Annual Meeting
Ohio Branch of the American Society for Microbiology

Miami University

MIAMI UNIVERSITY

Oxford, Ohio
April 14 – 15, 2023

Official Meeting Program
and
Conference Abstracts

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Acknowledgements

The assistance and dedication of the following have contributed to the success of OBASM 2023:

OBASM Executive Committee

Christine Weingart
Erin Murphy
D.J. Ferguson
Lubna Abu-Niaaj
Paul Hyman
Stephanie Strand
Harry Kestler
Chet Cooper
Laura Tuhela-Reuning
Stephanie Miller
Laura Saltman

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The Miami University Microbiology Department
The Miami University Microbiology Club
Chris Makaroff
Dean of the College of Arts and Science, Miami University
Kim Finer – Region 3 Branch Planning Coordinator
Steve Tuhela-Reuning – OBASM Webmaster
ASM Distinguished Lecturer Program
The American Society for Microbiology

Invited Speakers

Clay Fuqua
Jason Bartz
Annette Bollmann
Shaohua Wang
Jake McKinlay
Chet Cooper
Jason Blackard
Andrew Jones

Program Schedule for OBASM 2023

Friday, April 14

6:00 – 7:00 pm **Registration**

7:00 – 7:10 pm **Introduction –** Pearson 128
D.J. Ferguson, President of OBASM

Welcome Statement
Chris Makaroff
Dean of the College of Arts and Science, Miami University

7:15 – 8:15 **OBASM Keynote Lecture** – Pearson 128

"Navigating the way through sticky situations: Polar surface attachment and control in a model pathogen"

Clay Fuqua
Department of Biology
Indiana University

Saturday, April 15

8:30 am **Registration and Poster Set-up –** Pearson Hall, lower level

9:15 am **Introduction and Welcome** – Pearson Room 128

D.J. Ferguson
President of the Ohio Branch of the American Society for Microbiology

Concurrent Session I
Clinical and Medical Microbiology
Moderator: Chris Weingart

9:30 – 10:00 **Shaohua Wang** – Pearson, Room 128
Ohio University

"Gut microbiome and *Clostridioides difficile* Infection**"**

10:00 – 10:30 **Chet Cooper** – Pearson, Room 128
Youngstown State University

"**Survey of Dormitory Wastewater for SARS-CoV-2: The Real Poop**"

10:30 – 11:00 **Jason Blackard** – Pearson, Room 128
University of Cincinnati

"The drug crisis goes viral – how drugs of abuse are fueling the HIV and HCV epidemics"

<u>Concurrent Session II</u>
Applied and Environmental Microbiology
Moderator: Paul Hyman

9:30 – 10:00 **Annette Bollmann** – Pearson, Room 116
Miami University

"**Ammonia oxidation in bacteria**"

10:00 – 10:30 **Jake McKinlay** – Pearson, Room 116
Indiana University

"**Emergent and covert cross-feeding in a synthetic bacterial community**"

10:30 – 11:00 **Andrew Jones** – Pearson, Room 116
Miami University

"Engineering 'Magic' Microbes for the Production of Therapeutic Psychedelics"

11:00 – 11:15 am **Break**

11:15 – 1:00 pm **Podium presentations and judging** – Pearson, Room 128
Coordinator – Chet Cooper

<u>11:15 – 11:30 am</u>

Establishing a robust and stable light-driven microbial consortium for the synthesis of high-value compounds

Khondokar Nowshin Islam*[1], Shrameeta Shinde[1], Abhishek Sen[2], J Andrew Jones[3] and Xin Wang[1]
[1]Department of Microbiology, Miami University.
[2]Department of Cell, Molecular and Structural Biology, Miami University.
[3]Department of Chemical, Paper and Biomedical Engineering, Miami University

11:30 – 11:45 am

BK Polyomavirus Evolution in Hematopoietic Stem Cell Transplant Patients

Grace Zhang[1]*, Elizabeth A. Odegard[2], Anthony Sabulski[3,4], Sonata, Jodele[3,4], Assem Ziady[3,4], Alix E. Seif[5, 6], Stella M. Davies[3,4], Benjamin L. Laskin[5,7], and Jason T. Blackard[2]

[1] University of Cincinnati College of Medicine, Cincinnati, OH, United States

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[7]Division of Nephrology, The Children's Hospital of Philadelphia, Philadelphia, PA, United States

11:45 – 12:00 pm

Characterization of the Role of SarA and AgrA on the Temperature Dependent Production of the Metalloprotease Aureolysin

Raeven A. Bastock*[1,2,3], Paul Briaud[1], Ryan Steere[1], Ronan K. Carroll[1,2,3], and Erin R. Murphy[2,3,4,5]
[1]Department of Biological Sciences, Ohio University, Athens Ohio
[2]Molecular and Cellular Biology Program, Ohio University, Athens Ohio
[3]Infectious Tropical Disease Institute, Ohio University, Athens Ohio
[4]Department of Biomedical Sciences, Ohio University, Athens Ohio
[5]Herritage College of Osteopathic Medicine, Ohio University, Athens Ohio

12:00 – 12:15 pm – Break

12:15 – 12:30 pm

Oxidative stress alters the heterotrophic community structure of ammonia-oxidizing enrichment cultures

Madisen Kimbrel*, Madelynn D. Spencer, Annette Bollmann
Miami University

12:30 – 12:45 pm

Innate Immune dysregulation may lead to atherogenesis in severe Covid-19 patients

Manuja Gunasena*[1,2], Yasasvi Wijewantha[1], Will Mulhern[1], Dhanuja Kasturiratna[3], Joseph Bednash[2], Thorsten Demberg[4], Nicholas Funderburg[2] and Namal P.M Liyanage[1,2]
1 The Ohio State University, College of Veterinary Medicine, 2 The Ohio State University, College of Medicine, 3 Northern Kentucky University, 4 Marker Therapeutics Inc., Houston, TX, United States.

12:45 – 1:00 pm

Who goes there? Determining what and how microbes inhabit and influence a hyperalkaline spring

Leah Trutschel*[1], Brittany Kruger[2], Annette Rowe[1]
[1]Department of Biological Sciences, University of Cincinnati, Cincinnati OH
[2]Desert Research Institute-Las Vegas, Las Vegas NV

1:00 – 2:00 pm **Mid-Day Activities:**

Lunch: Box or "on your own"

For those that pre-ordered Box lunch, pickup in room 112

OBASM Business Meeting – Pearson, Room 32

2:15 – 3:15 pm **ASM Distinguished Lecture** – Pearson, Room 128

"Prions and Prion-Like Diseases"

Jason Bartz
Creighton University School of Medicine

3:15 – 3:30 pm **Break**

3:30 – 5:00 pm **Poster presentations and judging** – Pearson Hall, lower level
Coordinator – Chet Cooper

5:00 – 5:30 pm **Meet the Speaker –** Pearson, Room 128
An informal meeting with our speakers. Bring your questions and join in the conversation!

Clay Fuqua
Jake McKinlay
Jason Bartz
Andrew Jones
Jason Blackard

5:45 – 6:00 pm **Banquet and Student Awards Presentations** – 108/110 Marcum Conference Center
D.J. Ferguson – OBASM President

Abstracts of OBASM Poster Presentations
Saturday, April 15
3:30-5:00 pm

~~BOARD 1~~ – WITHDRAWN

Characterization of TisB Toxin Antitoxin system in *Shigella flexneri*

Waleed Khursheed*[1], David D. Sarpong[1], Peter W. Coschigano, PhD[2,3,4], and Erin R. Murphy PhD[2,3,4]

[1]Molecular and Cellular Biology, Ohio University, [2]Biomedical Sciences, Ohio University, [3]Ohio University Infectious and Tropical Diseases Institute, [4]Heritage College of Osteopathic Medicine

Shigella is a genus of Gram-negative, facultatively anaerobic pathogenic bacteria that causes Shigellosis in humans. The TisB toxin-antitoxin (TA) system initially characterized in *Escherichia coli,* a bacterial species closely related to *Shigella*. The TisB TA system is comprised of four genes, *tisB, tisA, istR1* and *istR2*, all located within a larger genomic island known as the high-pathogenicity island (HPI). The *tisB* gene encodes a small, membrane-associated, toxic peptide that inhibits cell growth by destabilizing the bacterial membrane, while the *istR1* gene encodes an antitoxin that neutralizes the TisB toxin. The function of the *tisA* and *istR2* genes remains unknown. The TisB TA system is highly conserved among enterobacteria, indicating that it has an important role in bacterial physiology. The expression of the TisB toxin is regulated by the transcriptional regulator H-NS, which represses TisB expression under normal growth conditions. However, during stress or nutrient limitation, H-NS is degraded, allowing for TisB expression and subsequent growth inhibition. In the absence of the antitoxin, the TisB toxin can cause cell death by inducing the formation of pores in the bacterial membrane. Recent studies have shown that the TisB TA system plays an important role in the virulence and persistence. Our lab is focused on studying pathogenic factors associated with *Shigella flexneri*. We are extending TisB TA studies in pathogenic Shigella strain and we have got data that shows overproduction of this toxin hinders cell growth. Our next goals will be to study TisB effect on membrane depolarization and ATP depletion that are characteristics of bacterial persistence in the presence of antibiotics. In conclusion, the TisB TA system is thought to play an important role in bacterial pathogenicity and antibiotic resistance. Further research into the TisB TA system may lead to the development of novel antimicrobial therapies against *Shigella flexneri.*

BOARD 2

Isolation of nitrogenase-like genes and discovery of their functional diversity for the production of industrially important hydrocarbons

Elizabeth A. Morgan*[1] and Justin A. North[1]
[1]The Ohio State University

All bacteria require sulfur for the synthesis of methionine, an essential amino acid, to grow and synthesize proteins. We recently discovered a novel nitrogenase-like enzyme called methylthio-alkane reductase (MAR), which cleaves volatile organic sulfur compounds (VOSCs) into biologically available sulfur for methionine synthesis. While initially discovered in the photosynthetic bacterium, *Rhodospirillum rubrum*, gene homologs were identified in numerous additional bacteria by our collaborators using bioinformatics of genome and metagenome libraries. To explore the diversity of bacteria with MAR activity as well as MAR substrate diversity, gene homologs identified in other bacteria were synthesized by the Joint Genome Institute and

assembled into a plasmid. Synthesized genes were then introduced into an *R. rubrum* MAR gene deletion strain and cells were grown in the presence of three known VOSC substrates for MAR, dimethylsulfide, ethylmethylsulfide, and methylthioethanol. These substrates produce methane, ethane, and ethylene, respectively. To measure VOSC cleavage by MAR activity, hydrocarbon production was quantified by gas chromatography, and we observed functional sequences from a diverse number of phyla, including clostridia, proteobacteria, and fibrobacter. Activity screens revealed that sequences with MAR activity for the three known VOSC substrates are localized to the nitrogenase-like clade IV-C.

Subsequently, we grew *R. rubrum* strains with select MAR sequences in the presence of possible nitrogen, sulfur, and phosphorus substrates. In total, forty-one compounds with varying alkane, alkene and benzene functional groups were assessed. Growth experiments with the library of nitrogen, sulfur, and phosphorous containing compounds reveals that MAR activity is specific for methylated VOSCs (i.e. CH_3-S-R) versus larger VOSCs (e.g. CH_3-CH_2-S-R), nitrogen or phosphorous compounds. This led to the identification of propane, propylene, isobutane, and butane products. Moving forward, identified MAR sequences with high activity for hydrocarbon production will be further employed in bioengineering efforts to produce large yields of these industrially important commodity chemicals.

BOARD 3

The impact of salinization on phytoplankton growth and diversity in local Ohio lakes

Hazel Higginbotham*[1], Rochelle Pereira[2], Bradley Krzysiak[2], Rachael Morgan-Kiss[2]
[1]University of Alabama at Birmingham, [2]Miami University

As one of the many places that experience harsh winters, Ohio is notorious for using salt on roads to aid in melting the snow and keeping the roads drivable. Despite its common use, the ecological impact of road salt is poorly understood. Stormwater runoff from these roads increases the salinization of local waterways, drastically affecting the aquatic ecosystems. Within these ecosystems, algal communities play a crucial role within the food chain, acting as some of the main primary producers. These algal communities are highly responsive to the changes in their environment, including changes in temperature, light, pH, salinity, and many other factors. The impact of salinization on native phytoplankton communities in Ohio lakes has not been thoroughly investigated. In order to study this impact, water samples were collected from Acton Lake, a local lake surrounded by prevalent anthropogenic activity impacting the water quality. In addition to the ambient group, there were three separate treatment groups that were tested within the experiment. Two treatments mimic a pulse environmental disturbance, one at low salt and one at high salt. Additionally, the final group represents a press environmental disturbance, where a saline solution was added every few days for the entirety of the experiment. Changes in the diversity and growth of algal communities were observed through daily sampling for two weeks. It was found that higher salinity levels contributed to a change in the algal communities, negatively affecting mixed groups and positively affecting the growth of green algae, cyanobacteria, and cryptophytes.

