Annual Meeting
Ohio Branch of the American Society for Microbiology

Ohio Branch American Society for Microbiology

Ohio Wesleyan University

Delaware, Ohio
April 5 – 6, 2024

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 2024:

OBASM Executive Committee

Christine Weingart
Chet Cooper
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Stephanie Miller
Paul Hyman

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ASM Distinguished Lecturer Program
The American Society for Microbiology

Invited Speakers

Andrea Suria
Jason Boock
Noelia Lander
Irene Newton
Brian Price
Darryl Wesener
Jennifer Bennett
Wun-Ju Shieh

Program Schedule for OBASM 2024
Schimmel-Conrades Science Center (SCSC)

Friday, April 5

6:00 – 7:00 pm **Registration**

7:00 – 7:10 pm **Introduction –** SCSC 161
D.J. Ferguson, President of OBASM

7:15 – 8:15 **OBASM Keynote Lecture** – SCSC 163

"Friends with benefits: protective microbial symbioses in the honey bee"

Irene Newton
Microbiology Section Associate Chair
Indiana University Bloomington

Saturday, April 6

8:00 am **Registration and Poster Set-up –** SCSC Atrium, lower level

8:30 am **Introduction and Welcome** – SCSC Room 163

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

Concurrent Session I, SCSC Room 163
Clinical and Medical Microbiology
Moderator: Shaohua Wang

8:45 – 9:15 **Darryl Wesener** – SCSC Room 163
Ohio State University

"Microbial glycobiology in the human gut microbiota**"**

9:15 – 9:45 **Noelia Lander** – SCSC Room 163
University of Cincinnati

"Compartmentalized cAMP signals in American trypanosomes**"**

9:45 – 10:15 **Brian Price** – SCSC Room 163
Tovero Bio, Columbus, OH

“Tovero Bio: Developing Vectorized RNAi Medicines for Neurological Disease**”**

<u>Concurrent Session II, SCSC Room 161</u>
Applied and Environmental Microbiology
Moderator: Paul Hyman

8:45 – 9:15 **Jason Boock** – SCSC Room 161
Miami University

"Characterization and engineering of *Priestia megaterium* for bioprocessing under supercritical carbon dioxide"

9:15 – 9:45 **Jennifer Bennett** – SCSC Room 161
Otterbein University

"Signals in the Soil: Studies of *Streptomyces* Second Messenger Signaling"

9:45 – 10:15 **Andrea Suria** – SCSC Room 161
Ohio Wesleyan University

"Genetics of bacterial competition in Hawaiian bobtail squid reproductive symbionts**”**

10:15 – 10:30 am **Break**

10:30 – 12:15 am **Podium presentations and judging** – SCSC Room 161
Coordinator – Chet Cooper

<u>10:30 – 10:45 am</u>
Increased KLRG1 expression on NK cells following BCG immunization may compromise their antiviral responses against HIV

Manuja Gunasena*[1,2], Mario Alles [1], Will Mulhern[1], Richard Robinson[1] and Namal P.M Liyanage[1,2]
[1]Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, [2]Department of Veterinary Bioscience, College of Veterinary Medicine, The Ohio State University

<u>10:45 – 11:00 am</u>
Single-cell RNA sequencing of the fungal pathogen*, Pneumocystis carinii,* for determination of its life cycle

Aaron W. Albee*[1,2], Steven G. Sayson[1,2], Alan Ashbaugh[1,2], Aleksey Porollo[3], Melanie T. Cushion[1,2]

[1]Department of Internal Medicine, University of Cincinnati, College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, [2]The Veterans Affairs Medical Center, 3200 Vine Street Cincinnati, OH 45220, [3]Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229

11:00 – 11:15 am

ZFP36L1 Suppresses Virus Replication Independent of Poly(A) Tail Deadenylation

Tooba Momin*[1], Malabika Bhowmik[1], Mrigendra Rajput[1]
[1]University of Dayton, Dayton, Ohio, USA 45469

11:15 – 11:30 pm – Break

11:30 – 11:45 am

Metabolic functional redundancies may dampen microbiome functional 'service outages' resulting from diet-induced species losses.

Kayla Cross*[1], Noelle Beckman[2], Benjamin Jahnes[1], Zakee Sabree[1]
[1]Ohio State University; [2]Utah State University

11:45 – 12:00 pm

Microfungal distribution & Interactions in Antarctic Lake Bonney across seasonal and spatial scales

***Eckhardt Karsten*[1]**, *Emily Reynebeau[2], Cristina Takacs-Vesbach[2], Rachael Morgan-Kiss[1]*
[1]Miami University, [2]University of New Mexico

12:00 – 12:15 pm

Identification of RNA:RNA Interaction Partners in *Staphylococcus aureus*

Julia Tennant*[1], Paul Briaud[1], Rachel Zapf[1], David Lalaouna[2], Ronan Carroll[1]
[1]Department of Biological Sciences, Ohio University, Athens, OH; [2]University of Strasbourg, IBMC-CNRS, Strasbourg, France

12:15 – 1:30 pm **Mid-Day Activities:**

Lunch: Box or "on your own"

Lunch will be available in

12:30 – 1:15 pm

Cancelled due to scheduling issues with speakers

~~**Microbiology Education Session** – SCSC Room 161~~

~~**Moderator:** Stephanie Strand~~

1:30 – 2:30 pm **ASM Distinguished Lecture** – SCSC Room 163

"Global Impacts and Challenges of Emerging Infections"

Wun-Ju Shieh, *MD, MPH, PhD, DrPH (h.c.)*,*FIDSA, FASCP, FRSM*

Centers for Disease Control and Prevention (CDC)

2:30 – 2:45 pm **Break**

2:45 – 5:00 pm **Poster presentations and judging** – SCSC Atrium, lower level
Coordinator – Chet Cooper

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

Andrea Suria – SCSC 161
Jason Boock – SCSC 161
Noelia Lander – SCSC 161
Brian Price – SCSC 161
Irene Newton – SCSC 163
Jennifer Bennett – SCSC 163
Darryl Wesener – SCSC 163
Wun-Ju Shieh – SCSC 163

5:45 – 7:00 pm **Banquet and Student Awards Presentations** – Room 300, Merrick Hall
D.J. Ferguson – OBASM President

Abstracts of OBASM Poster Presentations
Saturday, April 6
2:45-5:00 pm

BOARD 1

A CURE for Science Education: Antibiotic Production and Resistance in Environmental *Pseudomonas* strain TE50

Jasmine Light*[1], Mackenzie Krigbaum[1], **Dawson Holt***[1], **Aliya Berresford***[1], and Hans Wildschutte[1]
[1]Bowling Green State University

Antibiotic resistant infections are predicted to cause 10 million deaths per year by 2050, surpassing cancer. At Bowling Green State University we, as students, can participate in authentic antibiotic research discovery in collaboration with the Tiny Earth network. Biology 3130 Introduction to Microbiology and Biology 4260 Pathogenic Microbiology are each a semester-long class which follow a course-based undergraduate research experience format (CURE). For fall 2022 in Biology 3130, strain TE50 was isolated by a student from soil and experiments were performed to characterize the strain such as PCR, sequencing, BLAST analysis of the 16S rRNA gene, antibiotic production, and transposon mutagenesis to identify loss-of-killing mutants. For the spring 2023 semester in Biology 4260 Pathogenic Microbiology, we sequenced the genome and incorporated bioinformatic assignments to further characterize strain TE50. JGI IMG was used to annotate the genome and identify predicted genes that may confer antibiotic resistance. In parallel, the Kirby Bauer assay identified that TE50 was resistant to eight antibiotics thus verifying bioinformatic predictions. These results suggest the overuse of antibiotics selects for drug resistant strains in the environment. For antibiotic production, antiSMASH was used to identify biosynthetic gene clusters that may be involved in secondary metabolite production. From transposon mutagenesis and antiSMASH results, a 35 kb BGC was that identified and predicted to encode a product similar to MA026, which is a cyclic peptide that inhibit virals hepatitis-C replication. The TE antagonistic product is predicted to be novel and biochemical characterizations is being performed to purify and characterize the compound. Data from both of these courses was used to design this poster that we are presenting.

