietary fibers focus on single type with limited clinical benefits. Our previous work showed that a diet that contains diverse types of dietary fibers promoted a select group of gut bacteria in type 2 diabetic patients and improved clinical outcomes in diabetic patients. We hypothesized that the combination of diverse dietary fibers requires gut bacteria work collaboratively as a guild to degrade and ferment the substrates. An ex vivo fermentation system was used to characterize the response of gut microbiota to the combination of different fibers. Specifically, we tested how the combination of dietary fibers with different chemical structures (inulin vs bran mix) impact the gut microbiota composition. Fresh fecal sample from a healthy individual was homogenized in PBS buffer and cultured with the following combinations of dietary fibers: 1) 1% bran mix, 2) 1% inulin, 3) 1% bran mix + 1% inulin under anaerobic condition for up to 48 hours with no dietary fibers as a control. After 48 hours of fermentation, the inulin-containing groups induced the more dramatic reduction in pH compared with bran mix group. Principal coordinate analysis based on Bray-Curtis distance indicated that microbiota cultured with inulin-containing treatments clustered together. There were 9 unique Amplicon Sequence Variants (ASVs) significantly promoted only in the group with inulin and bran mix combined, indicating a synergistic effect of the combined dietary fibers on microbiota modulation. Among the 9 ASVs, members in *Bifidobacterium* and *Ruminococcaceae* are SCFA producers known to be beneficial to the human host. Further studies are needed to understand whether these ASVs represent a bacterial guild that only respond to the combination of diverse dietary fibers.

Lipid flippase subunit Cdc50 mediates drug resistance and fungal virulence in *Cryptococcus neoformans.*

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Cryptococcus neoformans is the most common cause of fungal meningitis in immunocompromised individuals. Treatment options for cryptococcosis are limited. Currently available antifungal drugs are either highly toxic (polyenes) or exert a fungistatic effect (triazoles). Drugs of the echinocandin class, which target the glucan synthase and are fungicidal against a number of other fungal pathogens, are ineffective against *C. neoformans*. Despite sensitivity of the target enzyme to drug, the reasons for the innate resistance of *C. neoformans* to echinocandins remain unknown. To understand the mechanism of echinocandin resistance in *C. neoformans*, we screened gene disruption and gene deletion libraries for mutants sensitive to echinocandin class drug caspofungin and identified a mutation of CDC50, which encodes the β-subunit of membrane lipid flippase. We found that the Cdc50 protein localized to membranes and that its absence led to plasma membrane defects and enhanced caspofungin binding and penetration into the cell. Loss of CDC50 also led to hypersensitivity to azole class drug fluconazole. Interestingly, in addition to functioning in drug resistance, CDC50 was also essential for fungal resistance to macrophage killing and for virulence in a murine model of cryptococcosis. Furthermore, the surface of cdc50Δ cells contained increased levels of phosphatidylserine, which has been proposed to act as a macrophage recognition signal. Together, these results reveal a previously unappreciated role of membrane lipid flippase in *C. neoformans* drug resistance and virulence.

Development of an in vivo site-specific photo-cross-linking and mass spectrometry-based approach to identify the small protein interactome.

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Small proteins are a class of polypeptides containing ≤ 50 amino acids discovered in both prokaryotes and eukaryotes. Several small proteins are being shown to play important roles in cell physiology expanding this field of small protein regulators of gene expression. Recent advances in RNA sequencing technologies, including ribosome sequencing, and genome annotation methods revealed many small open reading frames (small ORFs), which encode for small proteins, and are actively translated. To date, only a handful of the >150 small proteins annotated in *E. coli* have been functionally characterized and some of their protein targets have been identified. For example, the *Escherichia coli* small protein MgrB, a 47-amino acid protein, contains a transmembrane region that localizes to the inner cell membrane and negatively regulates the activity of the PhoQ sensor kinase by forming a complex with it, within the cell membrane. A single tryptophan residue within MgrB transmembrane region is essential for the formation of MgrB/PhoQ complex. To elucidate the small protein interacting partners of a continuously growing list of novel small proteins in an efficient and systematic manner, we developed a high throughput method that utilizes in vivo photo crosslinking, using a site specific, photoreactive amino acid analog p-benzoyl-phenylalanine (Bpa), combined with protein mass spectrometry to identify targets of a small protein. Our method takes advantage of a given small protein's size to generate scanning Bpa libraries, containing variants of the small protein where each amino acid position is replaced with Bpa. So, in addition to identifying an interaction partner, our method will allow for the determination of specific the amino acids involved in binding its target. Because Bpa in the small protein forms a covalent bond with the target protein, our method is sensitive in capturing both strong and transient interactions, which will be further validated using complementary approaches. Using a conserved small protein in *E. coli* – YkgO as a model, we demonstrated the proof of concept for our method. YkgO, a 46 amino acid protein is a paralog of RpmJ (38 amino acids long). RpmJ and YkgO are both highly conserved ribosomal proteins, found in bacteria, mitochondria, and chloroplasts. These two proteins are important for late 50S ribosome assembly, however not much is known about their interactions with other ribosomal factors. Preliminary crosslinking data for both proteins identified novel interactions with ribosomal structural proteins that surround the binding pocket of RpmJ/YkgO and proteins that bind and modulate the ribosome. Our method is broadly applicable to the studies of small protein interactions across prokaryotic and eukaryotic systems.