BOARD 4

Enzyme activity and genetic regulation of dihydroxyacetone phosphate shunt genes for carbon assimilation by pathogenic bacteria

Caitlin C. Wingerd*[1], Katherine A. Huening[1], Justin A. North[1]
[1]The Ohio State University Department of Microbiology

The dihydroxyacetone phosphate (DHAP) shunt pathway is a multifunctional pathway for carbon and sulfur acquisition from the inhibitory byproducts of S-adenosyl-L-methionine (SAM) utilization, 5'-methylthioadenosine (MTA) and 5'-deoxyadenosine (5dAdo). Originally identified in *Rhodospirillum rubrum,* and Extraintestinal Pathogenic *Escherichia coli* (ExPEC), the DHAP shunt has recently been observed in other pathogenic bacteria such as *Bacillus anthracis* and *B. cereus.* DHAP shunt genes are highly conserved between pathogenic species and include a nucleosidase, kinase, isomerase, and a novel class II aldolase that convert MTA or 5dAdo into adenine, DHAP, and either 2-methylthioacetaldehyde or acetaldehyde, respectively. While *R. rubrum* primarily uses the DHAP shunt for sulfur acquisition via 2-methylthioacetaldehyde, ExPEC use the pathway for carbon

assimilation and growth via DHAP. My work focuses on the characterization of gene products from pathogenic species and isolation of transcription factors regulating DHAP shunt expression specifically in ExPEC to understand the function and regulation of the DHAP shunt in pathogens. DHAP shunt enzymes from various pathogens are heterologously produced in *E. coli* from a plasmid with an IPTG-inducible promoter. Each protein is isolated by nickel affinity chromatography and activity is assayed spectrophotometrically following oxidation of NADH in a coupled enzymatic assay. Preliminary enzyme kinetics of the *Bacillus anthracis* isomerase revealed a K_M of 48 μM for 5-deoxyribulose-1-phosphate substrate which originates from 5dAdo. This is 100-fold more specific than previously characterized DHAP shunt isomerases, which exhibit a K_M of ~1 mM. To isolate the transcription factors involved in the regulation of the ExPEC DHAP shunt genes, we developed a pull-down method using biotinylated DHAP shunt promoter DNA sequences conjugated to streptavidin-coated paramagnetic beads. Initial assays have isolated 2 proteins with apparent molecular weight of 42 kDa and 32 kDa. Further experiments will fully characterize the pathogenic DHAP shunt enzymes and identify the isolated potential transcription factors.

BOARD 5

Friend or Foe? Investigating Interactions with the Antarctic Algal Phycosphere

Bradley Krzysiak[*1], and Dr. Rachael Morgan-Kiss[1]
[1]Miami University

The phycosphere is the interface in which many interactions between algae and their bacterial partners occur. These interactions primarily take the form of exchanged metabolites between both parties. Algae commonly share fixed organic carbon with their bacterial counterparts, often in exchange for compounds benefiting the algae such as phytohormones, vitamins, and other micronutrients required for algal growth. The phycosphere may also serve as a platform for negative interactions, in which the exchange of algicidal or antibacterial compounds allow for competition under conditions such as nutrient limitation. Phycosphere interactions are of high importance within the Antarctic lakes of the McMurdo Dry Valleys (MDV). These lakes represent some of the last perennially ice-covered lakes on the planet, because of this ice covering, the entirely microbial communities within are permanently stratified and receive minimal nutrient inputs. The lack of external inputs into the lake places a great deal of importance on the photoautotrophic algae within the lake as major organic carbon producers. Despite the importance of algae carbon production and the impact of phycosphere communities on algae, bacterial partnerships in MDV lakes remain understudied. In our work we seek to investigate the algae-bacteria partnerships in these lakes by identifying commonly associated bacterial and algal taxa along with defining the underpinning metabolic interactions governing these associations. In addition, we are investigating the stability of these communities in response to environmental disturbances.

BOARD 6

Analysis of Dormitory Wastewater for Levels of SARS-CoV-2

Kira Bowman*, Alexis Albrecht, Samantha Bachochin, Brooke Brocker, Julio Budde, Nadine Gabriel, Dhurviben Shah, and Chester Cooper
Youngstown State University

Prior studies have shown that a community's health can be monitored through wastewater-based epidemiology. These community-centered concepts are being used to monitor levels of the COVID-19 virus (SARS-CoV-2) from five Youngstown State University (YSU) residence halls. Initially, levels of SARS-CoV-2 from dormitory wastewater (WW) samples were analyzed by a commercial firm. However, the time from sample submission to the data delivery was determined to be untenable. Subsequently, an on-site digital polymerase chain reaction (dPCR) assay was employed to more efficiently and effectively detect levels of SARS-CoV-2 in WW. Doing so shortened the receipt of results from 3-6 days to less than 6 hours. To achieve this, autosamplers collected WW over a 24-hour period twice per week. When deemed necessary, “grab” samples were drawn between

scheduled autosampler collections. Using the "4S" method developed by Whitney *et al*. (*Environ. Sci. Technol*. 55: 4880-4888, 2021), nucleic acids were extracted from each WW sample. A portion of the extract was subjected to reverse-transcription polymerase chain reaction to detect SARS-CoV-2 nucleocapsid genes N1 and N2, as well as the human fecal control target, Pepper Mild Mottled Virus (PMMoV). The assay was conducted using the QuantStudio Absolute Q dPCR System. The resulting data were normalized to PMMoV as well as the stated census within a particular dormitory. Throughout the sampling periods, variations in SARS-CoV-2 levels were noted from each WW sample. However, no obvious associations were noted with semester breaks in which residents would be traveling (e.g., winter break). Unfortunately, the absence of concurrent COVID-19 testing precluded the establishment of a definitive relationship between active/asymptomatic cases and the WW presence of SARS-CoV-2.

BOARD 7

In vitro evaluation of potential HIV/AIDS gene therapy candidates

Eva Wanek*[1], Arianna E. Diaz[1], Muna M. Asraf[1], Aliya Z. Ali[1], Mehdi M. Ali[1], Noor Deif[1,2], Mariana A. Heru[1,2], Mallory Alvarez[1,2], Gary R. Dodson[1], Jilyan Hustic[1,2], Tate Love[1], Jocelyn N. Eldmire[1,2], Tatiana A. Fuentes[1,2], Robert Loper[1,2], Lyndsy R. Hamilton[1], Austin Doinidis[1,2], and Harry W. Kestler PhD[1]
[1]Lorain County Community College; [2]Early College High School

The primate lentivirus Human Immunodeficiency Virus Type-1 (HIV-1) is the cause of Acquired Immunodeficiency Syndrome (AIDS). HIV-1 infects a cell by interacting with one of two co-receptors, CCR5 or CXCR4, as well as the primary receptor CD4. CXCR4 and CCR5 are chemokine receptors involved with inflammatory responses. CD4 is used in humoral immune response signaling. A naturally occurring mutation in *ccr5*, known as *ccr5D32*, encodes a 32 base pair frameshift mutation that produces a truncated CCR5 protein that is localized in the cytoplasm instead of the membrane. The truncated protein also appears to downmodulate the cell surface expression of the full length CCR5 and CXCR4. The mutation was originally identified in individuals exposed to HIV but not infected. Individuals who are *ccr5D32* homozygous are resistant to HIV-1 infection. People who are *ccr5D32*/*ccr5* wildtype heterozygotes can be infected, but they progress to AIDS at a slower rate than homozygous wildtype. Five individuals with Leukemia and HIV-1 who received bone marrow transplant (BMT) from *ccr5D32* homozygous donors have undetectable levels of HIV.
Ccr5D32 will be evaluated as a gene therapy to reduce viral burden. A lentiviral vector system was used to construct viral particles containing *ccr5* wildtype, *ccr5D32, and ccr5D33*. The presence of the foreign genes was confirmed by PCR. HEK293-ft served as a packaging cell line by co-transfecting pLenti-*ccr5wildtype*, pLenti-*ccr5D32,* or pLenti-*ccr5D33* along withelper plasmids psPAX2 and pMD2.G. The plasmid psPAX2 encodes HIV metabolic genes and pMD2.G has a Vesicular Stomatitis Virus envelope gene. The pseudotyped viral particles produced were frozen and used to infect H9 and primary lymphocytes. The ability to down-modulate co-receptors by *ccr5D 32*, using lentiviral vectors has not been determined. If CCR5D32 can downmodulate wildtype CCR5 and CXCR4, an effective AIDS therapy that does not rely on lifelong chemotherapy with adverse side effects can be evaluated.

BOARD 8

Regulation of 5-deoxy sugar utilization for growth by extraintestinal pathogenic *E. coli*

Katelyn T. Kapusta*, Katherine A. Huening, Justin A. North
The Ohio State University

Extraintestinal pathogenic *E. coli* (ExPEC) is a growing health concern due to their prevalence in urinary tract and blood infections. We recently identified a metabolic pathway in ExPEC strains called the Dihydroxyacetone phosphate shunt that allows for the utilization of 5-deoxpentose sugars as carbon substrates for growth. This includes 5-deoxyribose and 5-methylthioribose, which cannot be used by commensal strains. *E. coli* utilizes an

expressional hierarchy of carbon assimilation pathways based on the preference of available sugars, with glucose typically being the most preferred. We hypothesized that the DHAP shunt in ExPEC strain ATCC 25922 is transcriptionally regulated based on available carbon substrates. To test this, we constructed a LacZ reporter plasmid in which fragments of the putative promoter region of the DHAP shunt gene cluster were cloned onto the 5' end of *lacZ*. ExPEC strain ATCC 25922 was transformed with the plasmids and grown in defined media with glucose or 5-deoxyribose as the carbon source. The resulting LacZ activity was measured in cell lysates spectrophotometrically. We observe that in the presence of glucose, LacZ activity and hence DHAP shunt expression from the putative DHAP shunt promoter is repressed. Conversely, when cells were grown in the presence of 5-deoxyriobse alone, LacZ activity and hence DHAP shunt expression was robust. Furthermore, deletion of a likely transcription regulatory protein located near the DHAP shunt gene cluster on the *E. coli* genome partially alleviated the repressive effects of glucose on DHAP shunt transcription. This work suggests that the DHAP shunt for 5-deoxypentose sugar utilization in ExPEC strains is transcriptionally repressed by glucose and is seemingly regulated in part by a transcriptional repressor protein. Future experiments will target potential transcription factor binding sites for pathway regulation using this LacZ reporter system and directed mutagenesis.

BOARD 9

Impact of long-term stress acclimation on response to short-term photoinhibition in the Antarctic alga, *Chlamydomonas priscuii*

Devon Popson*, Susanna D'Silva, and Rachael Morgan-Kiss
Miami University

The light reactions of photosynthesis convert light energy from the environment into chemical energy in the form of ATP and NADPH for downstream metabolism. Changes in environmental conditions can alter the amount of energy coming in from the environment or being utilized by metabolism leading to disruptions in energy homeostasis. This energy imbalance can result in oxidative stress and ultimately photoinhibition, or loss of photosynthetic activity. Consequently, the light reactions have evolved various mechanisms to defend against oxidative damage and reestablish energy balance between these processes. These photoprotective mechanisms occur on timescales of short-term acclimation (seconds to minutes), long-term acclimation (hours to days) and adaptation. It has previously been shown that long-term acclimation status can prime an organism's ability to survive short-term stress. However, these studies were performed on stress sensitive organisms such as the model green alga, *Chlamydomonas reinhardtii,* and *Cucumis sativus* (cucumber): there is significantly less known about the impact of stress adaptation on capacity to mediate short-term stress. *Chlamydomonas priscuii* is green alga isolated from the permanently ice-covered Lake Bonney located in the McMurdo Dry Valleys in Antarctica. *C. priscuii* exhibits a unique physiology, owing to adaptation to permanent low temperature, high salinity, and extreme shade conditions. Here we evaluated how long-term acclimation and adaptation impact the ability of *C. priscuii* to avoid photoinhibition. We found that the stress adapted physiology of *C. priscuii* makes it less susceptible to irreversible photoinhibition of PSI compared to *C. reinhardtii*. Additionally, acclimation to high light or low temperature prior to short-term high light stress increases resistance to photoinhibition, whereas high salt acclimation increases PSII photoinhibition in *C. priscuii*. Through this work we hope to gain a better understanding of how timescale regulates photoprotective mechanisms of the photosynthetic electron transport chain.

BOARD 10

Detecting Vancomycin Resistance Markers in Dormitory Wastewater

Jennifer Rakowski*, John Zimmerman, Isabel Schaefbaur, and Chester Cooper
Youngstown State University

Wastewater epidemiology is important in the detection of community-wide spread of infectious diseases (e.g., Covid-19) as well as antibiotic resistance. Vancomycin resistance has emerged as a serious concern in that this antibiotic is often considered the "drug of last resort" in the treatment of infections caused by antibiotic-resistant

strains of *Enterococcus*. Another consideration is the rise in vancomycin-resistant strains of *Staphylococcus aureus*, resulting from the genetic transfer of the vancomycin-resistance genes, *vanA* and *vanB*, from *Enterococcus*.
Using a digital polymerase chain reaction (dPCR) assay, the presence of vancomycin resistant genes was assessed in dormitory wastewater generated by residents living in Kilcawley House on the Youngstown State University campus. Wastewater from Kilcawley House was collected twice per week over a 24-hour period. DNA was extracted from each wastewater sample, from which an aliquot was assayed by dPCR to detect *vanA* and/or *vanB* genes. Experimental controls included known strains of Enterococcus possessing either *vanA* or *vanB*. Analysis of the wastewater from Kilcawley House showed that the presence *vanA* and *vanB* genes fluctuated over the past nine months. Despite the variation, the presence of *vanB* was generally greater than that of *vanA* indicating no apparent correlation between either gene. Collectively, these data suggest that vancomycin resistant bacterial strains are common among Kilcawley House residents and that rigorous hygienic practices should be followed to mitigate the potential spread of antibiotic-resistant microorganisms.