BOARD 2

FCRL1: A Regulator of Secondary Immune Responses

Malory M. Wolfe[1*], Timothy J. Wilson[1]
[1]Miami University, Department of Microbiology

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 adaptive immune responses has yet to be examined. This study seeks to determine if loss of FCRL1 hinders formation of an adaptive immune response when challenged with a model antigen. Current work has found that following an initial exposure to model antigen, $TNP_{(21)}$-KLH, there is no change in activated B cell populations in *Fcrl1*$^{-/-}$ mice. However, *Fcrl1*$^{-/-}$ mice demonstrated significant reductions in activated populations following re-exposure to the antigen on days 35 and 90, indicating a role in regeneration of an immune response from memory. Understanding FCRL1's role in the generation of adaptive immune responses is essential for furthering our knowledge on how dysregulation of surface receptors can result in impaired function of B cells resulting in an inability to appropriately respond to infection.

BOARD 3

The role of CodY in regulating LLO production and cell wall homeostasis by *Listeria monocytogenes* after anaerobic propionate exposure

Sydney Herzog*, Meaghan Evans, Allison Herceg, Jeanne Sering, Lizzy Herr, Troy Reisiner, Lauren Piper, Angela Murrin, Yvonne Sun
University of Dayton

Listeria is an intracellular foodborne pathogen with a variety of virulence factors. Listeriolysin O (LLO), in particular, is critical in phagosomal escape during the intracellular life cycle of *L. monocytogenes*. We have reported that anaerobic propionate exposure, which can take place during food storage as well as intestinal transit, significantly enhanced LLO production compared to no propionate controls. In this study, we began to explore the role of CodY, a transcription factor, in facilitating the effects of anaerobic propionate exposure. First, we discovered that CodY is required for the anaerobic enhancement effects on LLO production by propionate. Subsequently, using cell culture infection models, we noted that the codY deletion mutant exhibited a distinct response to anaerobic propionate exposure during intracellular growth in macrophages. Finally, to further investigate the extent of how CodY is involved in regulating *L. monocytogenes* pathogenesis, we performed several in vitro characterization experiments and observed a wide range of effects from anaerobic propionate exposure, from cell shape, lysozyme susceptibility, to peptidoglycan synthesis. These in vitro results suggest that anaerobic propionate exposure may greatly impact cell wall homeostasis and that CodY likely contributes, at least partially, to the signaling cascade. Moreover, this study highlights that anaerobic propionate exposure can have a long term impact on subsequent *L. monocytogenes* success during infections.

BOARD 4

Identification of novel SrtA-dependent sialic acid binding surface adhesins in adherence of *Streptococcus mitis*

Jnapika Palacharla*[1], Anu Narayana[1], Zahid Gani[1], and Samantha J. King[1,2]
[1]Center for Microbial Pathogenesis, Nationwide Children's Hospital, and [2]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH

Sub-acute infective endocarditis (IE), a deadly infection of the heart valves, is most often caused by viridans group streptococci. These bacteria enter the bloodstream from the oral cavity and attach to damaged heart valve surfaces. Subsequent bacterial growth and deposition of host material leads to vegetation formation which can cause congestive heart failure. Hence, bacterial binding to the valve is a critical step in infection.
Streptococcus mitis is a significant cause of IE with no established adhesion mechanisms. Our study aims to define essential binding mechanisms in *S. mitis*-induced IE. Many other streptococci infect the heart through adhesion to sialic acid on platelets that bind to the site of damage. In previous studies, we discovered that, apart from the established interaction between serine-rich repeat proteins (SRRPs) and sialic acid, a novel adhesin called AsaA plays a crucial role in the binding of *Streptococcus* o*ralis* to sialic acid. We also identified AsaA orthologs across species, including *S. mitis.*
The distribution of predicted sialic acid binding proteins varies amongst *S. mitis* strains but includes both orthologs of SRRPs and AsaA and novel adhesins**.** These data support the hypothesis that *S. mitis* binds sialic acid through multiple surface proteins that include both orthologs of previously identified adhesins and novel adhesins. To test this hypothesis, we are constructing mutants of *S. mitis* strains lacking these putative adhesins mechanisms and to compare their sialic acid adherence against wild-type strains.
Our research is broadening the field's understanding of sialic acid binding and points to the presence of unexplored adhesins. By unraveling these mechanisms, we hope to pave the way for targeted therapeutic interventions for streptococcal IE.

BOARD 5

Probiotics modulation of gut microbiota protects against *Clostridioides difficile* infection.

Bijay Gurung*[1,2], Shaohua Wang[1,2,3,4]
[1] Molecular and Cellular Biology Program, Ohio University, Athens, OH, USA. [2] Department of Biological Sciences, Ohio University, Athens, OH, USA. [3] Department of Biomedical Sciences, Ohio University, Ohio University, Athens, OH, USA. [4] Infectious and Tropical Disease Institute, Ohio University, Athens, OH, 45701 USA.

Clostridioides difficile (*C. difficile)* is a gram-positive spore-forming anaerobic bacteria that produces toxins A and B, which are major virulence factors associated with *C. difficile* infection (CDI). CDI is the leading cause of hospital-associated diarrhea and can cause severe issues like pseudomembranous colitis and even death in some cases. The primary reason underlying the CDI is the dysbiosis of the normal gut microbiota, which can occur due to antibiotic usage or proton pump inhibitors during hospitalization. Gut microbiota dysbiosis induced gut metabolites alteration can also enhance the pathogenicity of *C. difficile.* Probiotics have been indicated about their effects on gut microbiota-gut metabolites modulation. However, to date, there have no probiotics been proven to be an effective option for CDI therapy. In this study, we isolated human-original probiotics strains from infant feces and developed a novel probiotics cocktail containing 11 different probiotics species screened specifically based on their inhibition activity against *C. difficile*. This most diverse probiotic cocktail protected mice well against CDI with lower clinical scores and higher survival rates. Less *C. difficile* burden and toxins production in colon were detected from mice fed with probiotics, resulting in ameliorated gut permeability (less secreted sCD14 in serum) and gut inflammation. Moreover, increased expression of mucin and tight junction proteins further verified effects of our probiotics cocktail on strengthening gut barrier. In addition, gut microbiome analysis demonstrated that our probiotics cocktail increased gut microbiota diversity and beneficial species. Along with gut microbiota modulation, gut metabolites especially butyrate was significantly increased in the probiotics group. Results from this study indicated that probiotics could be a promising CDI therapy as gut microbiota modulator, which will lay the foundation to translate probiotics in mitigating CDI and other intestinal pathogens for clinical use.