Using the minion to rapidly profile the oyster microbiome.

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Nanopore sequencing is a rapidly-evolving technology that is useful for profiling communities of bacteria. By sequencing the rRNA operon (4200 + bp), species- and strain-level resolution can be achieved. Long-read methods are important since it provides a much more detailed picture of oyster microbiome dynamics, which can by obscured by short-read approaches with limited resolution to the genus level. For this study, extraction and sequencing methods were adapted to the unique challenges posed by oysters reared in different settings, including semi-sterile lab conditions, an oyster farm, and wild or restored oyster reefs. The Oxford Nanopore MinION was used to profile rRNA operons and assess how the oyster microbiome varies geospatially, as well as the potential connections to oyster disease status (*Perkinsus marinus* infection).

Detection of sepsis-causing pathogens using an innovative and rapid molecular diagnostic test.

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Background: Sepsis is a burdensome condition with almost 49 million cases annually. Sepsis globally accounts for almost 20% of all deaths. Blood cultures are recognized as the gold standard for diagnosing bloodstream infections. However, there are some disadvantages with these tests. For example, blood culture-based species identification may take a few days, which can delay appropriate targeted therapy. In addition, antimicrobial administration may also result in negative culture results. Prolonged administration of broad-spectrum antibiotic therapy is associated with antimicrobial resistance. Early identification and treatment of sepsis is essential; for every hour of delay in appropriate therapy, survival decreases by almost 8%. T2Bacteria and T2Candida panels are culture independent tests that provide species identification directly from whole blood samples in 3 to 5 hours. The T2 panels are FDA approved and CE marked. They utilize T2 Magnetic Resonance (T2MR) technology which demonstrates highly sensitive (>90%) and specific (> 98%) results. Usage of culture independent tests can improve clinical outcomes by detecting sepsis-causing pathogens sooner resulting in earlier administration of appropriate targeted antimicrobial therapy.

Methods: A literature review was conducted to assess the impact and accuracy of T2 Panels in detecting sepsis-causing pathogens in bloodstream infections. The clinical outcomes of T2 panels were compared to blood cultures along with an impact assessment on antimicrobial stewardship.

Results: T2Bacteria and T2Candida panels demonstrated faster time to detection and time to species identification along with earlier time to targeted antimicrobial therapy as compared to blood cultures. In addition, de-escalation of antimicrobial therapy was sooner in patients with negative results. Utilization of the T2 panels shortened length of hospital stay, including in the intensive care unit.

Conclusion: T2Bacteria and T2Candida are rapid culture independent tests and are highly sensitive as well as specific in detecting sepsis-causing pathogens in bloodstream infections. T2MR technology may facilitate antimicrobial stewardship initiatives by providing faster species identification, improvements in time to targeted therapy, de-escalation of antimicrobial agents, and shorter length of stays for patients.

Microplastics as hubs enriching antibiotic-resistant bacteria and pathogens in municipal activated sludge.

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Microplastics can serve as carriers of antibiotic-resistant bacteria (ARB) and pathogens, representing a pressing concern to aquatic biota and human health. Activated sludge units at municipal wastewater treatment plants (WWTPs) are “hotspots” converging microplastics and antibiotics. In this batch study with activated sludge samples from three domestic WWTPs, we demonstrated both polyethylene (PE) and polystyrene (PS) microplastics can acclimate biofilms enriched with sulfonamide resistance genes (sul1 and sul2) and the associated mobile genetic element (intI1) in comparison with fine sands as control particles. Absolute abundances of these genes were further elevated by 1.2∼4.5 fold when sulfamethoxazole was initially spiked as a representative sulfonamide. The combination of 16S rRNA amplicon sequencing and differential ranking analysis revealed that microplastics selectively promoted antibiotic-resistant and pathogenic taxa (e.g., *Raoultella ornithinolytica* and *Stenotrophomonas maltophilia*) with enrichment indices ranging from 1.6 to 3.3. Furthermore, heterotrophic *Novosphingobium* and filamentous *Flectobacillus* accounted for 14.6 % and 3.3 % on average in microplastic biofilms, respectively, which were up to 2.8 and 11.1 times higher than those in sand biofilms. Dominance of these bacterial species may contribute to initial biofilm formation that facilitates subsequent colonization and proliferation of ARB and pathogens, thus amplifying their risks in the receiving environments and beyond.

RNA structure-dependent activation of the innate immune response by influenza virus mini-viral RNAs.