~~BOARD 11~~ - WITHDRAWN

Characterization of RNA Thermometers in the Heme Uptake System of *Shigella dysenteriae*

Anneliese Wagoner*[1,2], Erin R. Murphy, PhD[1,2,3,4]
[1]Molecular and Cellular Biology, Ohio University; [2]Ohio University Tropical and Infectious Diseases Institute; Dept. of Biomedical Sciences, Ohio University; [3]Heritage College of Osteopathic Medicine, [4]Dept. of Biomedical Sciences

Shigella dysenteriae is a pathogenic bacterium that, like all pathogenic bacteria, requires key nutrients from its environment to survive and cause disease. One of those required nutrients is iron, and the main source of which within the human body is heme. *Shigella* utilized essential iron from heme using the multi-component heme uptake (Shu) system. Gene encoding component of the Shu system, including: *shuA*, *shuS*, *shuT, shuW, shuY, shuU and shuV*. Of these genes, expression of *shuT* and *shuA* has been shown to be regulated by the activity of RNA thermometers (RNATs). This project focuses on characterizing and comparing the activity of these RNATs. The *shuT* and *shuA* RNATs were initially characterized in a virulent strain of *Shigella dysenteriae* and their activity compared to that in the non-native bacterial host *Escherichia coli.* Surprisingly, the activity of the *shuT* RNAT was different in *S. dysenteriae* and *E. coli.* One of the major differences between virulence *S. dysenteriae* and *E. coli* is the presence of the virulence plasmid. We are now working to characterize the activity of the *shuS* RNAT in a strain of *S. dysenteriae* lacking the virulence plasmid in order to determine if activity of the RNAT is influenced by a plasmid-encoded factor. As RNAT activity is not traditionally thought to be influenced by other cellular factors, the implication of a plasmid encoded factor in influencing the regulatory activity of the *shuS* RNAT would represent an advancement in our foundational knowledge of bacterial RNA thermometers.

BOARD 12

Phage-antibiotic synergy decreases *Burkholderia cenocepacia* populations

Erin Kistler*, Angie Cuiffo, and Christine Weingart
Denison University

Antibiotic resistance has been on the rise and leads to increasing difficulties with treating bacterial infections. *Burkholderia cenocepacia* is part of a group that includes over 22 species of bacteria that exhibit innate antibiotic resistance mechanisms making treatment increasingly difficult. *B. cenocepacia* is particularly harmful for cystic fibrosis patients due to the sticky mucus in the lungs that it is particularly adept at colonizing. Due to their antibiotic resistance, alternative treatment methods are needed. Bacteriophages are viruses that infect bacteria and are a potential alternative treatment method. Phage-antibiotic synergy (PAS) has been observed when phages are used in the presence of antibiotics, leading to enhanced outcomes in treating bacterial infections. To determine if PAS is effective at decreasing *B. cenocepacia* populations, we infected an artificial sputum model with *B. cenocepacia,* and exposed it to either phage (KP1), antibiotic (meropenem), or both

(PAS) in the absence and presence of A549 lung epithelial cells. We determined that PAS significantly decreases bacterial population but there is no difference in phage production between phage alone and phage in the presence of antibiotic. While there was a large reduction after 24 hours in the PAS treatment, there was then a slight increase in population. Mutant colonies generated from *B. cenocepacia* exposed to PAS for 72 hours exhibited phage resistance but antibiotic sensitivity. Subsequent PAS assays with the mutant *B. cenocepacia* determined that their growth was somewhat reduced by PAS and was significantly reduced by the antibiotic alone. In summary, the combination of phage and antibiotic is effective at reducing *B. cenocepacia* populations and could be a potential treatment. The mutants generated are a potential challenge for this treatment method so understanding their change in sensitivities is important.

BOARD 13

Probing interactions between adhesins and attachment organelle core proteins in *Mycoplasma pneumoniae*

Kristina A. Gara*, Mitchell F. Balish
Miami University

The human respiratory pathogen *Mycoplasma pneumoniae* uses a polar extension of the cell, termed the attachment organelle (AO), to adhere to host cells and engage in gliding motility along surfaces. The AO is essential for this pathogen's virulence and contains some of the most immunodominant proteins of the cell, making it an appealing target for the development of therapeutic agents. Various novel AO-specific proteins localize to certain regions of the AO, but it is unclear how these proteins interact to form a cohesive and functional AO. Protein complexes required for adherence, called nap particles, consist of a pair each of the homologous transmembrane proteins P1 and P40/P90. These structures densely line the entire membrane of the AO are the main factors in the functionality of the AO, while the proteins found in the dense internal core are necessary for the formation of the AO and its structural integrity. Destabilization of the core protein HMW1 prevents localization of the components of the nap particle to the AO, suggesting there is a link between this protein and the membrane adhesins. We tested the hypothesis that the localization of the nap particle to the AO is driven by interactions between its components, like P1, and core proteins like HMW1. Specific charged domains of P1 and HMW1 appear to be the most likely to interact based on the organization of the AO and the topology of P1. Multiple protein-protein interaction assays, including far western blotting, affinity chromatography, and a bacterial two-hybrid screen were used to probe whether these two domains interacted. All were negative, suggesting that either the interaction is weak or indirect, or that other interactions are required for AO function in *M. pneumoniae.*

BOARD 14

Laser-Assisted Killing of Antibiotic-Resistant *Escherichia coli* Using Gold Nanoparticles

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The emergence and spread of antibiotic-resistant microbes is a significant problem across health-care settings, the community, and the environment. Among bacteria of particular concern are those possessing extended-spectrum beta-lactamases (ESBLs). ESBL enzymes inactivate some commonly used antibiotics (e.g., penicillins and cephalosporins), thereby making these drugs ineffective for treating infections. The focus of this study is the development of a novel treatment method for ESBL strains of *Escherichia coli*.
We investigated the use of a novel laser-assisted treatment strategy employing gold nanoparticles (GNPs) to kill 18 strains of ESBL *E. coli*. These isolates were obtained from **the** Center for Disease Control's Antimicrobial Resistance Laboratory Network. A screening approach was implemented whereby 3-4 individual strains were combined prior to experimental treatment. These bacterial suspensions were mixed with 40 nm diameter GNPs, then exposed to laser radiation for 10 minutes. Following laser treatment, the suspensions were serially diluted and plated on nutrient media to assess survival. Control experiments in which bacterial suspensions were mixed with GNPs only, as well as those that were laser treated in the absence of GNPs, showed no decrease in

viability. However, suspensions that contained 30,000 and 10,000 GNPs per cell, then subjected to laser treatment exhibited 85% and 95% decreases in viability, respectively. Selected clones of surviving bacteria were isolated and tested in the same manner. The results were similar to the original treatment regimen, suggesting that these clones were not resistant to laser-assisted killing in the presence of GNPs. We conclude that this laser-assisted GNP treatment strategy **represents a potentially novel approach to treat infections** by antibiotic-resistant bacteria.
[This work is supported by the Halberd Corporation]

BOARD 15

Role of ZFP36L1 in suppressing human coronavirus replication

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ZFP36L1 is a CCCH-type zinc figure protein (ZFP) where zinc ions coordinate the protein structure in a tetrahedral geometry by binding to cystine-cystine or cysteine-histidine amino acids. ZFP36L1's unique structure enables it to interact with a wide variety of molecules including RNA; thus, it could modulate several cellular processes including virus replication. Several CCCH-type ZFPs have shown their antiviral efficacy against various DNA and RNA viruses. However, the role of ZFP36L1 in the human coronavirus is little explored. We used human coronavirus (HCoV)-OC43 to determine the role of ZFP36L1 on its replication. We overexpressed and knockdown ZFP36L1 in HCT-8 cells individually using lentivirus transduction. Wild type, ZFP36L1 overexpressed, and ZFP36L1 knockdown cells were each infected with HCoV-OC43, and the virus titer in each cell line was measured over 96 hours post-infection (p.i.). Our results show that HCoV-OC43 replication was significantly reduced with ZFP36L1 overexpression while ZFP36L1 knockdown significantly enhanced virus replication. ZFP36L1 knockdown HCT-8 cells started producing infectious virus at 48 hours p.i. which was an earlier timepoint as compared to wild-type and ZFP36L1 overexpressed cells. Wild-type and ZFP36L1 overexpressed HCT-8 cells started producing infectious virus at 72 hours p.i. Overall, the current study showed that overexpression of ZFP36L1 suppressed human coronavirus (OC43) production.

BOARD 16

Differential Expressions of Human-like Gene Transcripts in Non-pathogenic *Naegleria* Amoeba

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When humans contract Primary Amebic Meningoencephalitis (PAM) from freshwater amoeba *Naegleria fowleri*, death is almost always imminent. From 1962 to 2021, only 2.60% of people in the United States survived the infection, in part because there is no established mechanism to manage or cure PAM. In quest of potential drug targets, we have conducted a BLAST homolog search against human BRCA2 and CFTR genes since both gene products are involved in cell proliferation. The search identified CFTR (cystic fibrosis transmembrane conductance regulator)-like and BRCA2 (breast cancer susceptibility gene 2)-like genes in *N. fowleri* and *Naegleria gruberi*, a nonpathogenic relative. In humans, a mutation in CFTR, the membrane ion channel, results in cystic fibrosis, while a mutation in BRCA2, a member of DNA repair machinery, increases the risk of developing malignant neoplasia such as breast cancer.

Due to safety reasons *N. gruberi* was used for further analyses and biological assays. Protein sequence alignments revealed that human CFTR has 29.53% and 31.35% identities with the CFTR-like protein in *N. gruberi* and *N. fowleri*, respectively, and human BRCA2, on the other hand, has 29.66% and 27.27% identities with *N. gruberi* and *N. fowleri*, respectively. With this level of variation between the proteins in humans and the amoeba, eventual targeting of these gene products may have fewer adverse effects in humans. Importantly, our phosphoproteomics data also show the CFTR-like protein in both *Naegleria* species is phosphorylated at the

common serine residue, which is not conserved in human CFTR. Currently, we are investigating the differential expressions of CFTR-like and BRCA2-like gene transcripts in *N. gruberi* by quantitative PCR.

BOARD 17

Nutritionally unbalanced diets reduce gut microbial diversity yet maintain functional

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Many animals, including humans, contain a species rich and diverse gut microbiota that participates in many functions that assist the host, including dietary nutrient and toxin breakdown. Research has shown that commensal gut bacterial communities will shift in composition and diversity when there are host perturbations such as dietary changes, infection, or exposure to antimicrobials. However, shifts in metabolic functional capability is not as clear following perturbations. The objective is to understand to what extent dietary perturbations alter gut microbial community diversity, composition, and functional capability. The American cockroach (*Periplaneta americana*) is an appropriate model to study host microbiomes since they are dietarily flexible, maintain a species rich microbial community, and gut microbe inhabitants are congeneric with human gut microbial members. Insects were nondestructively sampled through weekly poop collection during an eight-week feeding period on a balanced, protein-enriched, or cellulose-enriched diet. Quantification of bacterial taxa were completed through 16S rRNA gene sequencing and metagenomic functional capability was predicted using PICRUSt2. A protein and cellulose-enriched diet shifted bacterial community composition and significantly reduced family-level phylotypes that resulted in diet-defined community profiles. Predicted functional MetaCyc pathways and KEGG orthologues reduced in diversity, however, less than 15% of pathways in an unbalanced diet were significantly different from a balanced diet (absolute effect size > 0.8 and Benjamini-Hochberg adjusted p value < 0.05). Of the significantly different pathways, almost half have unchanged pathways that perform the same function. These results suggest that despite diet induced taxonomic variability, the gut microbial community can maintain functions through functional redundancy. Future research should identify if host gut microbial community is selected based on specific taxa, functions, or both.

BOARD 18

Investigating Expression of Integrin β-1-like Proteins Found in *N. gruberi* as Possible Future Drug Treatment Targets for Primary Amoebic Meningoencephalitis (PAM) Induced by *N. fowleri*

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Primary amebic meningoencephalitis (PAM) is a rare brain infection caused by the amoeba *Naegleria fowleri* and is fatal in over 90% of cases. As precious little is known about the molecular mechanism of how *N. fowleri* attacks human brain tissue, we seek to expand this understanding by working with similar proteins found in nonpathogenic *Naegleria gruberi.* We began our search with human integrins – a group of proteins that form α/β heterodimer on the plasma membrane and allow cells to communicate with the connective tissue around them. We compared these integrins by BLAST homolog search for *Naegleria* and found no good candidates among the α-integrins but identified several among the β-integrins, ultimately narrowing these down to two experimental candidates. One is a partially annotated integrin-like serine/threonine kinase – a protein that may be involved in cellular communication and regulating cellular proliferation, programmed cell death, and differentiation in human cells. The other integrin-like protein possibly carries seven-transmembrane helical structures, like G protein-

coupled receptors (GPCR). GPCRs are a hugely diverse group of receptor proteins with functions such as in nervous and endocrine systems, and about half of drugs on the market target GPCR receptors.