BOARD 6

Characterization of cyclic-di-GMP metabolizing genes in *Streptomyces scabies*

Ashni Patel*, Jennifer Bennett
Otterbein University

Streptomyces scabies is a gram-positive bacterium that causes a scab disease in potatoes.
The *rmdA*, *rmdB,* and *cdgC* genes in *S. scabies* are used in the cyclic-di-GMP signaling pathway. This pathway is responsible for regulating many processes including cell cycle progression and differentiation. *RmdA* and *B* are phosphodiesterases that break down the second messenger, cyclic-di-GMP. *CdgC* is thought to relate to the cyclic di-GMP pathway by making cyclic dimeric guanosine monophosphate. Cyclic dimeric guanosine monophosphate is a second messenger that helps to regulate cellular processes within bacteria. The specific question addressed is what role *rmdA*, *rmdB,* and *cdgC* play in the development of *S. scabies* and their ability to cause disease in potatoes. One aim of the project will result in the deletion of the *rmdA* gene. This deletion has been constructed using the lambda Red recombinase system (REDIRECT). REDIRECT works by first designing a forward primer sequence that will provide a region of homology for 39 bases that includes the start codon and flanking DNA directly upstream of the gene. A reverse primer was then designed to provide homology to the stop codon and downstream sequence of *rmdA*. The cosmid containing the wild type *rmdA* gene has been transformed and the apramycin resistance cassette that has flanking regions of homology to the gene to be deleted into *Escherichia coli* to create the deletion. The new cosmid containing the deletion of *rmdA* (pAP1) has been introduced into the mating strain of *E. coli* and will subsequently be conjugated into the wild-type and *rmdB* mutant to construct single and double mutant strains, respectively. Here, Multiple time courses on various media types have been performed to determine phenotypic differences between wild-type and *rmdB* and *cdgC* mutants. Finally, phenotypic differences were analyzed using radish seedling and potato infection assays.

BOARD 7

Hawaiian bobtail squid symbiont interspecies competition is mediated by a diffusible antimicrobial and the type VI secretion system

Ivan A. Vore* & Andrea M. Suria
Ohio Wesleyan University

Leisingera bacteria (phylum Alphaproteobacteria) are the dominant symbionts in the accessory nidamental gland (ANG) symbiosis of the Hawaiian bobtail squid, *Euprymna scolopes*. The ANG is a female reproductive gland which inoculates eggs with healthy microbiota to protect against pathogens during development. Microbiome species composition is hypothesized to have significant impacts on overall host health and reproductive success in *E. scolopes*. Species dominance is hypothesized to result from competition between symbionts during colonization. Previous research has shown an ANG symbiont, *Leisingera* sp. ANG-M7, expresses a diffusible antimicrobial compound (DAC) *in vitro*, which may allow it to compete with other strains in the host. However, genetic deactivation of DAC expression demonstrated persistent competitive activity. Another symbiont of *E. scolopes*, *Vibrio fischeri*, is known to use the Type VI Secretion System (T6SS), a toxin delivery system common in diverse bacterial species and habitats, as a killing mechanism during colonization of the squid light organ. Since two discrete T6SS gene clusters are present in *Leisingera* ANG-M7, we hypothesize that the T6SS may play a role in competitive activity in this strain. Key structural protein genes in the two T6SS clusters of ANG-M7 were disrupted individually in a mutant background that no longer expresses the DAC. The T6SS- strains were tested in an *in vitro* coincubation assay against another ANG symbiont, *Leisingerra* sp. ANG-DT, to screen for total loss of killing. Data from seven trials suggests that disruption of the T6SS-2 in ANG-M7 may lead to a slight decrease in inhibitory activity against ANG-DT. These data suggest that a third competitive mechanism may exist in ANG-M7, but the T6SS-2 may play a role in contact-dependent bacterial competition. Further research will require the generation of a triple mutant inhibiting both T6SS-1 and T6SS-2 within a DAC mutant background to discern additional significant competitive inhibitory mechanisms.

BOARD 8

Growth and Stress Physiology of Two Novel Antarctic Algal Isolates from the McMurdo Dry Valleys (Antarctica)

Benjamin Nagle*[1], Rochelle Pereira[1], Drew Wolf[1], Rachael Morgan-Kiss[1]
[1]Miami University

The permanently ice-covered lakes of the McMurdo Dry Valleys (MDV) in Antarctica have been the subject of three decades of ecological research (mcmlter.org). These lakes harbor diverse and unique microbial communities which are exposed to permanent extreme conditions including cold, high salinity, and variable light levels. This ecosystem is largely supported by cold-adapted (psychrophilic) phytoplankton, many of which have yet to be isolated. Despite the remote location of the MDV, it still experiences the effects of climate change; however, it is not well understood how microbial communities will respond. To contribute to the preservation and characterization of novel microorganisms, this research focuses on two novel algae isolates that were recently collected from Lake Fryxell. Sequencing of 18S rRNA identified the strains as Ulothrix and Chlorella. Both strains are novel in this lake, moreover, the Ulothrix isolate is the first filamentous algae to be isolated from Lake Fryxell. The algal isolates both show exponential growth in defined medium under batch culture conditions. Preliminary growth physiology indicates that both strains are psychrophilic. Growth stress physiology under environmentally relevant conditions will be discussed to better understand these novel isolates and their response to a variety of abiotic environmental stressors.

BOARD 9

The Effects of Spaceflight on the Immune System and Subsequent Portrayal in the Media

Emily McIntire*
Heidelberg University

Recent advancements in technology mean sending astronauts to planets like Mars is a greater possibility. However, as this is a much longer flight than has ever been undertaken, there are a number of questions and concerns. The effects of microgravity, radiation, and other factors on the immune system (and the body in general) while in space mean that considerable research needs to be performed prior to sending astronauts to Mars. One of these fields is that of immunology. The environment of space has been shown to impact how the immune system functions, including decreased numbers and functions of some types of immune system cells, and reactivation of viruses such as herpes and Epstein-Barr virus. As society looks to a new frontier to conquer, it is important to understand how it is marketed to the public. Media such as movies and television shows often do not show how spaceflight affects the immune system. Instead, they provide the public with a glorified version of spaceflight, which negates the hazardous physiological effects.

BOARD 10

Variable Dose Scheduling and Antibiotic Resistance Evolution in the EVolutionary biorEactor (EVE)

John Joyce*[1], Rowan Barker-Clarke[2], and Jacob Scott[2]
[1]Hawken High School; [2]Cleveland Clinic Lerner Research Institute, Translational Hematology & Oncology Research

Multi-antibiotic resistance, occurring when a bacteria becomes tolerant to standard treatment through evolution and selection pressure, is a worldwide problem. The evolution of resistance has the potential to nullify both current and future treatments, spilling over beyond the type of drug used. An essential component of combating antibiotic resistance requires understanding multiple factors: ecological competition between bacteria, the prevalence and profiles of pre-existing resistance, and selection pressure variability due to both varying drug uptake and schedule adherence to prescribed antibiotics. Thus, to better model and address these factors in the lab, we looked to advance the in-silico and in-vitro simulations of variable dose uptake and scheduling.
In our work we used the EVE, a small, experimental, raspberry-pi based bioreactor that can pump drug or nutrient media into bacterial culture units. This study focuses on dose scheduling, and we used Python to develop mathematical models antibiotic resistance evolution to form predictions. These predictions aimed to optimize various factors like dose timing, drug concentration, and dose concentration. At the same time, we developed new adaptive EVE algorithms to simulate, in-vitro, different pharmacodynamics and dosing schedules and their adherence. To study the effect dosing regimens have on the evolution of antibiotic resistance, we created three separate control algorithms for the EVE to simulate dosing processes, all of which work to automate dosing processes in a controlled setting. These algorithms are widely customizable, and we tailored them respectively to our models. We then experimentally verified our predictions about the effects of different cefotaxime dosing regimens on the co-evolution of combinations of different wildtype and resistant Weinreich E. coli strains in the EVE.

BOARD 11

Comparison of the gut microbiome of canines from residential homes and mill-bred dogs

Ireland A. Nowak*[1], Kiley Lewin[1]
[1]Ohio Wesleyan University

The gut microbiome in canines is an under-researched topic in veterinary medicine, with a growing number of published papers in recent years. Quantification of the gut microbiome in different dog breeds has led to the discovery that there is some variance in the species diversity of each breed where bacterial populations can be influenced by diet, environmental factors, genetics, and age. Differences in the environment a canine was raised can have a major effect on a healthy microbiome, leading to an increased risk for infection of the intestinal tract.