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Innate immune activation during influenza virus infection involves the binding of viral RNAs to host pathogen receptor retinoic acid-inducible gene I (RIG-I). However, not all influenza virus RNAs are strong RIG-I agonists. Here we reveal an RNA structure-dependent innate immune activation mechanism that underlies how the most potent influenza virus RNA agonists, mini viral RNAs (mvRNA), induce RIG-I-dependent signaling. Specifically, we find that mvRNA formation leads to the creation of template loops (t-loop) capable of stalling viral RNA polymerase activity and inducing IFN-beta promoter activation. The effect of t-loops is dependent on the stability of their double-stranded stem and the polymerase residues that guide the template mvRNA out of the RNA polymerase. Overall, these findings offer new insights into how active viral replication contributes to the activation of innate immune response, and they advance our understanding of how our innate immune system detects influenza A virus infections.

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.

IGP synthase in *Mycobacterium tuberculosis* mutant E57Q

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Mycobacterium tuberculosis is the bacterium that causes tuberculosis. Recently, this pathogen has started to show signs of antibiotic resistance. IGP synthase is an enzyme that catalyzes a key step in the tryptophan biosynthetic pathway. We suspect that IGP synthase is an essential enzyme in *Mycobacterium tuberculosis* and that it could perhaps be targeted by inhibitors to cut off the bacteria's ability to create tryptophan. The goal of the project is to understand the roles of the different amino acids in the active site by creating single point mutations. The more we understand how this enzyme works, the better we will be able to design effective inhibitors. We created a single point mutation, known as E57Q, where we replaced the glutamic acid (E), a negative charged amino acid, with glutamine (Q), a neutral amino acid. In the wildtype, we hypothesize that the glutamic acid acts as a catalytic base. By creating the single point mutation that has a similar structure but only differs in charge, we can examine the role of this residue in IGP synthase catalysis.

DNA uptake in the deep-sea Epsilonproteobacterium, *Caminibacter mediatlanticus*

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DNA transformation due to natural competence is one of the major mechanisms for Horizontal Gene Transfer (HGT), which in turn drives bacterial evolution. Omics-based studies have shown that the genes involved in competence and recombination are present and even actively expressed in Epsilonproteobacteria from deep-sea hydrothermal vents. But there have been no studies attempting to characterize natural competence in these organisms. The aim of this study is to demonstrate that *Caminibacter mediatlanticus*, a deep-sea vent Epsilonproteobacterium, can take up free DNA at its optimum growth conditions. Actively growing cultures of *C. mediatlanticus* were exposed to an exogenous, PCR-amplified, Cy3-labeled dsDNA fragment during their exponential and stationary phases. Post exposure, the DNase-treated, DAPI-stained cells were visualized using fluorescent microscopy. Cy3-fluorescence was observed in both exponential and stationary phase cultures, indicating the presence of exogenous DNA in the cells. About 20% of the cells were able to take up exogenous DNA in both exponential and stationary phase. These observations suggest that these organisms are naturally competent, and their competence is growth-phase independent. This is the first set of experiments to understand if deep-sea vent Epsilonproteobacteria are capable of taking up free DNA, with the ultimate goal of developing a transformation protocol to generate knock-out mutants. Further experiments will be aimed to: 1) quantify the expression of DNA uptake-related genes, and 2) demonstrate transformation of *C. mediatlanticus* with a gene cassette and a selective marker.

Malonate utilization regulates quorum sensing and virulence factors of *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa is an opportunistic pathogen that uses several carbon sources for its metabolism including malonate. We recently reported that upon its growth in blood from trauma patients, *P. aeruginosa* upregulated the expression of malonate utilization genes. In this study, we describe several experiments designed to explore the role of malonate utilization in *P. aeruginosa* virulence. We grew *P. aeruginosa* strain UCBPP-PA14 (PA14) in M9 minimal medium containing either malonate (MM9) or glycerol (GM9) as a sole carbon source and assessed the effect of the growth on the quorum sensing systems, virulence factors, and antibiotic resistance. Compared with its growth in GM9, the growth of PA14 in MM9 reduced the production of elastases, rhamnolipids, and pyoverdine but enhanced the production of pyocyanin, catalase, and fluopsin C. It also increased PA14 sensitivity to norfloxacin. Furthermore, the growth of PA14 in MM9 decreased the extracellular levels of N-acyl homoserine lactone autoinducers, an effect likely associated with the increased pH of the culture medium. However, this pH change had a limited effect on the extracellular level of the *Pseudomonas* quinolone signal. At late stages of growth in MM9, PA14 formed aggregates or biofilm like structures, which were associated with magnesium-related biomineralization related to the increased pH. These results suggest that malonate significantly impacts *P. aeruginosa* pathogenesis by influencing the activity of quorum sensing systems, the production of virulence factors, biofilm formation, and antibiotic resistance.