The two proteins of interest in *Naegleria* may participate in cellular functions such as foraging behavior or adhesion to substrates in its environment. Current studies on the expression of mRNA transcripts for these proteins with qPCR in *N. gruberi* have shown that mRNA transcripts of the GPCR-like protein are expressed in adhesion conditions, while the annotated Serine/Threonine Kinase protein mRNA was not found to be expressed.

BOARD 19

Hepatitis B virus genotype E/A recombinants from blood donors in Beira, Mozambique

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Hepatitis B virus (HBV) is a viral infection of the liver. Chronic infection can lead to severe health effects such as liver cirrhosis and hepatocellular carcinoma. Despite the availability of a safe and effective vaccine, HBV infection remains endemic in many sub-Saharan African countries, including Mozambique.

Currently, there are 10 known genotypes (A through J), each with unique geographic distributions. Novel variants are generated by recombination events between the various genotypes. The effects of recombination events are not entirely understood, however, certain mutations arising from recombination may be more associated with disease progression.

The lab previously partially sequenced 57 samples from blood donors from the city of Beira, located in central Mozambique. Of the 57 samples, 6 were suspected recombinants. In the present study, PCR was performed with full-length primers for the HBV genome and then run on a gel. Extracted DNA was then sent for next-generation sequencing (NGS) and full-length consensus sequences were evaluated for HBV genotype and recombination.

Of the 6 suspected recombinants, 5 were successfully amplified. All sequences clustered separately from reference genotype E sequences on a phylogenetic tree. Prediction recombination profiles suggest that the sequences were E/A recombinant variants. The present study provides insight into HBV genotype diversity of blood donors in Beira, Mozambique.

BOARD 20

Inhibition of *Pseudomonas aeruginosa* Attachment by Native-Mucin

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Pseudomonas aeruginosa is adept at establishing chronic infections thanks to its biofilm-forming abilities. This process typically begins with surface attachment and in a host, these surfaces are usually covered in a protective mucosal layer. The main structural component of mucus, the glycoprotein mucin, prevents surface attachment of *P. aeruginosa*. The glycan components of mucin, but not the individual monosaccharides, can additionally inhibit attachment, suggesting that *P. aeruginosa* is prevented from attaching due to specific biochemical structures found in mucin. The mechanism behind this inhibition is independent of the viscoelastic and lubricative properties of mucin. We have determined mucin-glycans are impacting a heat-labile protein that does not require transcription for attachment inhibition by mucin. Flagella mutants resulted in a modest increase

in attachment, but a flagella pili double mutant is completely unable to attach in mucin, suggesting mucin is impacting additional attachment mechanisms. Mucin interestingly lowers secondary messenger levels, but restoration of c-di-GMP and cAMP did not result in increased attachment in mucin. Other attachment-associated pathways, such as Las quorum sensing, chemotaxis, the GacS/RsmA system, and rhamnolipid production do not appear to be involved either. Transient mucin exposure still reduces *P. aeruginosa* attachment, suggesting that mucin causes *P. aeruginosa* to become surface adverse or blind in some unknown manner. Mucin also does not appear to physically prevent *P. aeruginosa* surface localization, as pre-coating with mucin did not inhibit attachment. Attachment inhibition by mucin additionally appears to be well conserved as lab-adapted strains of *P. aeruginosa* presented varied levels of attachment in the presence of mucin, with PAK being the most resistant and PA103 the most sensitive. Our work thus far suggests mucin inhibits surface attachment in a mechanism independent of those primarily associated with adhesion. Completion of this study will further our understanding of innate host defenses in preventing biofilm establishment.

BOARD 21

A Meta-Analysis Reveals Distinct Host-Associated Microbial Communities Across the Insect Phylogeny

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Insects are one of the most biologically diverse eukaryotic classes in the world, representing >250 million years of evolution. Insect microbiomes are essential for health and development, similarly as to humans. While individual studies have characterized the microbiomes of different insect species, questions remain as to how inter-species differences in microbiome structure are maintained by the host. Observable taxonomic compositional differences between insect microbiomes have led us to the question, can ecology or phylogeny better explain insect microbiome composition? We expect host ecology, such as diet, lifestyle, and environment, to be drivers of microbiome composition; but additionally, we expect host factors such as the innate immune system to play a large role in shaping and controlling the microbiome. Innate immunity gene divergences across the host phylogeny might be responsible for the observable diversification of microbiomes. Here we begin to interrogate this question further by conducting a meta-analysis using publicly available sequencing data from insect microbiomes across the host phylogeny. We characterized the microbiome of different insects using alpha diversity metrics and examined differences in the most abundant bacterial taxa. Additionally, we compared microbiome composition between phylogenetically close, and distant, insect species finding that overall, between species differences are greater than within species differences.

BOARD 22

Taking on Multiple Research Projects in a NSF Research Experience for Undergraduates (REU) Summer Program as a Disabled Undergraduate Student

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The UMaine REU ANEW program is NSF funded and takes in undergraduates to gain mentored research experience over 10 weeks. As a disabled student, I came in with a disadvantage due to challenges at my small home school. During my REU time, I worked on three projects and sought ways to improve the accessibility of the research.
Cryptosporidium in Farm Calves: allowed room for discovering accessibility in time-dependent research as I was able to collaborate with team members utilizing each other's strengths. We worked on an online folder to share information accessibly. I worked on joint ergonomics involving lab techniques. *Cryptosporidium parvum* is a protozoan that lives in freshwater, survives for long periods in harsh conditions, and causes gastroenteritis in mammals. Calves that suffer from cryptosporidiosis have slower growth rates, life-threatening diarrhea, require

intensive care, and this results in a loss of profit. This project seeks to find the best method to efficiently identify it across sample types for student projects. Three sample types were collected and processed with multiple microscopy stains. Water samples were tested with Crypt-A-Glo, a fluorescent dye and a Modified Ziehl-Neelsen (MZN) dye procedure. Fecal and soil samples were tested using fecal flotations stained with Gram's Iodine. We found that MZN Method is the easiest method for water, Crypt-a-Glo can be used with and without fluorescent microscopy but can be difficult to see, and fecal flotations using Gram's Iodine is a fast and easy way to quickly find protozoa in feces. Improvement can be made in the project by identifying *Cryptosporidium* with PCR to verify visual identification.

Camel Rumen Transcriptome: provided insight on remote accessibility as the project is in a data-processing stage. I was able to use the online team folder, assess and interpret the data, view past graphs, and build on them. I built on past ideas and made sure figures would be accessible for those with visual disabilities (e.g., font, color palette).

Microbes and Social Equity Symposium: allowed for a chance where I could work in the background of a scientific conference featuring large group conversations. I helped take notes that were made accessible to the group all in real time, and everyone was welcome to contribute virtually by either speaking or writing their thoughts on collaboratively written notes. I gathered a consensus on issues of social equitability, involving access to resources. This was informative on equity in science, and helped me learn the process behind designing research, coursework, or conversations in science.

Many disabled students are held back in science because of a lack of logistics, resources, or approaches to performing research that allows them to participate equally. This project has found insights that can provide different concepts to help work with disabilities in a research setting to help break these barriers.

BOARD 23

Clostridioides difficile evade neutrophil response *in vitro* and *in vivo*

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Clostridioides difficile is the most common cause of nosocomial infections in the US. The bacterium secretes toxins (TcdA, TcdB, and in some strains, CDT) which damage intestinal epithelial cells and drive *C. difficile* infection (CDI) pathology. Epithelial injury cause release of inflammatory mediators leading to recruitment of innate immune cells, including neutrophils, to the site of infection. Current literature suggests that neutrophil response in CDI fail at clearing the pathogen. For example, in a murine model of CDI, we have shown that number of neutrophils at the site of infection (cecal tissue) did not affect *C. difficile* burden, suggesting that *C. difficile* avoid destruction by neutrophils. Here, we have examined the impact of neutrophils on *C. difficile* burden *in-vitro* and *in-vivo.*

We co-cultured magnetically sorted (MACS negative selection) neutrophils from mouse bone marrow and with *C. difficile* in Columbia broth for 6hrs under anaerobic conditions. Colony forming unit (CFU) assays and microblot replicator stamping onto CCFA plates indicated that neither inactivated nor LPS-activated neutrophils altered *C. difficile* number compared to controls (*C. difficile* alone). Optical density (OD600) reading and flow cytometry evaluation of propidium iodide staining verified this observation. We further examined the effect of neutrophils on *C. difficile* burden *in-vivo*. For this purpose, we used a transgenic mouse (PMN^{DTR}) in which peripheral and tissue neutrophils can be depleted by intraperitoneal injections of diphtheria toxin. PMN^{DTR} mice were challenged with *C. difficile* spores by oral gavage. Cecal contents collected from $iDTR^{+/+}$ Cre^{+} mice (neutrophil deficient) and $iDTR^{+/+}$ Cre^{-} mice (neutrophil sufficient) 24hrs after the spore challenge had a similar pathogen burden. Taken together, these data indicate that *C. difficile* evades neutrophil response. Future studies will focus on elucidating mechanisms employed by *C. difficile* to circumvent killing by neutrophils.

BOARD 24

Host-adaptive features set the stage for cladogenesis of insect-associated Lachnospiraceae

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Diverse bacterial communities are found in the guts of animals of every lifestyle and level of complexity, often contributing to host health through nutrient provision. Lachnospiraceae are a family of anaerobic bacteria that are abundant in mammalian guts. Elsewhere in the environment and in invertebrate guts, Lachnospiraceae are rarer and lacking in characterization. Two Lachnospiraceae isolates cultivated from the gut of the omnivorous model insect *Periplaneta americana* exhibited cladogenesis, with multi-locus phylogenetic and gene content analysis suggesting they diverge significantly from near neighbor taxa within the family and represent two novel genera. Whole genomes of the isolates and a group of relatives were obtained, which underwent detailed gene annotation and comparative genomic analysis. The isolates encode an array of gene products that may facilitate colonization of the insect gut environment and potentially play a role in host diet, such as those involved in plant fiber degradation, short chain fatty acid synthesis, flagellar synthesis, and mucin and chitin catabolism. For oxygen-sensitive commensal microbiota, dispersal outside the gut is largely limited to transmission between insects via coprophagy. We hypothesize that isolation within the host can lead to genetic drift that could drive allopatric speciation (as opposed to coevolution of a mutualistic relationship), resulting in cladogenesis of host-associated bacterial lineages. Further work is currently being conducted to expand the known cultivable constituents of the *P. americana* gut microbiome and their functional capabilities, and to ascertain how lineage and function of microbiota are related to support of the host

BOARD 25

Towards identification and investigation of Sentinel Organisms in Antarctic Lake Fryxell

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McMurdo Dry Valley, Antarctica houses a variety of lakes that consist of a food web that is entirely microbially dependent, with photosynthetic algae forming the base of the food web. The microorganisms that live in this environment are accustomed to a variety of harsh stress conditions including low temperatures, low light, and high salt concentrations. These lakes have a stratified water column under permanent ice that are devoid of mixing. However, with the increase in temperatures due to global warming, we suspect the increase in the ice melting of the permanent ice layer or increase in stream flow due to glacial melts, shall cause a disruption to the stratified water column under the permanent ice. We hypothesize that this disruption to their ecosystem will thus cause a shift in the microbial communities and change the structure of the food web.
Our research aims at investigating these shifts and determining potential Sentinel Taxa as candidates to detect early warning signals for environmental disturbance. These organisms play an important role in shaping the microbial food web and exhibit clear responses to environmental variability or change. Our current working definition is that Sentinel taxa are a group of organisms that are integral to the microbial food web, sensitive to environmental disturbance, and exhibit predictable spatial or temporal patterns in abundance or activity. The goal of our research is to identify Sentinel taxa in Antarctic Lake Fryxell.

BOARD 26

The impact of spacer length on sigma factor-controlled gene expression in *E. coli*

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Genetic circuits provide precise control of gene expression. Sigma factors play a main role in regulating gene expression by controlling mRNA transcription initiation by binding the -10 site and -35 sites in the promoter region. In this study, we investigated the impact of spacer length, region between -35 and -10 sites, on gene expression controlled by non-native sigma factors in *E. coli*. We designed promoter regions recognized by sigma factor B (SigB) from *Bacillus subtillis* with spacer lengths ranging from 10 to 18 base pairs (bp). The promoter recognized by SigB was located upstream of a gene encoding green fluorescent protein (*gfp*), which was used as a reporter of expression. The gene for SigB was introduced to *E. coli* on a separate plasmid. Control experiments showed that SigB could recognize one of its native binding sites from *B. subtilis*, which has a spacer length of 12 bp, resulting in 61-fold more fluorescence than a control which lacked SigB. The spacer length of 15 bp produced the highest level of GFP expression, followed by spacer lengths of 14 bp and 13 bp. However, the 15 bp promoter showed high fluorescence for cells that lack SigB, suggesting this promoter is not orthogonal to native *E. coli* sigma factors. The 14 bp spacer results in 29-fold and 52-fold increase in expression compared to those with 10 bp and 17 bp, respectively. Interestingly, the expression pattern for SigB spacers follows the native distribution of sigma factor spacer length found in promoters in *B. subtilis* with 14 bp being the most popular. These findings provide insights into optimizing gene expression in bacterial systems and suggest the potential for incorporating other sigma factors to control transcription.