Dogs in home environments with routine care, space, and exposure to other animals and humans can have a different bacterial makeup in their gut microbiomes compared to mill-bred dogs. Canines that grow up in cramped spaces can develop alterations in their gut microbiomes due to high stress living situations, lack of environmental socialization, and space to grow. To learn more about the microbiome and the possible differences in bacteria involved, fecal samples of dogs raised in a home environment and mill-bred dogs were analyzed to identify differences between the bacterial species diversity, metabolic function, and genetic makeup of their gut microbiome. The comparison of these microbiomes will contribute to a better understanding of potential links between gastrointestinal issues observed in mill-dogs and differences in diversity or metabolic capability of these bacteria compared to canines in residential homes. Fecal samples were gathered from a local veterinary hospital and the genomic DNA was isolated and shipped to a laboratory for shotgun sequencing. Sequencing results allowed the abundance and species of bacteria present in the gut from these two groups to be determined alongside metabolic capabilities. Differences in bacteria found between the two groups can provide essential information to the veterinarian community about possible factors, such as stress, that can change canine microbiomes.

BOARD 12

Novel non-atherogenic breakdown of quaternary amine by a gut bacterium:CJ25

Roshan Timsina*[1], Ryan Gora[1], and D.J. Ferguson Jr.[1,2]
[1]Miami University, Oxford, OH; [2]Miami University, Hamilton, OH

Human gut is dynamic in nature exhibiting a complex interaction between gut microbiome and human physiology. This complex interaction can influence susceptibility to disease through immunological and metabolic activities. Gut metabolism of quaternary amines (QA), such as choline and carnitine have been shown to influence cardiovascular as well as renal health in an individual (1).Choline is cleaved by a glycyl radical enzyme choline TMA Lyase (*CutCD*) (2) and carnitine is cleaved by *CaiABCD* operon followed by *bbu* gene utilization cluster in human gut (3). These breakdown of QA yields Trimethylamine (TMA), a metabolite known to influence cardiovascular and renal health in individuals. TMA in the gut travels to the liver and further oxidizes to TMAO by FMO3 enzyme, this accumulation of TMAO plays a critical role in causing atherosclerosis in humans. Interestingly, CJ25, the strain isolated and characterized by our lab have been shown to grow on choline but doesnot have a canonical CutCD enzymes as well as no known methyltransferases from COG5598 Superfamily that are known to demethylate QAs. Since the genome doesnot have *CutCD* and known methyltransferases, we tried to answer the choline and carnitine metabolism using combined metabolomics and proteomics approach. The metabolomics showed us significant accumulation of glycine betaine (GB) and the proteomics showed putative dehydrogenases that could be oxidizing choline and carnitine to GB. We *hypothesize* that CJ25 is using dehydrogenase homologs to breakdown choline and carnitine into non-atherogenic product, GB. This hypothesis has been formulated based on the observations from proteomics and metabolomics data. The identification of initial pathways for choline and carnitine breakdown may also exist in other gut microbiota, which could amplify the effects of these pathways significantly possibly reducing the risk of atherosclerotic cardiovascular disease.

BOARD 13

ZFP36L1 suppresses Rotavirus and Norovirus replication, moderates the virus-induced hyperinflammation and suppresses host cell damage.

Malabika Bhowmik*[1], Tooba Momin[2], and Mrigendra Rajput[1]
[1] Department of Biology, University of Dayton

Most acute viral infections cause damage in the host body either directly when the virus hijacks the host cell machinery, changes cell physiology, causes cell death by viral proteins, or indirectly by hyperinflation. Zinc finger proteins (ZFP) are one of the highly abundant proteins in eukaryotes. Due to their distinctive structure, ZFPs bind with different cellular components such as DNA, RNA, lipids, or other proteins. A specific type of ZFP, ZFP36L1, belongs to the CCCH-type ZFP, which has been identified as a regulator of RNA metabolism. It is known to control the overall turnover of cellular

mRNA including mRNA of cytokine mediators and cytokines by breaking it down through poly A tail using the deadenylation mechanism. The current study was designed to investigate the role of ZFP36L1 on virus replication and moderating virus-induced inflammation. Our results showed that stable overexpression of ZFP36L1 through lentivirus transduction significantly reduces Rotavirus and Norovirus titre as well as it moderated virus-induced proinflammatory cytokines like IFN-α and TNF-α with reduced virus-induced cytopathic effect in cells. While knockdown of ZFP36L1 significantly enhanced viral titre, virus-induced cytokines with more cytopathic effect. Our results also showed that ZFP36L1 overexpression does not affect macrophage (RAW 264.7) migration as compared to control while knockdown of ZFP36L1 significantly enhanced the macrophage migration in trans-wells. Overall, our study showed that overexpression of ZFP36L1 suppresses the virus (Rotavirus and Norovirus) replication and moderates the virus-induced hyperinflammation and thus mitigates virus-induced damage in the host.

BOARD 14

Characterization of Bacteria Isolated from Soil for Their Antibiotic Resistance and Antibiotic-Producing Properties

Monishka Major*[1], Lubna Abu-Niaaj[1]
[1]Central State University

The microbial antibiotic resistance has been on the rise and has become a major health problem. The emergence of such microbes in soil might affect the soil health and consequently the quality of crops, in addition to being a potential threat to farmers' health if injured. The aim of this study is to isolate and characterize bacteria from soil especially those resistant to antibiotics and those producing antibiotic-like metabolites. Five soil samples were collected from Ohio. The samples were suspended in nutrient agar (1:10 w/v) supplemented with an antifungal solution (Amphotericin B) with or without 10µg/mL of Ampicillin or Tetracycline. The characterization was done using traditional methods including Gram staining, selective culturing, and biochemical tests. The morphology of the isolated colonies showed twenty-seven bacilli and twelve cocci. Thirty-nine isolates were purified and two of which were Gram positive bacteria susceptible to both antibiotics. The remaining thirty-seven isolates were Gram negative, twenty-four colonies (67%of total) of which were only Ampicillin resistant, two of which identified as *Pseudomonas species*. In addition, three Gram-negative isolates were only Tetracycline resistant, two colonies were resistant to both antibiotics, and the remaining eight colonies were susceptible to Ampicillin and Tetracycline. The identification Gram-negative bacilli and oxidase negative was confirmed using the designated RapID one system kit. Using the well-diffusion method, screening of the antibacterial activity of the supernatants of overnight bacterial cultures was against *Pseudomonas species (P.aeruginosa and P.putida)* and *Enterococcus faecalis.* The screening of supernatants of seven colonies exhibited inhibition against all bacteria. One of these isolates was susceptible to antibiotics, and the kit confirmed it as *Enterobacter gergoviae.* The screening and identification of the remaining isolates is in progress. In conclusion, soil shows an increase in the antibiotic-resistant bacteria, though, some of which may have the potential to secrete antibiotic-like metabolites that could impact the bacterial interaction in the soil.

BOARD 15

Identification of candidate enzymes involved in novel gut microbial metabolism of quaternary amines

Ryan Gora*[1], Roshan Timsina[1], and D.J. Ferguson Jr.[1,2]
[1]Miami University; [2]Miami University Regionals

The human gut microbiome has quickly become known for its diversity, broad implications on human health, and complexity. Factors such as diet and stress can significantly impact both the composition of the microbiome and metabolism of the microbes themselves. The molecules choline and carnitine, which are quaternary amines commonly found in red meat and seafood products, are typically metabolized to a byproduct called trimethylamine (TMA) by gut microbes. TMA is commonly associated with an increased risk of cardiovascular diseases. By growing an isolated strain of *Citrobacter amalonaticus*, named CJ25, we showed that this organism can use either carnitine or choline as a sole carbon and energy source without producing TMA.