BOARD 27

Expanding promoter options to engineer an environmental-isolate of *Bacillus megaterium*.

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Engineering microbes for sustainable bioproduction is a promising avenue to tackle societal problems in pollution, medicine and energy. Traditional model organisms have received much attention due to their easily manipulated genetics and well characterized growth. However, there are a wealth of microbes with attributes that are beneficial for biotechnological application, but genetic tools must first be developed to engineer them by incorporating foreign DNA and characterizing promoters for controlled expression. In this work, we are looking to expand the genetic toolbox for a strain of *B. megaterium*, called SR7, that was isolated from an underground carbon dioxide reservoir. High pressures of CO_2 are typically sterilizing, but SR7 is able to withstand these. Further, supercritical CO_2 is useful in extracting compounds, such as short-chained alcohols, potentially removing product inhibition. For these reasons, SR7 was previously engineered to produce isobutanol, a second-generation biofuel, under supercritical CO_2. We next sought to develop constitutive promoters for our bioproduction pathway by mining a transcriptome of SR7 grown in different environments. Several promoters were identified and characterized, finding particularly high expression from the native citrate synthase promoter. Genetic manipulation of SR7 was possible using a protoplast-osmotic shock method, but was highly inefficient, rendering SR7 incompatible with modern genetic manipulation using libraries. To solve this, we are investigating electroporation transformation protocols. DNA methylation compatibility was thought to be key to ensuring successful plasmid incorporation and replication. A strain of *E. coli* was constructed which lacks native methylation (*dam, dcm mcrA, mcrC*) genes and contains a methyltransferase gene from SR7 under the control of an arabinose promoter. Using the methylation strain as well as other common methods to enhance

electroporation efficiency, such as osmotic balancing agents, we believe that we will achieve more transformants leading to further development of promoters and metabolic engineering of SR7.

BOARD 28

FCRL1 mediated inhibition of ERK phosphorylation through recruitment of SHIP-1

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FCRL1 (Fc-receptor like 1) is a surface protein expressed on B cells, comprised of extracellular immunoglobulin domains and 2 cytoplasmic immunoreceptor tyrosine-based activation (ITAM)- like motifs. With a similar structure to Fc receptors, whose immunomodulatory functions consist of both positively and negatively regulating B cell receptor (BCR) activation, FCRL1's role in BCR signaling has been examined. In vivo and in vitro studies have demonstrated FCRL1 negatively mediates ERK phosphorylation in a Grb2 dependent manner following BCR stimulation. The cytoplasmic tyrosines are essential for recruitment of phosphatases such as Src homology region 2 domain-containing phosphatase (SHIP1). This study seeks to identify the individual domain of SHIP1 involved in the recruitment to FCRL1 and the downstream modulation of ERK phosphorylation. Fusion proteins with the individual domains of SHIP1 have been generated with GFP and tyrosine mutated constructs of FCRL1 to examine both recruitment and downstream modulation of ERK phosphorylation following BCR activation, respectively.

BOARD 29

Characterization of the *cvn7* Conservon Operon in *Streptomyces coelicolor*

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Streptomyces coelicolor is a gram-positive bacterium that is commonly found in soil. This bacterium has proven its use and importance with its antibiotic production and other secondary metabolites. Recently, *S. coelicolor* research has focused on a conserved system of thirteen operons, called the conservon. Each operon consists of a gene encoding a probable Ras-like GTPase in addition to three or four other genes. Some conservon operons within *S. coelicolor* have proven to be interesting due to the effects of mutating such genes, causing *S. coelicolor* to produce altered pigmentation patterns of antibiotics when exposed to other species of actinomycetes. Within our lab, we have found the genes within the *cvn7* operon to be of particular interest, as not much literature has been published about this operon yet. We have conducted work on four of the five genes from this operon: *cvnB7* (SCO6795), *cvnC7* (SCO6796), *cvnD7* (SCO6797), and *cvnF7* (SCO6798). We obtained the transposon mutated versions of each gene within cosmid 1A2 of *Escherichia coli.* These strains of *E. coli* were later conjugated with wild-type *S. coelicolor* selecting for the apramycin resistance gene of the transposon. Apramycin-resistant colonies were picked and plated onto media containing either kanamycin or apramycin. We chose apramycin-resistant, kanamycin-sensitive candidates that indicated a double homologous recombination event had occurred resulting in the disruption of each gene of interest. We identified six candidates for SCO6795, five for SCO6796, nine for SCO6797, and five for SCO6798. These strains are being grown on plates that feature all candidates from each mutated gene, including wild type for phenotypic reference. By studying the effects of altering the genes within the *cvn7* operon, we could possibly discover how these alterations can benefit our understanding of microbial interactions, metabolic processes, and even antibiotic production.

BOARD 30

Citrobacter amalonaticus CJ25: Is it gut friendly?

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Gut microbiota affects human biology through their biochemical functions and have significant correlation with both health and disease status. Quaternary amines such as choline and carnitine are proatherogenically broken down in human gut. Choline is broken down to produce TMA by canonical CutCD and carnitine is broken down to produce TMA via an intermediate, gamma-butyrobetaine by the products of the *bbu* gene cluster. TMA travels to the liver and is oxidized to TMAO by FMO3 in the liver and increased TMAO concentration in bloodstream promotes the development of atherosclerosis. Interestingly, *Citrobacter amalonaticus* CJ25 (CJ25), an organism isolated and characterized in our lab does not encode *cutCD* genes as well as any COG5598 methyltransferase family members that are known to demethylate quaternary amines. The degradation of choline and carnitine has been confirmed to be a non-atherogenic because no production of TMA was detected in the spent medium via LC-MS. Since the genome does not have *CutCD* and no TMA was detected, we tried to examine the choline and carnitine metabolism in CJ25 using a combined metabolomic and proteomic approach. The study of metabolites when CJ25 was grown on choline showed the significant presence of glycine betaine (GB) in the mid-log phase compared to the glucose growth condition. Preliminary proteomic data suggest the presence of a putative alcohol dehydrogenase enzyme that shares 35% identity with a choline dehydrogenase enzyme. The discovery of this possibly non-atherogenic choline and carnitine degradation pathway supports the potential of CJ25 as a probiotic agent in human gut.

BOARD 31

Wsp-dependent fitness phenotypes of *Pseudomonas aeruginosa* Rugose Small Colony Variants

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Pseudomonas aeruginosa is a nosocomial, opportunistic pathogen implicated in several acute and chronic infections. During chronic infection, the persistence of *P. aeruginosa* is attributed to its ability to form biofilms. As *P. aeruginosa* adapts to the host environment, it acquires mutations that enhance its biofilm forming capabilities. This is reflected by the emergence of rugose small colony variants (RSCVs), hyper-aggregating mutants of *P. aeruginosa*, during chronic infection. RSCVs form robust biofilms and this phenotype is due to their elevated, intracellular concentrations of the secondary messenger cyclic di-GMP which promotes biofilm formation. Several genetic mutations can result in the RSCV phenotype, including mutations within the *wsp* operon which encodes a surface-sensing system that upon activation, produces cyclic di-GMP. Our work has revealed fitness differences between *wsp* mutants of different *P. aeruginosa* strain backgrounds in planktonic and biofilm conditions after 48 hours. Furthermore, mutations within the *wsp* operon were observed to be selected for in some but not all *P. aeruginosa* strain backgrounds during chronic infection. Therefore, we are interested in (1) identifying molecular determinants that mediate the fitness of *P. aeruginosa wsp* mutants and (2) investigating the influence of environmental factors on the fitness of these mutants. Our work will shed light on the mechanisms underlying the fitness advantage of *P. aeruginosa wsp* mutants.

BOARD 32

Inhibition of Biofilm Formation and Quorum Sensing by Bioactive Compounds

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Biofilm is a consortium of microorganisms that stick to each other while being attached to a non-living surface, which could cause serious health problems. The microbial cells secrete polymeric substances and become embedded within a slimy extracellular matrix. A biofilm can be indicative of an unsanitary environment in the food industry, often contaminating water filters and medical devices. The development of antibiotic resistance in such microbial communities mandates discovering new antimicrobial agents. This study evaluates the antibacterial activity of selected plants′ extracts against three biofilm-forming bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Pseudomonas putida.* Methanolic plant extracts were prepared as 5% (w/v) at room temperature for hibiscus, orange peel and red cactus peel, and the well-diffusion method was used for screening their antimicrobial activity. Wells were made into the agar before streaking a standardized 24hr bacterial culture. Each well had 100ul of the desired extract or methanol as a control. The plates were incubated overnight at 30°C and inhibition zones (IZ) were measured in millimeters. Results showed all tested extracts exhibited bacterial growth inhibition (IZ 10mm- 24mm), which was comparable to the tested standard antibiotics: Streptomycin, Penicillin, Ampicillin and Clindamycin. The highest inhibitory effect observed on *S. aureus* were by red cactus peel (22mm), followed by hibiscus (15.5mm) and orange peel (15mm). The hibiscus, orange peel, and red cactus peel showed a higher inhibition on *P. putida* (IZ 20mm, 17mm, 14.5mm, respectively) compared to their inhibition against *P. aeruginosa* (IZ of 14.5 mm, 15mm, 12mm, respectively). The antimicrobial inhibition was equal to or higher than that caused by the standard antibiotics. Work is in progress to determine the minimum inhibitory concentration for extracts, and to determine the inhibition of quorum sensing for these bacteria. Overall, the plants′ extracts have the potential to be used as eco-friendly antimicrobials for different applications.

BOARD 33

A Novel Adjuvant to Enhance NK Cell Response in HIV Preventive Vaccines

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Despite taking over 650,000 lives and infecting 1.5 million individuals annually, an efficacious HIV vaccine regimen remains elusive. The RV144 clinical trial remains the superior vaccine strategy, utilizing an ALVAC-HIV vector and alum adjuvanted AIDSVAX B/E gp120 protein, with 31.2% efficacy. This vaccine regimen demonstrated limited yet significant protection, serving as an intriguing avenue for further research. Prior rhesus macaque studies have identified NK cell and ILC subsets as correlates of protection against SIV infection. Usage of novel adjuvants in concomitance with this ALVAC-HIV vector may serve as an effective strategy in improving vaccine efficacy by stimulating the expression of protective correlates. *In vitro* studies demonstrate LL001's, a novel adjuvant, ability to elicit a strong mucosal innate immune response. This study investigates NK cell responses to the RV144 vaccine, plus LL001 adjuvant, in mice spleen, liver, blood, lung, and bone marrow. C57BL/6 mice were categorized into four treatment groups (n=6): PBS, ALVAC, ALVAC+LL001, and LL001 alone. Mice were sacrificed after two weeks of vaccination and immune cell frequency and functions were studied via high dimensional flow cytometry. Results demonstrated that the novel adjuvant LL001 altered

receptor expression and the functional capacity of NK cells. In groups treated with LL001, mature NK cell (KLRG1+) populations significantly increased in all tissues ($p<0.05$). Decreased exhausted NK cells (PD1+) were present in LL001-treated groups ($p<0.05$). These NK cells further enhanced the expression of CXCR5 and CCR2 receptors, suggesting lymph-node migration ($p<0.05$). Increased proinflammatory cytokines (IL6, TNFα, IFNγ, and IL1β) production were found in NK cell subsets in all tissues in LL001 vaccinated mice. Memory-like NK cell populations (Ly49H+) demonstrated similar increased functional capacity compared to total NK cell populations. These results suggest that LL001 may serve as an efficacious adjuvant, enhancing the NK cell responses of the RV144 HIV vaccine strategy *in vivo*.

BOARD 34

In vivo and in vitro analyses of BK polyomavirus diversity after stem cell transplantation

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BK polyomavirus (BKPyV) reactivation occurs in 57-87% of hematopoietic stem cell transplantation (HSCT) patients, resulting in hemorrhagic cystitis (HC) in up to 25% of pediatric HSCT recipients. Symptomatic HC results in prolonged hospitalization, increased hospital costs, and higher mortality rates. In other viruses, viral diversity plays a major role in pathogenesis, clinical manifestations, and outcomes; however, the extent to which BKPyV diversity impact viral pathogenesis and clinical outcomes is unknown. Thus, we examined the genomic diversity of BKPyV in a pediatric HSCT cohort and used these findings to develop in vitro experiments to access the consequences of the mutations identified.
Urine samples from a cohort of 147 pediatric allogeneic HSCT recipients at Cincinnati Children's Hospital Medical Center and Children's Hospital of Philadelphia at 1-month post-transplant were sent for quantitative BKPyV testing. The BKPyV from positive samples were amplified using rolling circle amplification, PCR, and next-generation sequencing (NGS). Phylogenetic analysis was performed to determine the BKPyV subtype. We observed higher median viral loads in subtype Ia compared to all other subtypes at multiple timepoints post-transplant. For subtype 1a samples, hypermutation analysis revealed 23 sites with APOBEC-induced mutations present, across all protein coding regions. Four of these sites – two within VP2, one within VP1 and one within TAg – were non-synonymous. Future in vitro work focuses on the production of virions with these identified mutations, subsequent infection and measurement of transcript and protein levels to access the consequences on replication.
This study is the largest BKPyV diversity study with HSCT patients and furthers the knowledge of the genomic variation exists within BKPyV. The NGS data generated showed nucleotides of interest will be studied for functional consequences, furthering the understanding of the role of BKPyV diversity in disease progression.