Genomic analysis revealed the absence of genes encoding known enzymes involved in catabolism of choline and carnitine. To investigate these reactions, untargeted metabolomics and proteomics were used to guide the identification of candidate enzymes. Thus, we believe these pathways in CJ25 to be novel and unexplored. Analysis of proteins identified during proteomic analysis, using the NCBI Blastp tool with an adjusted scoring matrix to account for evolutionary diversity coupled with proteomic data, has identified candidate enzymes for breakdown of choline and carnitine by CJ25. Future work will include overexpression and purification of these enzymes followed by enzyme assays to test our hypotheses.

BOARD 16

Enzymatic Activity & Detection of Nitrifying Microorganisms in Purple Pitcher Plants on Beaver Island, Michigan

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Purple pitcher plants (Sarracenia purpurea) are a species of plants that use carnivory to obtain essential nutrients in environments of scarcity. Insect prey, which act as a major source of nitrogen, are digested by microorganisms within the pitcher fluid. Previous research has not investigated nitrification within the pitcher fluid and the microbes that participate in the conversion of ammonium to nitrate, a key form of nitrogen that can be utilized by the host plant and other microbes. During five weeks of sampling, pitcher fluid samples were measured for chitinase activity and DNA extractions, PCR, and gel electrophoresis were performed to detect nitrifying microbes. The investigation uncovered ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and nitrite-oxidizing bacteria (NOB) within the pitcher fluid for the first time which gives insights into an unstudied aspect of nitrogen cycling within pitcher plants. More evidence is needed to determine the relationship between digestion enzyme activity that releases nitrogen into the system, and the presence of nitrifying microbes that convert its products into nitrate. Future sequencing of DNA samples can be conducted to confirm results and to identify the detected microorganisms.

BOARD 17

Evaluation of novel HIV/AIDS therapies

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The cause of AIDS was shown to be the primate lentivirus Human Immunodeficiency Virus (HIV). Most individuals become antibody positive several weeks after exposure; some individuals known as Exposed Seronegative (ESN) do not. Other individuals become seropositive but do not progress to AIDS (LTANP). There are multiple explanations for both situations.
HIV-1 infects a cell by interacting with CD4 followed by a fusion interaction with one of two co-receptors, CCR5 or CXCR4. A naturally occurring mutation in *ccr5*, known as *ccr5delta32*, encodes a 32 base-pair frameshift mutation. That mutation encodes a truncated CCR5 protein that is not detected on the membrane. The truncated protein was shown to also down-modulate full length CCR5 and CXCR4. Some ESN individuals are *ccr5delta32* homozygous. Some LTANP are heterozygotes for *ccr5* (*ccr5delta32*/*ccr5* wild-type). Some Individuals with Leukemia and HIV-1 have undetectable levels of HIV after receiving bone marrow transplants from *ccr5delta32* homozygous donors. Some LTANP and ESN have wild-type *ccr5* genes yet have low levels of surface CCR5. These individuals produce antibodies to the first extracellular loop (ECL1) of CCR5 which cause CCR5 endocytosis[1].
A gene therapy can be envisioned that targets CCR5 surface expression. *Ccr5delta32* will be evaluated as a gene therapy to reduce viral burden. A lentiviral vector system (pLenti puro HA-Ubiquitin) was used to construct

viral particles containing *ccr5* wildtype, *ccr5delta32, and ccr5delta33*. *Ccr5* wild-type and *ccr5delta32* were constructed by amplifying the genes of a heterozygous individual. The gene *ccr5delta33* was constructed by PCR mutagenesis. The packaging cell line HEK293-ft was co-transfected with pLenti-*ccr5wildtype*, or pLenti-*ccr5delta32,* or pLenti-*ccr5delta33* and helper plasmids psPAX2, pMD2.G. Pseudotyped viral particles were frozen and used to infect H9 and primary lymphocytes. If *ccr5delta32* can downmodulate wildtype CCR5 and CXCR4 *in vivo*, an effective AIDS therapy can be evaluated. This therapy could be combined with antibodies to ECL1.

BOARD 18

Isolation and Characterization Antibiotic-Resistant Bacteria from soils with the Potential to Produce antibacterial Compounds

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Prokaryotes, found widely in environments such as soil and sediment, play crucial roles in biogeochemical cycles and offer potential for medical and industrial applications. Given the growing resistance to existing drugs, the hunt for new antimicrobial-producing strains in soil is vital. Soil complexity, influenced by factors like texture and structure, affects microbial distribution, impacting accessibility. Antimicrobial resistance (AMR) is a global threat exacerbated. Our project's goal is to identify antibiotic resistant bacteria with a focus on those producing antibiotic-like metabolites. Two soil samples were suspended in nutrient broth overnight at 30oC. Serial dilutions were prepared and 100uL of each was spread on Tetracyclin (Tet) plates (3.75 µg/mL). Eleven colonies were picked up from the Tet-plates, and were characterized by the morphological determination, biochemical tests, and the use of differential and selective media. Ten isolates fere Gram-negative and one isolate was Gram-positive. Five out of the eleven isolates (45%) were resistant to Ampicillin (50 µg/mL) while two of these isolates were ampicillin-resistant up to 100ug/mL. Using the well-diffusion method, screening of the antibacterial activity of the supernatants of the overnight cultures of all bacteria was evaluated against Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis. Using a RapID one system kit targeting Gram-negative oxidase negative bacilli, the identification of two colonies was confirmed to be Acinobacteria calcoaceticus and Salmonella choleraeuis. Both of these isolates showed antibiotic resistance to Ampicillin up to a concentration of 100 µg/mL. The identified Acinobacter exhibited a moderate antimicrobial activity against the three tested selected bacteria, despite its known to be an environmental species. The identified Salmonella (ampicillin-resistance) exhibited no effect on the studied bacteria, its of importance because of its antibiotic resistance. The identification of the remaining isolates is still in progress. In conclusion, the isolated colonies from soil showed MDR (Tet and Amp) at the tested concentrations, and some showed an antimicrobial activity against the tested Gram-negative bacteria.

BOARD 19

AmpG regulates the expression of L1 and L2 penicillin resistance genes in *Stenotrophomonas maltophilia* Oak Ridge strain 02

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Stenotrophomonas maltophilia Oak Ridge strain 02 (*S. maltophilia* 02) is a gram-negative bacterium from a metal-contaminated site in Oak Ridge, TN. In addition to being resistant to several different metals, it is also resistant to ampicillin, a type of penicillin, with a minimal inhibitory concentration (MIC) of over 1,000 µg/ml. Genomic sequencing of the *S. maltophilia* 02 genome revealed that it contains two penicillin resistance genes, L1 and L2. Both genes encode β-lactamase, which inactivates penicillin by cleaving its β-lactam ring. Previous research using transposon mutagenesis produced a mutant, AJ22, with a reduced ampicillin MIC of 400. Sanger sequencing revealed that the mutation was in a gene for AmpG, a trans-membrane permease involved in transporting degraded cell wall components from the periplasm to the cytoplasm. The import of damaged cell components signals the cell to express the L1 and L2 β-lactamases. The study hypothesizes that the mutation in *ampG* inhibits the expression of the β-lactamases. Samples for RNA extraction were collected from *S. maltophilia* 02 and the AJ22 mutant before and after exposing them to 100 µg/ml ampicillin. Then, RT-

PCR was performed to test for the expression of resistant genes. Although an *ampG* transcript appeared to be present in the mutant, the transcripts of the L1 and L2 genes appeared to be expressed at lower levels in the mutant than in the wild type strain. Thus, it appears that AmpG plays a role in the expression of both β-lactamases.