BOARD 35

Antimicrobial Activity of Plants' extracts on *Pseudomonas* species

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The increased bacterial resistance to antibiotics is a threat to public health and disease management. It is caused by the misuse of antibiotics to treat infections and use in animal feed. Thus, there is a need to discover new antibiotics. The use of plants to treat infections has been known since the Ancient Egyptians. This study aims to determine if the tested plants' extracts are effective inhibitory agents against two *Pseudomonas* species knowns for their biofilm formation*: P. aeruginosa and P. putida*. The methanolic extracts of three plants were

evaluated for antimicrobial activity using the well-diffusion method: mountain sage, pomegranate peel and avocado peel. The stability of the extracts was determined by measuring the pH over eight weeks. Agar plates were prepared, wells were made into agar, and bacteria were streaked on plates before placing extracts in wells (100ul), while the control was methanol. The plates were incubated at 30°C overnight, and the inhibition zone (IZ) was measured in millimeters. The screening was done in triplicate and the average was calculated. The pH of the methanolic extracts was almost the same over time indicating the stability of their bioactive compounds. The tested methnolic extracts exhibited antimicrobial activity against both *Pseudomonas* species. The inhibition of *P. aeruginosa* with mountain sage was the most effective (IZ 20mm), followed by pomegranate peel (17mm) and avocado peel (15mm). The inhibition of *Pseudomonas putida* was highest by pomegranate peel (22mm), followed by mountain sage and avocado peel (both 19mm). The results indicate that the methanolic extracts of the tested plants can be used as a source for bioactive compounds that exhibits antimicrobial activity against *Pseudomonas* species.

BOARD 36

Two tandem sRNAs act as Antitoxins in a putative Type 1 Toxin-Antitoxin system in *Shigella flexneri*

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Shigella is a pathogenic bacterium responsible for shigellosis, a severe diarrheal disease that claims the lives of immunocompromised individuals worldwide. To develop novel therapeutics against this disease, an understanding of the molecular mechanisms underlying the pathogens physiology is crucial. Small non-coding RNAs (sRNAs) have emerged as important regulators of bacterial physiology, including as components of toxin-antitoxin systems. In this study, we investigated the role of RyfA in *S. flexneri* physiology and virulence. RyfA, originally identified as an sRNA in *Escherichia coli* is conserved within the *Enterobacteriacea*e family, including *Shigella*. Whereas two copies of *ryfA* are present in *S. dysenteriae*, all other *Shigella* species contain only one copy of the gene. Additionally, we identified a putative open reading frame within the RyfA transcript, suggesting that it may be a dual-functioning gene, encoding a small protein in addition to its sRNA function. To study *ryfA in vitro*, we cloned the gene into an inducible plasmid and observed the effect on bacterial growth. Here, we report that RyfA production inhibits the growth of *S. flexneri*, and this inhibition is dependent on the contained open reading frame. *In-silico* analyses have revealed the presence of two divergently transcribed sRNAs, RyfB1 and RyfB2, which share nucleotide complementarity with RyfA, and thus are predicted to function as anti-toxins. Our data demonstrate that RyfB2 has a stronger antitoxin effect than RyfB1. This regulatory pattern suggests a novel form of a toxin-antitoxin system, in which the activity of a single toxin is inhibited to varying degrees by two sRNA antitoxins. Studies are ongoing to investigate the regulatory mechanism(s) of the antitoxin genes, as well as the downstream targets and mechanism of growth inhibition by the RyfA toxin. This study offers new insights into the regulatory mechanisms underlying *Shigella* physiology and may inform the development of new anti-Shigella therapeutics.

BOARD 37

Novel role of the histidine kinase in cell division

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Streptomyces coelicolor is a Gram-positive filamentous soil-dwelling bacterium. The genus *Streptomyces* is utilized to produce over two-thirds of the commercially available antibiotics, and its growth is similar to that of

fungi. *S. coelicolor* grows using a mycelium-like structure which produces aerial hyphae above the media surface for sporulation. During cell division of these aerial filaments, evenly-spaced crosswalls are developed using division genes, including *ftsZ* and *ftsQ*, which are essential for growth in common bacteria. If these genes are silenced or deleted in *Streptomyces*, they will retain their ability for growth. When *ftsZ* or *ftsQ* are deleted in *Streptomyces*, it causes a loss of septum formation in the aerial hyphae, and therefore a loss in spore formation. Research performed in this project has contributed to the discovery and characterization of three new *ftsQ*-null suppressor strains using visual phenotyping and bioinformatics. These strains were demonstrated to partially compensate for the loss of division in the *ftsQ*-null mutant. Whole genome sequencing confirmed that all three strains contained a mutation within the same gene, *sqnA* (suppressor of *ftsQ*-null). Bioinformatic databases confirmed that this gene encodes a histidine kinase and is located next to a gene encoding a response regulator. These two genes encode proteins that potentially function as a two-component regulatory system, which has been implicated to play a role in bacterial stress response. Extended bioinformatic analyses for homologues demonstrated significant similarities between a wide variety of bacteria. S*. coelicolor* deletion strains for these genes of interest are being constructed using the Lambda-REDIRECT recombinase system in both the wild type and *ftsQ*-null mutant background. These strains will be compared phenotypically on plates with different media types and phase-contrast microscopy. Novel information produced from this study will further elucidate the cell division process by identifying a new role in division for these genes of interest.

BOARD 38

Gut Microbiota of Female Bean Beetles *Callosobruchus Maculatus*

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Beetles are hundreds of species that feed on different matters including wood, organic matters and beans. *Callosobruchus Maculatus* feed on beans causing a great loss to this nutritious source. Evaluation of gut microbiota of beetles can be helpful to discover an eco-friendly method for their management. This study compares the gut microbiomes of female beetles that are fed on black-eye beans and chick pea beans. The beetles were crushed in 450 µL 0.9% NaCl, and 100ul was spread on each agar; basic nutrient agar as Luria Betani for bacteria, potato agar for fungi. The differential agars used were eosin methylene blue (EMB) and phenylethyl alcohol (PEA) agar to select Gram negative and Gram positive bacteria, respectively. Agar plates were incubated at 30^{o}C overnight. Purification of colonies was done by sub culturing, and bacteria were identified by Gram staining, biochemical testing and culturing on selective agars. Gram staining indicated the presence of cocci and bacilli bacteria. The catalase test indicated that the selected bacteria were catalase positive. The blood agar did not indicate the presence of hemolytic streptococci in the gut microbiomes of beetles. The McConkey agar indicated some bacilli are lactose fermenters. Colonies isolated from gut microbiomes of beetles fed on black-eye beans were stickier than colonies isolated from those fed on chickpeas especially those grown on PEA. This differs from another study in our lab that showed the gut microbiomes of males where sticky colonies were isolated from beetles fed on black-eye or chickpeas beans. More investigation is ongoing to determine the microbiota difference caused by the type of beans the beetles feed on. This work is important for agricultural applications specially for pests′ managements during crops' storage and transportation.

BOARD 39

Analysis of microbial activity associated with cable bacteria activity using a metatranscriptomic approach

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Cable bacteria (*Ca*. *Electrothrix spp and Ca. Electronema spp*) are long filamentous bacteria observed in sediments globally and notable for performing electrogenic sulfur oxidation. Spanning the distance between the oxic and suboxic zone, cable bacteria oxidize sulfide in sediments by translocating electrons along their longitudinal axis. As a result of their prolific growth and unusual metabolic activity, cable bacteria activity affects the sediment-associated microbial community, through impacts on sediment geochemistry or by direct interactions. Despite their global distribution and high local abundances, how cable bacteria activity impacts the sediment-associated microbial community is not well known. In this study, we selectively arrested cable bacteria activity to investigate metabolisms associated with cable bacteria activity using a metatranscriptomics approach. Estuarine sediments from Chesapeake Bay, USA were enriched for cable bacteria in laboratory incubations. Cable bacteria activity was arrested in a subset of cores by cutting horizontally below the oxic zone. Sediment at multiple depths was harvested after 18 hours for metatranscriptomic analysis. After accounting for changes in cable bacteria transcription, additional changes in gene expression are being studied using negative binomial distribution analysis of *de novo* assembled reads. Additionally, microbial taxa expressing differentially expressed genes are being investigated using a phylogenetic approach to determine how the groups performing these processes change with cable bacteria activity. We find that differences in gene expression were influenced by cable bacteria activity and depth. Preliminary results indicate differential transcription in genes associated with sulfur oxidation and chemoautotrophy, dissimilatory sulfate reduction, and denitrification between sediments with and without cable bacteria activity arrested. The study aims to reveal the role of cable bacteria activity on sediment microbes and their metabolism.

BOARD 40

Deletion of Potential Chromosome Segregation Gene in *Streptomyces coelicolor*

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Streptomyces coelicolor is a gram-positive bacterium located in the soil, commonly used to study antibiotic production and as a model for multicellular prokaryotic development. *S. coelicolor* has a complex life cycle, forming filaments with multi-genomic compartments leading to single linear chromosomes within mature sporular compartments. In this bacterium, chromosomes segregate through the ParABS system. ParA and ParB proteins organize the subcellular space to play a role in chromosome segregation. While ParA and ParB play a major role in chromosome segregation, mutations of each gene only contributed to small phenotypic growth changes. Using transposon mutagenesis, a novel mutant was previously identified in our lab with a chromosome segregation defect using propidium iodide staining with fluorescence microscopy. The mutated gene resulted in 22% anucleated spores compared to the less than 1% anucleated spores found in wild type. Extending this study into *S. venezuelae*, propidium iodide staining with fluorescence microscopy was used on a single deletion strain for the novel gene ortholog and a double deletion mutant of the novel gene ortholog and adjacent gene positioned in a potential operon. Preliminary results showed the *S. venezuelae* single mutant and the wild type had 0.78% anucleated spores, whereas the double mutant had 1.18% of anucleated spores. The subtle chromosome segregation phenotype may indicate a species difference pertaining to this gene's role. Currently, the novel gene is in the process of being deleted in *S. coelicolor* by using REDIRECT. The deletion mutant will be compared to the wild-type and transposon insertion strains. This study will allow us to determine if the identified gene is the cause of the *S. coelicolor* chromosome defect or to proceed with whole genome. In addition, the deletion of the novel gene will lead to further understanding of the chromosome segregation process in filamentous bacteria.

BOARD 41

Gut Microbiomes of *Callosobruchus Maculatus* Fed either on Black-Eye Beans or Chickpeas Beans

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A microbiome is the collection of all microbes, such as bacteria, fungi, viruses, and their genes that naturally live in or on the bodies. This is a comparative study to investigate the gut microbiomes of male bean beetles that are fed on two different beans: black-eye beans and chick peas beans. The living male beetles were identified and sterilized before crushing them in a saline solution. 100ul of the microbiome suspension was streaked on Luria bertani (LB) and differential agar such as Eosin methylene blue (EMB), phenylethyl alcohol agar(PEA). All plates were labeled and placed in the incubator upside for a minimum of 24 hours at 37^{o}C. Colonies were picked up from different plates and Gram staining was done to confirm the type of cells. Purification of colonies was done using biochemical tests and additional selective agar as blood agar, McConkey etc. Results indicated the presence of cocci with different arrangements, and bacilli in the gut of beetles fed on both types of beans. There is no apparent difference in the gut microbiomes of beetles fed on different beans other than the presence of some sticky colonies for Gram positive bacteria isolated only from microbiomes of beetles fed on both types of beans. Further investigation is ongoing to identify the bacterial species. This study is important to determine control methods for pests negatively impacting the agriculture and crops production.

BOARD 42

Characterization of genes involved in extracellular electron uptake in *Shewanella oneidensis* MR-1

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The process of extracellular electron transfer (EET) from the cell internal metabolic machinery to extracellular surfaces, which allows *Shewanella* species to reduce redox active surfaces such as minerals or electrodes, has been well characterized. However, *S. oneidensis* (MR-1) is capable of bi-directional EET. Though much of the characterized EET pathway, including the canonical multiheme cytochromes, have been shown to be reversible (1), how electrons from the reverse pathway interact with the cells' other respiratory pathways (I.e., aerobic electron transport chain) remains unclear. To identify genes involved in extracellular electron uptake, an MR-1 transposon library knocking out all non-essential genes was screened for potential electron uptake deficiencies (2). Several candidates identified in this screen were further investigated for an extracellular electron uptake phenotype when using an electrode as an electron donor (2). Four genes have been identified as potentially novel components of extracellular electron uptake. However, the role of these novel genes in extracellular electron uptake is still unclear. To further investigate the role these genes play in extracellular uptake, we will insert these genes into arabinose-driven overexpression vectors in MR-1 and quantify the effects of overexpressing these genes on electron uptake (i.e., the production of cathodic current). We hypothesize that two of these genes (SO0400 and SO3662) are directly involved in electron transfer in the periplasm, connecting the anaerobic EET pathway with aerobic respiration. As such, we expect over expression of these genes will increase biological electron uptake in MR-1. This work will help further the understanding of extracellular electron uptake in MR-1 and help us understand how microbes exchange electrons with their surroundings.