BOARD 20

Novel Antibiotic Resistance Gene Identified in *Mycobacterium* Bacteriophage PSullivan

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Mycobacterium smegmatis is an opportunistic pathogen known to cause infections in humans. With the increasing prevalence of antibiotic resistance in bacterial pathogens, a better understanding of the mechanisms by which bacteria develop resistance is needed. While lytic bacteriophages are being investigated as a potential therapeutic to treat antibiotic-resistant bacteria, lysogenic phages have been shown to act as a reservoir for bacterial genes, including virulence factors. PSullivan is a 50kbp mycobacteriophage with a temperate lifestyle. Annotation of PSullvian using PECANN, GeneMark, Glimmer, and DNA master revealed the presence of a putative beta-lactamase enzyme, a virulence factor associated with antibiotic resistance. Computational models of the 59.4kDa putative beta-lactamase generated using AlphaFold demonstrated a strong predicted binding affinity with benzylpenicillin using SwisDock ligand docking software. The predicted coding region of the gene is between position 26,487 and 27,731 bp. A better understanding of the lifestyle of PSullivan and other temperate mycobacteriophages could improve our understanding of the spread of antibiotic-resistance genes.

BOARD 21

Enhancing the production of ethylene in *Rhodospirillum rubrum* for sustainable plastics through expression of novel S-adenosyl-L-methionine synthase enzymes\

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Ethylene is a key feedstock in the chemical industry and the direct precursor to polyethylene. Conventional ethylene production involves an energy-intensive process that releases substantial amounts of carbon dioxide and pollution. Alternatively, our lab has characterized a novel methionine salvage pathway (MSP), in the facultative anaerobe *Rhodosprillum rubrum*, presenting a greener ethylene production process. SAM synthetase catalyzes the reaction of methionine with ATP to produce S-adenosyl-L-methionine (SAM). Here, we evaluate the capacity of different SAM synthetases in regenerating the biologically available SAM pool, increasing flux through the MSP cycle, and increasing ethylene production. We identified four SAM synthetase genes: Sce_YDR502C from *S. cerevisiae,* Eco_B2942 *(metK)* from *E. coli* K12, and genes Rru_A0917 and Rru_A3776 from *R. rubrum*. These were cloned into a plasmid with a constitutive promoter and a viral SAM hydrolase gene, transformed into Top10 *E. coli* cells, and transferred by triparental conjugation into *R. rubrum*. Transconjugants possessing each plasmid were isolated by antibiotic selection, then cultured anaerobically under five different sulfate concentrations (75 µM, 250 µM, 500 µM, 750 µM, 1 mM). Ethylene from stationary phase cultures was characterized by gas chromatograph to quantify activity of the MSP. Testing of each SAM synthetase was performed in biological triplicate for all five sulfate concentrations. Expression of *E. coli* SAM synthetase with viral SAM hydrolase produced over 3-fold the ethylene (umol/L/OD) of empty plasmid at their respective maxima. Furthermore, while expression of SAM hydrolase alone produced no ethylene above 500 µM sulfate, the SAM hydrolase with *E. coli* SAM synthetase produced ethylene in all sulfate concentrations, overcoming typical pathway inhibition. By performing a systematic analysis using several SAM synthetase genes in combination with SAM hydrolase, we were able to identify a promising *E. coli* SAM synthetase that provides increased and sustained ethylene production.

BOARD 22

Determining the role of a potential antimicrobial gene cluster in Hawaiian bobtail squid symbiont, *Leisingera* sp. ANG1

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The accessory nidamental gland (ANG) of female Hawaiian bobtail squid, *Euprymna scolopes*, contains a diverse symbiotic bacterial community that has been shown to prevent biofouling of the eggs. Many of these bacteria belong to the roseobacter clade, which contains species known to produce secondary metabolites, including antimicrobials. It is not yet known exactly how these antimicrobials are produced. Analysis of roseobacter genomes using the Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) revealed potential biosynthetic gene clusters in roseobacter species. No classical antibiotic pathways were identified, suggesting any antimicrobial activity described may be novel. One symbiont, *Leisingera* sp. ANG1, contains a potential Type 1 Polyketide Synthase (T1PKS) / Non-Ribosomal Peptide Synthetase (NRPS) hybrid gene cluster. Previous metatranscriptome analysis showed that this type of gene cluster is expressed in both the *E. scolopes* ANG and the jelly coat surrounding their eggs. Multiple known antimicrobials are synthesized through hybrid PKS/NRPS pathways, including indigoidine in another *Leisingera* ANG symbiont. This study aims to determine the function of the T1PKS/NRPS in ANG1 by creating a disruption mutant in the largest gene of the cluster, a polyketide synthase. We used pEVS118 as the plasmid backbone, to which we inserted a PCR-amplified portion of the targeted gene. Plasmid construction was confirmed by DNA sequencing. The plasmid, pAJI001, was successfully introduced into *E. coli* RHO3, which we are currently conjugating into ANG1. This will cause a targeted disruption via homologous recombination. Future research will screen for phenotypic changes in the mutant ANG strain through competitive assays against other marine bacteria. This research is significant because the T1PKS/NRPS gene cluster present in ANG1 is highly conserved among other *Leisingera* species, but no known products are described. Revealing a function for this cluster will broaden our understanding of secondary metabolism in a common group of marine bacteria.

BOARD 23

Environmental Conditions Affecting *Listeria monocytogenes* Biofilm Formation

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Biofilm formation poses a unique challenge for food processing plants seeking to avoid contamination. *Listeria monocytogenes* threatens public safety in these environments due to its ability to form a biofilm, resulting in Listeriosis cases caused by contamination. Identifying various environmental conditions and their effect on *Listeria monocytogenes* biofilm formation can highlight high-risk areas within food processing plants for contamination.

Some conditions that affect *Listeria monocytogenes* biofilm formation include temperature, nutrient deficiency, benzalkonium chloride (BAC) presence during biofilm formation, and introduction of BAC following biofilm formation. Listeria displayed more biofilm formation at higher temperatures. The amount of biofilm formed decreased as temperature decreased. Listeria biofilm formation under nutrient-deficient conditions was dependent on the presence of oxygen. Aerobic conditions enhance biofilm formation, while anaerobic conditions decrease biofilm formation when Listeria is grown in reduced media.

BAC is a commonly used surfactant that has antimicrobial properties. Biofilm formation in the presence of BAC displays concentration-dependent growth. Biofilm formation is favored at 1% BAC compared to 0.1% and 5% BAC. BAC showed low effectiveness at clearing Listeria biofilm following initial formation. The 0.1% and 1% concentrations showed significantly more biofilm formation than Listeria biofilms not exposed to BAC. BAC introduction following biofilm growth enhances Listeria biofilm formation.

BOARD 24

Growth Inhibition of Gram-Negative Bacteria by Avocado Peel and Hibiscus

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Foodborne illnesses represent a large portion of bacterial infections annually, and the increased microbial resistance to antibiotic resistance has created a novel public health threat for treating bacterial infection. Natural products identified from plants have shown various bioactivities including antimicrobial effects against different types of bacteria. Some plants are known as a rich source for bioactive compounds including orange, avocado, and hibiscus. This study selects the avocado peel and petals of hibiscus to evaluate their antibacterial activity against three Gram negative bacteria associated with food infection. These are *Pseudomonas aeruginosa, Enterococcus faecalis, and Escherichia coli*. Aqueous and methanol extracts were prepared, filtered and refrigerated. An overnight bacterial culture was streaked on nutrient agar plates and 7mm wells were made into the agar. Using the well diffusion method, one-hundred microliters of each extract was placed in a well and left to soak into the agar. The plates were incubated at 30oC overnight before measuring the inhibition zones in millimeters (mm). The average of triple wells was calculated and used for assessment, and a control was a well containing the extraction solvent. Results show that both extracts of hibiscus and avocado peel had inhibited the growth of the three bacteria up to 20mm and 25mm, respectively. When compared to some standard antibiotics using the disc method, the inhibition of the studied plants was higher including Penicillin (10ug) and Streptomycin (10mcg). The study is in progress to determine the MIC values for the concentrate of the tested extracts. The results indicate that the flower hibiscus and avocado peel as promising sources for natural compounds that inhibit the growth of Gram-negative bacteria such as *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli*, and other Gram-negative bacteria associated with food spoilage. Further studies are recommended to determine if they can be used as natural food additives or preservatives to inhibit foodborne bacteria.