BOARD 43

The Synthetic Opioid Fentanyl Increases HIV-1 Infection In Vitro

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Aim: The opioid epidemic has had a large burden on global health. Opioid use is associated with increased risk of infectious diseases, including HIV, through the use of intravenous drugs. Several *in vitro* studies have demonstrated the immunosuppressive effects of opioids on multiple immune cell types. However, how synthetic opioid fentanyl impact HIV infection / replication is not well understood. In this study, we investigated the effect of synthetic opioid fentanyl on HIV replication and chemokine co-receptors expression *in vitro*.
Methods: HIV susceptible and infected lymphocyte cells were incubated with fentanyl at different concentrations. Levels of the chemokine co-receptors and HIV p24 antigen were quantified by ELISA. HIV proviral DNA was quantified using SYBR RT-PCR. RNAseq was performed to characterize cellular gene regulation. To gain further insight into the action of fentanyl on HIV-infected primary cell types, we isolated peripheral blood mononuclear cells, purified and activated CD4+ T cells and the activated cells were infected with HIV_{NL4-3} and treated with fentanyl.
Results: In HIV-susceptible and infected cell lines, fentanyl increased expression of both chemokine receptors in a dose-dependent manner. Fentanyl also enhanced viral expression in lymphocyte cell lines and TZM-bl cells exposed to HIV. Multiple genes associated with apoptosis, antiviral / interferon response, chemokine signaling, and NFκB signaling were differentially regulated. Further evaluation of primary CD4+ T cells treated with fentanyl revealed increased HIVp24 expression and proviral DNA relative to untreated cells.
Conclusion: Fentanyl affects the expression of chemokine co-receptors and HIV replication. Elevated HIV levels suggest that opioid usage may hasten disease progression and the likelihood of transmission.

BOARD 44

Overcoming sulfur-mediated inhibition of ethylene biosynthesis in *Rhodospirillum rubrum* through altered transcriptional regulator expression

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Rhodospirillum rubrum produces ethylene anaerobically through a novel methionine biosynthetic pathway that uses volatile organic sulfur compounds as substrates. However, exogeneous preferred sulfur sources such as sulfate at concentrations of ≥200uM have been found to inhibit the pathway and ethylene production. Biologically produced ethylene is an attractive replacement of petroleum-based ethylene for manufacturing plastics to reduce the environmental impact of using fossil-fuels. In this methionine biosynthetic pathway that produces ethylene, at least 6 key enzymes are under transcriptional regulation of SalR, a member of the LysR-type transcriptional regulator family. When SalR is deleted from the chromosome, the methionine biosynthetic pathway is no longer expressed, and no ethylene is made. When SalR is reintroduced by constitutive expression from a plasmid (pMTAP), the methionine biosynthetic pathway is restored in the complemented strain and ethylene is continuously produced, even in presence of 1000 µM of exogeneous sulfur sources like sulfate and methionine. This observation shows that SalR is an activator of the novel methionine biosynthetic pathway and controlled expression of SalR can help maintain uninterrupted ethylene production irrespective of

sulfate concentrations for renewable production of this commodity chemical. Interestingly, exogenous cysteine, when present at ≥500uM concentration was found to still inhibit the ethylene production in the complemented strain as observed when SalR is natively expressed form the chromosome. This differential ethylene production in the presence of cysteine versus sulfate and methionine as exogenous sulfur source suggests that cysteine or a metabolite directly produced therefrom could be a negative effector molecule of SalR for regulation of pathway expression**.** The predicted tertiary structure of SalR shows presence of two cysteine residues within the conserved LysR-family regulatory domain. We hypothesize these residues are involved in sensing cysteine or similar effector molecules to regulate SalR activity and thus expression of the methionine biosynthetic pathway for ethylene biosynthesis.

BOARD 45

Utilizing the esophageal microbiome to ameliorate eosinophilic esophagitis

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Eosinophilic esophagitis (EoE) is a chronic, food antigen-driven disease associated with epithelial barrier impairment, interleukin 13 (IL-13)-induced response, and loss of the protease inhibitor, serine peptidase inhibitor kazal type 7 (SPINK7). *SPINK7* loss leads to uncontrolled proteolytic activity in the esophagus, which drives epithelial barrier dysfunction and inflammation. Oral treatment of esophageal diseases is challenging due to a short transit time, salivation, and nonadherent mucus in the esophagus. There is a need to generate a direct delivery platform for the esophagus with increased therapeutic bioavailability and reduced off-target effects. The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that senses environmental cues to enact ligand-specific downstream transcriptional programs. Activating AHR partially reverses the expression of key dysregulated genes in EoE, including induction of *SPINK7 expression* and attenuation of inflammatory IL-13–induced responses. Thus, AHR is a potential therapeutic target for EoE. AHR is activated by many ligands, including indole-3-lactic acid and butyrate, which are produced by several bacterial species. The esophagus has a distinct microbiome that can modulate the epithelial barrier and immune response and offers a potential platform for directly delivering therapeutics to the esophagus. Therefore, we hypothesize that: 1) colonizing the esophagus with AHR-ligand-producing bacteria (AHRB) will increase the availability of AHR ligands in the esophagus, which will locally activate AHR; and 2) AHRB will stimulate signaling pathways that will subsequently induce expression of protective epithelial genes, such as *SPINK7*, in an AHR-dependent manner. We found that AHR ligands upregulate AHR-specific genes in mouse esophagi *ex vivo.* Additionally, we observed that AHRB co-cultured with human esophageal epithelial cells stimulate AHR-specific genes, including *SPINK7*. Therefore, we suggest that the AHR pathway offers a novel therapeutic target for EoE and that microbiome colonization offers a novel direct delivery method for EoE and other esophageal diseases.

BOARD 46

Investigating Mechanisms of Extracellular Electron Uptake in the Marine Heterotroph, *Thalassospira sp.* SN3W

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Extracellular electron transfer (EET) is the process utilized by some microorganisms to transfer electrons across cell membrane(s) to/from solid-phase materials, such as minerals or electrodes. Much of our knowledge of this process has been gleaned from model iron reducing bacteria, such as *Shewanella* and *Geobacter*, both of which can utilize iron oxides and anodes as terminal electron acceptors for respiration. Although the mechanisms of electron deposition have been relatively well-characterized from studies in these and other metal-reducing microorganisms, the process of electron uptake – whereby some microorganisms obtain electrons by oxidizing reduced insoluble substrates – remains poorly understood. To better elucidate the

mechanisms and ecological implications of EET in oxidative processes, we present a genomic and bioelectrochemical investigation of *Thalassospira sp.* SN3W, an bacterium previously isolated from marine sediment cathode enrichments. Genomic and physiological investigations into the metabolic capacity of this organism suggest that SN3W is an obligate heterotroph and lacks the capacity for lithotrophic and autotrophic growth. Despite this finding, SN3W cells are capable of coupling cathodic electron uptake with aerobic and anaerobic respiration (reduction of oxygen or nitrate). Media exchange experiments and electrochemical analyses of spent media demonstrate contact-dependent extracellular electron uptake as one mechanism for cathode oxidation employed by SN3W. Given that the genome of SN3W lacks genes canonically involved in the EET pathways of several model organisms, this indicates that this organism may employ a novel mechanism for EET. Continued biochemical and genetic investigations will aid in elucidating the molecular mechanism of oxidative EET in SN3W and will further our understanding of the ecology of EET-capable heterotrophic bacteria in marine sediments.

BOARD 47

Dynamics of Natural Killer cells in perinatally HIV-acquired adolescents in Uganda: a possible role in non-AIDS-related co-morbidities

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Owing to anti-retroviral therapy (ART)-mediated viral suppression, the average life span of HIV-infected individuals has improved. However, there is increasing evidence of non-AIDS-related co-morbidities among HIV-infected individuals compared to uninfected individuals. This may result from persistent immune activation due to virus persistence, gut microbial translocation, co-pathogens, or ART. Natural killer (NK) cells play a major role in immune activation against viral infections. More recently these have been found to possess antigen specificity and memory features. In this cross-sectional study, using high dimensional spectral flow cytometry, plasma biomarker profiling, and transcriptomics, we compared immune signatures in cryopreserved peripheral blood mononuclear cells and plasma from adolescents with perinatally acquired HIV (PHIV) (n=21) and unexposed adolescents (n=22) in Uganda. We found significantly elevated activation (CD69, HLA-DR, NKp44) markers in cytokine-producing ($CD56^{dim}CD16^{-}$) and elevated maturation (CD57) markers in cytotoxic ($CD56^{dim}CD16^{dim}$) NK cells. CXCR5 chemokine receptor, which mediates NK cell migration to lymph nodes, was elevated in the $CD56^{dim}CD16^{dim}$ subset. Incidentally, both follicular helper T cells and activated B cells were significantly reduced in PHIVs. CXCR3, which mediates NK cell migration to vascular endothelium was significantly elevated in the $CD56^{dim}CD16^{dim}/CD57^{+}$ NK subset. Simultaneously oxidized LDL (which is taken up by vessel wall macrophages in atherosclerosis) levels were significantly lower in the plasma compartment among PHIVs. Further, a negative correlation was found between $CD56^{dim}CD16^{dim}$/CD57+ NK subset and plasma oxidized LDL levels among PHIVs ($p<0.05$ for all). Bulk-RNA sequencing data showed activation of multiple innate immune pathways in the PHIV group. Collectively, our data demonstrate persistent activation, maturation and alterations in tissue homing potential of NK subsets among PHIVs. These modifications may increase the risk of atherosclerosis-mediated cardiovascular diseases, blunt lymph node-associated responses to infection and vaccines and influence other inflammatory disorders. We are currently performing mechanistic and longitudinal studies to confirm these findings.

BOARD 48

The role of Natural Killer B cells (NKB) during SARS-CoV-2 infection

Will Mulhern*[1], Manuja Gunasena1[,2], Yasasvi Wijewantha[1], Dhanuja Kasturiratna[3], Joseph Bednas[2], Thorsten Demberg[4], Nicholas Funderburg[2] and Namal P.M Liyanage[1,2]
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Since December 2019, Covid-19 infection caused by SARS-CoV-2 has spread globally. The resultant pandemic has affected most countries, and despite the influx of research on the rapidly spreading disease, the pathogenesis of Covid-19 is still relatively unknown. Some virological, epidemiological, clinical, and management outcome features of patients with COVID-19 have been defined, but the immune system's response remains incompletely understood. Natural killer B (NKB) cells are a novel immune cell subset recently identified in non-human primates (NHP), humans, and mice. They have unique features differentiating themselves from T and B cells. They produce IL-18 and IL-12 at an early phase of infections and may play a critical role in the early stage of innate immune responses. However, the role of NKB cells during COVID-19 is currently unknown. In this study, we investigate phenotypic and functional changes of peripheral blood NKB cells in COVID-19 patients with moderate (n=27), severe (ICU-admitted) (n=44), and patients who had recovered (n=57) compared to healthy individuals (n=17) using high dimensional flow cytometry.We found significantly elevated levels of activation (CD69+) markers and increased expression of CXCR2 in NK cells of severe COVID-19 patients ($p<0.05$). We also found a significant decrease of CXCR5+ NKB cells ($p<0.05$) in moderate and severe COVID-19 patients, signifying migration of CXCR5+ NKB cells to the lymph nodes in moderate to severe COVID-19 infection. These activation and migratory changes in NKB cells were observed longitudinally. Our data suggest that NKB cells show characteristics of both NK and B cells. Further studies are needed to identify the exact role of NKB during COVID-19 infection.

Abstracts of OBASM Podium Presentations

Saturday, April 15
11:15 am – 1:00 pm

11:15 – 11:30 am

Establishing a robust and stable light-driven microbial consortium for the synthesis of high-value compounds

Khondokar Nowshin Islam*[1], Shrameeta Shinde[1], Abhishek Sen[2], J Andrew Jones[3] and Xin Wang[1]
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The direct conversion of CO_2 to high-value compounds by photosynthetic microbes, particularly cyanobacteria, has gained much attention due to their rapid cell growth, minimal nutritional requirements, and genetically modifiable nature. Although numerous engineering strategies have been utilized to manufacture a wide range of target molecules in cyanobacteria, the titers attained are magnitudes lower than those of their heterotrophic counterparts. Our recent study indicated that high titers of chemical production in phototrophs is constrained by thermodynamics (substrate availability) as well as absence of a strong sink to overcome this thermodynamics limitation. As a solution, we propose the employment of co-culture engineering to deliver a high thermodynamic driving force for CO_2 to chemical conversion. There have been multiple proof-of-concept studies indicating the success of modular photo-heterotroph consortia-driven bioproduction of chemicals. To date, the chemicals produced from various consortia have been restricted to high-flux metabolites such as acetyl-CoA derivatives namely, polyhydroxybutyrate (PHB) and 3-hydroxypropionic acid (3-HP). In this study, we designed and explored the applicability of a phototroph-heterotroph consortium to produce low-flux natural products like terpenes. Our hypothesis is that sugar production in cyanobacteria integrates the high-flux central metabolism to form a strong carbon sink, leading to increased photosynthesis and carbon fixation, and that the high carbon flux in cyanobacteria can be inherited by the terpene producer *E. coli* to achieve high limonene titer in co-culture fermentation. Furthermore, we have employed promoter engineering to control the sucrose uptake ability of the heterotroph to create a robust and stable synthetic consortium for efficient chemical production. Future efforts in stabilizing the consortium will provide important insight to apply coculture engineering to produce various secondary metabolites from carbon dioxide.