BOARD 25

Analysis of HBV Viral Load Persistence Despite Tenofovir (TDF)-Containing Treatment Regimens in HBV-HIV Coinfected Patients in Ghana

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Globally, almost 300 million people are chronically infected with hepatitis B virus (HBV), and ~820,000 people die each year from HBV-related complications[1]. Approximately 10% of people with HIV are coinfected with HBV, and tenofovir disoproxil fumarate (TDF) is often a key component in antiretroviral treatment (ART) regimens[2]. However, incomplete HBV suppression in HBV/HIV coinfected patients on TDF-containing ART and tenofovir resistance have been described[3].

The present investigation is a subgroup analysis of a cross-sectional study of viral suppression in 138 HBV-HIV coinfected patients on TDF-containing ART in Ghana[4]. In this study, serum samples from nine patients with incomplete HBV suppression / detectable HBV DNA levels were analyzed for the presence of previously-reported resistance-associated HBV mutations using next-generation sequencing. Adherence to ART was measured using TDF levels in peripheral blood mononuclear cells which were consistent with seven doses per week in seven of the nine subjects.

Four of nine patients were male with a median age of 40 years. The average ALT and AST levels were 36.2 and 47 U/L, respectively. Pre-enrollment CD4+ counts ranged from 37 to 849 cells/mm^3. Three patients also had positive HIV viral loads. Consensus nucleotide sequences from eight patients were identified as HBV genotype E – the dominant genotype in western Africa – while one sequence

was a genotype E/A recombinant. Nine distinct point mutations within the HBV reverse transcriptase gene that were previously reported as being associated with tenofovir resistance were present in at least one of the samples. The resistance-associated point mutations rtY9H, rtH126Y, rtF221Y, and rtC256S were present in all nine sequences.

These findings support the previously-posited conclusions that tenofovir resistance-associated HBV mutations may exist and be of clinical importance. Analysis of drug resistance mutations in HBV-HIV coinfected individuals will enable optimized HBV treatment strategies in areas where genomic analysis of HBV is feasible.

BOARD 26

Antimicrobial Effects of Some Plant Extracts on Pseudomonas aeruginosa

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Plants have been known for a long time to contain compounds that have bioactivity against different types of bacteria, fungi and viruses. In this study, methanol and ethanol extracts of peppermint, and pomegranate rind were prepared to be evaluated against Pseudomonas aeruginosa using the well-diffusion method. The overnight bacterial culture was streaked on nutrient agar plates and 7mm wells were made and 100uL of each extract was placed in the well. The plates were let to stand so the agar would absorb the extract before they were incubated up-side down at 30°C overnight then the inhibition zones were measured in millimeters (mm). The assessment for each extract was done in triplicates, and the control was the solvent used in the extraction. The initial results showed that both methanol and ethanol extracts of pomegranate rind showed a moderate inhibition of Pseudomonas growth. The inhibition was in the range of 16mm-20 mm which seems to be promising. Neither of the extracts of peppermint showed inhibition of the growth of this bacterium. The measurement of the pH was to determine their stability through study. The data shows that extracts of the studied plants were stable with a net pH change of less than 10% over four weeks. The primary results show the potential of using pomegranate rind as a source for natural inhibitors for bacterial growth. The work is still in progress to determine the minimum inhibitory concentration of pomegranate rind on P. aeruginosa and compare it with standard antibiotics.

BOARD 27

Altered Profiles of NK Cells and Monocytes During Severe COVID-19 May Heighten the RIsk of Cardiovascular Disease

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The post-acute sequelae of SARS-CoV-2, commonly called LONG COVID, have been associated with many lingering symptoms. Altered immune responses and persistent inflammation could impact vascular health, leading to long-term cardiovascular complications. Evidence indicates heightened susceptibility to atherosclerotic cardiovascular diseases (CVDs) in COVID-19 patients, with less understood mechanisms.

Traditionally monocytes and macrophages contribute to atherosclerosis; however, the role of natural killer (NK) cells in specifically COVID-19 associated atherosclerosis is unclear. In this study, we compared immune subset phenotypes and plasma biomarkers in severe COVID-19 patients (n=29), recovered individuals (n=30), and uninfected controls. Two groups were compared using the Wilcoxon rank-sum test and three groups were compared through a one-way ANOVA followed by Dunn's multiple comparison test for pairwise comparisons (significance at $p<0.05$). We found that severe cases exhibited dysregulated monocyte subsets, indicated by boosted frequencies of circulating pro-inflammatory intermediate monocytes (which were also observed in recovered patients) and lowered levels of non-classical monocytes which are known for their potential to perform trans-endothelial migration. In the severe group, all NK subsets (determined on CD56 and CD16 expression) exhibited reduced frequencies, while many simultaneously displayed an activated phenotype through increased expression of activation markers such as CD69. We also discovered increased levels of plasma biomarkers associated with cardiovascular risk in severe patients. Levels of circulating oxidized low-density lipoprotein (Ox-LDL) and its chaperone lipoprotein-associated phospholipase A2 (LpPLA2) were decreased in both severe and recovered groups. Our in vitro assays further confirmed the role of activated NK cells for Ox-LDL uptake by monocyte-derived macrophages. Transcriptome analysis supported enriched pro-inflammatory responses and lipid dysregulation, coupled with epigenetic modifications in monocytes and NK cells during severe COVID-19 ($p<0.05$ for all). Our study offers novel insights into the interplay between monocytes and NK cells, shedding light on the immunopathogenesis of CVD risk in COVID-19 infection.

BOARD 28

The Role of Rps26-Deficient Ribosomes in Sugar Metabolism

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Ribosomes are complex macromolecules composed of seventeen ribosomal proteins. One of these proteins is Rps26, which has been shown to be paired with chaperone protein TSR2 and taken off the ribosome during cellular stress. The accumulation of Rps26-deficient ribosomes leads to an entirely new set of mRNAs being preferentially bound to the ribosome, indicating an alternate function for these deficient ribosomes. This led to the question of whether or not Rps26 deficient ribosomes play a role in sugar metabolism. To test this, a series of growth curves on yeast cells were conducted in which cells were grown on a fermentable carbon source- dextrose- or non-fermentable carbon sources- glycerol or ethanol- to test the optimization of non-fermentable carbon source metabolism in Rps26-normal cells and Rps2-deficient cells. Rps26-deficient ribosomes were found to have a significant fold-change growth recovery on non-fermentable carbon sources compared to their counterparts, indicating a direct role in sugar metabolism. Further study is necessary to determine the mechanism of this growth recovery, and to ensure that Rps26-deficiency is the cause.

BOARD 29

Freshwater ammonia oxidizing bacteria show decreased growth rates under copper and nickel stress

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Nitrification is an important process in the nitrogen cycle. During nitrification, ammonium is converted to nitrite then further to nitrate. Ammonia-oxidizing bacteria (AOB) perform the first-rate limiting step of the nitrification by oxidizing ammonium to nitrite. AOBs are ubiquitous and play a crucial role in terrestrial, marine, freshwater and wastewater systems. Within freshwater and wastewater systems, heavy metals such as copper and nickel from either anthropogenic or environmental sources impede growth of bacteria including AOBs and can thus inhibit nitrification. To study the effect of heavy metal stress on AOBs, six different strains were grown under varying concentrations of both copper and

nickel. All six strains were isolated from different freshwater environments, such as Lake Acton (Ohio), Lake Superior (Michigan) and Lake Erie (Ohio). The growth rates were calculated and used to determine inhibition of the growth by copper and nickel. The presence of both copper and nickel caused decreased growth rates of all AOBs, although at differing levels of severity. Copper (Cu^{2+}) concentrations as low as 35 µM, up to 500 µM caused inhibition of growth with differential responses observed across the tested strains. Nickel (Ni^{2+}) inhibited growth of the AOBs at lower concentrations than copper. Two of the six strains were inhibited at 2.5µM nickel while the remaining strains were inhibited at 25 µM nickel. The six tested strains responded differently to copper and nickel stress vastly, highlighting that heavy metal contamination can have varying responses on nitrification based on the AOBs present, and their adaptations to the environment.