11:30 – 11:45 am

BK Polyomavirus Evolution in Hematopoietic Stem Cell Transplant Patients

Grace Zhang[1]*, Elizabeth A. Odegard[2], Anthony Sabulski[3,4], Sonata, Jodele[3,4], Assem Ziady[3,4], Alix E. Seif[5, 6], Stella M. Davies[3,4], Benjamin L. Laskin[5,7], and Jason T. Blackard[2]

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Introduction: BK polyomavirus (BKPyV) is a ubiquitous, circular DNA virus that typically establishes asymptomatic infection in immunocompetent individuals. However, in hematopoietic stem cell transplantation

(HSCT) patients, BKPyV replicates unchecked and can lead to BKPyV-associated hemorrhagic cystitis—increasing patient morbidity and mortality. We hypothesized that intra-patient viral diversity may occur after HSCT.
Methods: From a cohort of 193 patients who received allogeneic HSCT at Cincinnati Children's Hospital Medical Center or the Children's Hospital of Philadelphia, patients with positive viruria at months 1 and 12 post-HSCT were included. BKPyV DNA full-length or VP1 structural gene partial genomes were amplified and evaluated by next generation sequencing (NGS). NGS reads were aligned to the Dunlop BKPyV reference, generating a consensus sequence for each patient/timepoint. Study sequences were aligned with references to create a phylogenetic tree to determine genomic diversity among individuals and between time points from the same individual.
Results: 27 participants with detectable BKPyV viruria at 1 and 12 months post-HSCT were enrolled. Eleven samples could not be sequenced. 12-month and 1-month post-HSCT sequenced samples were compared to detect viral evolution. Of 16 evaluable subjects, 8 (50%) paired samples had 100% genome similarity, 5 (31%) pairs had 1 mutation difference, 1 (6%) pair had 19 differences, and 2 (13%) individuals had different BKPyV subtypes.
Conclusions: While nearly half our patients had at least one nucleotide mutation, two paired samples showed distinct BKPyV subtypes suggesting re-infection of the host. BKPyV re-infection may be more common than expected; therefore, BKPyV genotyping at a single time point may miss new infections. As BKPyV polymorphisms may impact virus function, understanding BKPyV pathogenesis and diversity may yield clinically significant outcomes in pre-symptomatic diagnosis of hemorrhagic cystitis and improved understanding of viral replication, disease, and treatment.

11:45 – 12:00 pm

Characterization of the Role of SarA and AgrA on the Temperature Dependent Production of the Metalloprotease Aureolysin

Raeven A. Bastock*[1,2,3], Paul Briaud[1], Ryan Steere[1], Ronan K. Carroll[1,2,3], and Erin R. Murphy[2,3,4,5]
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Staphylococcus aureus is a Gram-positive opportunistic pathogen that chronically colonizes the anterior nares of ~30% of the global population. Colonization is often asymptomatic, however, it is associated with recurrent infections and increased risk of surgical site infections. These risks are attributed to the ability of the bacterium to transition from the nares to the deeper tissues of the body and blood stream. Transition of the bacterium can result in potentially fatal infections such as endocarditis, toxic-shock syndrome, and septicemia. Previous work in our lab investigated the role of the temperature change experienced by the bacterium during the aforementioned transition. A global screen of the *S. aureus* transcriptome as well as the secreted and cytoplasmic proteome, was performed on WT cultures incubated at 34˚C, representative of the nares, 37˚C, the core body temperature, and 40˚C, representative of pyrexia. These studies revealed that the production of the metalloprotease aureolysin was inversely correlated to temperature (increased production at the lower temperature and decreased production at the higher temperature). In the current study we investigate the factors mediating the temperature dependent expression of aureolysin. We identify influence of temperature on the *aur* transcript stability using an RNA stability assay. We also explore the role of two major regulatory proteins, SarA and AgrA, in the temperature dependent expression of aureolysin. In the absence of SarA and AgrA, the impact of temperature on *aur* promoter activity was examined using promoter fusion β-galactosidase assays. Additionally, temperature dependent production of the aureolysin protein was investigated in the SarA and AgrA mutant backgrounds through western blotting and secreted proteomics. Using casein agar plates, we also examined the temperature dependent caseinase activity of aureolysin in the SarA and AgrA mutant backgrounds. The current study shows the temperature dependent production of aureolysin is regulated by the transcriptional regulators SarA and AgrA.

12:00 – 12:15 pm - Break

12:15 – 12:30 pm

Oxidative stress alters the heterotrophic community structure of ammonia-oxidizing enrichment cultures

Madisen Kimbrel*, Madelynn D. Spencer, Annette Bollmann
Miami University

Ammonia-oxidizing bacteria (AOB) are important contributors to the global nitrogen cycle, carrying out the first step of nitrification. They are found in a range of environments, including freshwater lakes and wastewater treatment plants, where they live in communities with heterotrophic microorganisms. Bacteria living in aquatic environments are frequently exposed to oxidative stress, from byproducts of their natural metabolism or photochemical processes like photosynthesis. Oxidative stress is harmful to bacteria, causing damage to DNA, lipids, and proteins in the cell. Some bacteria possess defense mechanisms, such as superoxide dismutase, catalase, and peroxidase, to protect themselves against oxidative stress. A previous proteomics study showed that the presence of heterotrophic bacteria reduces the effects of oxidative stress on AOB (Sedlacek et al., 2016). Here we investigated the effects of hydrogen peroxide (H_2O_2) exposure on the heterotrophic community of an AOB enrichment culture. The culture was originally enriched from Acton Lake, a freshwater eutrophic lake in Ohio. The culture received varying exposure to different concentrations of H_2O_2 for up to six growth cycles. Growth and activity of AOB was monitored by measuring nitrite production and the bacterial community composition was determined by 16S rRNA gene amplicon sequencing. Exposure to H_2O_2 impacted growth and altered the community structure of the heterotrophic bacteria. The most drastic changes in community structure were observed when the enrichment was incubated at high H_2O_2 concentrations. The relative abundance of multiple taxa was significantly different at high H_2O_2 compared to the control and the incubations at low H_2O_2. Intermittent incubation with H_2O_2 resulted in different bacterial communities compared to control and constant exposure. Communities with intermittent or constant exposure to high H_2O_2 concentrations displayed low resistance and resilience. This study provides insight into heterotrophic members that might provide support to AOB under oxidative stresses in their native environment.

12:30 – 12:45 pm

Innate Immune dysregulation may lead to atherogenesis in severe Covid-19 patients

Manuja Gunasena[1,2], Yasasvi Wijewantha[1], Will Mulhern[1], Dhanuja Kasturiratna[3], Joseph Bednash[2], Thorsten Demberg[4], Nicholas Funderburg[2] and Namal P.M Liyanage[1,2]
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COVID-19 infection, caused by SARS-CoV-2, has led to a global health emergency. Patients exhibit various clinical manifestations, ranging from asymptomatic to severe disease and death. Disease severity may be related to a dysregulated immune response and pre-existing health conditions. Clinical data shows that COVID-19 may promote the development of cardiovascular disorders (CVDs). However, a lack of mechanistic and systematic studies on the association of Covid-19 and CVDs hinder early risk identification and therapeutic interventions. In this study, we investigated selected biomarkers of CVD risk in COVID-19 patients at different disease states. Using high-dimensional flow cytometry and plasma biomarker assays, we investigated changes in immune subsets in whole blood and plasma biomarkers of CVDs in healthy (n=17), severe Covid-19 (n=28), and recovered individuals (n=30). Neutrophils and Intermediate monocytes (ITM) were significantly higher in severe Covid-19 patients ($p<0.05$). Interestingly, HLA-DR expressing neutrophils ($p<0.05$) were elevated even

after recovery. Among those with severe Covid-19, ITMs expressed high levels of CXCR5 ($p<0.05$), which may react with activated platelets to increase the risk of atherosclerosis. In severe patients, an increased number of activated (CD69+) NK cells were observed in three of the five NK cell subsets defined using CD56 and CD16 expression. Lower levels of CXCR3 expressing NK cells ($p<0.05$) were observed in severe disease, suggesting migration of these cells to infected sites. With regard to cardiometabolic markers, CRP (C-Reactive Protein), MCP-1 (Monocyte chemotactic protein-1) and, FABP4 (Fatty acid-binding protein 4) were significantly elevated ($p<0.05$) in severe Covid-19 patients suggesting an increased risk of atherogenesis in these patients. Interestingly, decreased OxLDL ($p<0.05$) levels were noted even in recovered individuals suggesting increased uptake of OxLDL from macrophages and increased risk of foam cell formation signaling long-term risk for atherogenesis. These changes in immune responses may contribute to an increased risk of CVDs in Covid-19 patients.

12:45 – 1:00 pm

Who goes there? Determining what and how microbes inhabit and influence a hyperalkaline spring

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Despite the prevalence of both natural and anthropogenic high pH environments, our understanding of the microbial ecology of these systems is far less investigated than that of their low pH counterparts. As such, our understanding of the microorganisms that survive and flourish under highly alkaline conditions remains limited. I will be presenting my research on the microbial ecology of Ney Springs, a small serpentinizing spring in northern California notable for its high pH (>12), high sulfide (700-1000 mg/L), ammonia (70-120 mg/), and gaseous methane (83%) composition. Using seasonal geochemical sampling and 16S rRNA gene surveys coupled with metagenomics, we determined some of the adaptations and metabolic capabilities Ney Springs' core microbial community members employ to persist in these polyextreme conditions year-round. We also investigated the relationships of core community members with key geochemical constituents, which allowed for further insight into sulfur and nitrogen cycling in this system. This data suggests that core community sulfur-oxidizing taxa such as *Halomonas*, *Thiomicrospira,* and members of the *Rhodobacteraceae* influence the abundance of sulfur-oxidation intermediates like thiosulfate and tetrathionate measured in the system. We also discovered that the most abundant members in the system, *Tindallia* spp. belonging to Clostridia, may be the source of excessive ammonia that has been unexplained in this spring for over a century.

Description of Awards for Presentations

N. Paul Hudson Award for Research Excellence

N. Paul Hudson, MD, was chairman of the Microbiology Department at Ohio State University from 1935 – 1950. Dr. Hudson was recruited from the University of Chicago Medical School where he already had established a research/teaching reputation and was told to bring several colleagues with him to serve as faculty members in the department. In 1950 he was appointed Dean of the Graduate School at Ohio State University and served until his retirement in 1957. During the 1930's he was responsible for the successful development of a vaccine against Yellow Fever, which was necessary for the war effort in the tropical countries during the 1940's. Dr. Hudson died at the age of 95 in 1993 in Florida after retiring to his second home in Sarasota in 1970.

Donald C. Cox Award for Research Excellence

Donald C. Cox, PhD, was professor and chair of the Department of Microbiology at Miami University from 1978-1989. After he earned his PhD at University of Michigan in 1965, he joined the microbiology faculty at University of Oklahoma. He later moved to Miami, where he became well known as a charismatic and highly effective teacher, researcher and leader. Dr. Cox received Miami's Distinguished Educator Award, and fostered the growth and development of the Department of Microbiology. Throughout his research career, he focused on the biochemistry and molecular biology of replication of human viruses, and ultimately studied utilization of reovirus in cancer therapy. Dr. Cox was a strong advocate for attracting young people into scientific careers and mentored many students who have gone on to highly significant research careers.

J. Robie Vestal Award for Research Excellence

J. Robie Vestal, PhD, was professor of both biological sciences and environmental health at the University of Cincinnati. He earned his MS in Microbiology at Miami University and his PhD in Microbiology at North Carolina State University. His postdoctoral research at Syracuse University involved the biochemistry of *Thiobacillus ferrooxidans*. Dr. Vestal's research interests focused on how microbial communities function in nature. He studied microbial communities in Arctic lakes and in soils contaminated with hazardous waste, cryptoendolithic (hidden within rock) communities in Antarctica, mangrove-degrading communities in the Bahamas, and decomposer communities in municipal solid waste compost. He also investigated microbial survival under simulated Martian conditions. Dr. Vestal served on many local and national committees and chaired the Divisional Advisory Committee of the National Science Foundation's Division of Polar Programs.

Ohio Branch ASM Award for Research Excellence

This award traditionally recognizes excellence in graduate research and presentation at the annual Ohio Branch ASM meeting.

Allan A. Ichida Undergraduate Research Award

Allan Ai Ichida, PhD, earned his BA from Ohio Wesleyan University in 1953 and went on to study botany, mycology, and bacteriology at the University of Tennessee where he earned his MS in 1955 and the University of Wisconsin in Madison where he earned his PhD in 1960. Dr. Ichida returned to Ohio Wesleyan in 1961 as a faculty member in the Department of Botany and Microbiology where he taught botany and mycology until he retired in 1995. During his career, Dr. Ichida served as president and advisor of the Ohio Branch of the American Society for Microbiology and on the Olentangy Scenic River Commission where his water quality research helped to secure the river's "Scenic River" status. Dr. Ichida also conducted research in the OWU Bohannan and Kraus nature preserves and mentored numerous undergraduates who went on to become research scientists.

The Ohio Branch ASM Award for Pre-college Research Excellence

Established in 2017, this award recognizes excellence in pre-college research and presentation at the annual Ohio Branch ASM meeting.

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