BOARD 30

Evaluating the Role of SCO1215 in *Streptomyces* Bacteria

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Using various bioinformatics tools, investigation of an unknown gene of interest for *Streptomyces* bacteria that was identified by transposon mutagenesis as producing less gray spore pigment has translated into research to uncover more about the gene's function. SCO1215 has nine-hundred and fifty nucleotides and is located in a potential operon with SCO1216. SCO1215 encodes a three-hundred and seventeen amino acid protein that is a probable integral membrane protein. It contains a Pfam Caa3_CtaG domain that is found in the CtaG protein of *Bacillus subtilis* responsible for the formation of active cytochrome caa(3). The protein is highly conserved among *Streptomyces* species as well as outside the genus. The Phyre2 Protein Fold Recognition Server predicted it to best match to the crystal structure of heme a synthase from *Bacillus subtilis* and domain mapping revealed six transmembrane helices spread out between the N-Terminal and C-Terminal domains. All computer models applied to the study of SCO1215 benefit further lab research and support its role in the diversity of cytochrome *c* oxidase proteins in bacteria.

BOARD 31

Patterns of symbiotic bacterial community composition and innate immune system complexity across insects

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All animals, including humans, evolved within a microbial world, resulting in metazoans maintaining a myriad of symbiotic relationships with microbes that range from antagonistic to mutualistic, single obligate endosymbionts to complex, species-rich microbiomes. Host-associated bacterial microbiomes have demonstrated multifaceted importance to animal health and development due to essential functions such the ability to degrade dietary components into beneficial metabolites, remove toxic compounds, and provide protection from pathogens. Despite near universal essentiality, symbiotic microbiomes show very different compositions and functions across host diversity. We chose to examine insects, which among metazoans exhibit the greatest amount of diversity along the following axes: innate immune gene family repertoire, ecological niche occupancy, microbiome compositionality (i.e., diversity and abundance) and host-microbe relationships (i.e., mutualism, parasitism, commensalism). At the interface between insect host and symbiont is the innate immune system where these direct interactions influence microbiome assembly and maintenance. Innate immunity is present in all metazoans, with several features being conserved over deep evolutionary time, and, while its role in pathogen defense is acknowledged, its role in the maintenance of microbiome homeostasis is emergent. Previous studies have demonstrated that many animals maintain a microbiome with high specificity in composition, however the host genetics underpinning this maintenance is unclear. Therefore, we hypothesize that evolutionarily conserved interactions between host and microbiome are identifiable through the differences in microbiome diversity and innate immune system gene complexity between different species. Here we begin to address this hypothesis by examining patterns of both microbiome composition and immune gene evolution

across 48 insect genera. We have taken a meta-analysis approach leveraging existing bacterial community and host genome sequencing efforts. We demonstrate the phylogenetic signal present across insect microbiome compositions, and how this intersects with patterns of innate immune gene evolution.

BOARD 32

Feather-Degrading Bacteria: Screening and Characterization of Isolates from Microbial Collections

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Bird feathers can be incorporated into various products from agriculture, textiles, cosmetics, biomedicine, and environmental remediation, thus presenting a valuable opportunity for sustainable use. While previous studies have identified microbial communities capable of feather degradation, research on their enzymatic properties is still needed. Therefore, our study aimed to investigate the keratinolytic activity of bacteria isolated from avian feathers.

OWU has a collection of over 3000 bacterial strains isolated from wild songbirds. Screening 50 bacterial strains for their feather-degrading capabilities involved qualitative assessment (observing the remaining feathers) and quantitative analysis (measuring oligopeptide levels). *Bacillus licheniformis* O.W.U.138B, known for its keratinase production and feather degradation capacity, was utilized as a control. Thus, on day seven, strain 4587T demonstrated the highest oligopeptide concentration, approximately 4.3 times greater than the lowest concentration observed. The control strain B138 exhibited an oligopeptide concentration approximately twice as high as the lowest recorded concentration. Keratinase activity varied considerably among the strains, with strain 5159B exhibiting the highest activity on the second day, reaching a concentration approximately ten times greater than the lowest concentration recorded for strain B138. Additionally, the assessment of percentage feather degradation based on featherweight loss on day four revealed 94% degradation for strain 5159B, 75.91% for the control strain B138, and 66.22% for strain 4587T. Physiological assessments of carbohydrate consumption and other biochemical traits showed metabolic variation between the strains, which could influence their keratinolytic activity.

The bacterial strains were identified through the analysis of the 16S rRNA gene sequence. BLAST analysis revealed a high percentage of sequence homology with related entries in the NCBI GenBank, leading to the identification of the strains as *Bacillus cereus*, *B. subtilis*, and *B. licheniformis*.

Further studies are needed to elucidate molecular mechanisms underlying keratin degradation and explore their applications in bioremediation and bioconversion processes.

BOARD 33

Viral Predictors of BK Polyomavirus Associated Hemorrhagic Cystitis

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Introduction: BK Polyomavirus (BKPyV) associated hemorrhagic cystitis (HC) is a common complication in hematopoietic cell transplant (HCT) recipients. Though viruria occurs in 80% of individuals, only 25% will develop HC. BKPyV exists as 4 genotypes and multiple subtypes. Further investigation is needed to determine how BKPyV diversity impacts the pathogenesis of HC. This study aimed to determine circulating BKPyV genotypes in a cohort of pediatric allogeneic HCT recipients to validate previous results reported by our group.
Methods: Viral DNA was extracted from urine samples and linearized. PCR amplification of the full-length BKPyV genome was performed, and PCR products were run on a 1% agarose gel. If a sample yielded no visible product, nested PCR amplification of the viral protein 1 (VP1) region was performed. All PCR positive bands

were gel extracted and sent for next-generation sequencing. A phylogenetic tree including GenBank references was used to determine the sample genotype.
Results: 61 urine samples from subjects with median age of 9.1 years were included. 23 (38%) were positive for the full-length BKPyV genome, and 1 sample was positive for VP1, yielding a total positivity rate of 39% (24 of 61). 37 samples could not be amplified (61%). Genotype Ia was present in 11 of the 24 amplified samples (46%). Genotype Ib1 was present in 7 samples (29%). Genotype Ib2 was present in 3 samples (13%). Genotype II was present in 2 samples (8%). Genotype IV was present in 1 sample (4%)
Conclusions: The most common genotype in this pediatric HCT cohort was Ia, followed by Ib1, Ib2, II, and then IV. The presence of two genotype II samples and one genotype IV sample is of particular interest as these genotypes are rarely observed in the USA.

BOARD 34

Characterization and investigation of some oligotrophic actinobacterial species in Lake Fryxell

Joseph J. Morgan*, Rachael Morgan-Kiss
Miami University

Antarctic lakes are an extremely unique environment. Many stressors exist here that do not exist in this combination anywhere else on Earth. Lakes in the Taylor Dry Valleys share these conditions and therefore force microbes which live there to adapt to a wide array of variables, some of which include extreme cold, low precipitation, seasonal lack/excess of sunlight, and above average ionizing radiation. Some bacteria seemingly predisposed to such a harsh environment are aquatic actinobacteria, some of which are extremely oligotrophic and rely on other bacteria to create necessary components to survive. Actinobacteria, or actinomycetota, are, in general, saprotrophic bacteria similar to fungi, for which they are named. They are usually found in the soil and have capabilities involved with the decomposition of plant and animal matter. However, in the aquatic food web, certain actinobacteria display a different phenotype based around oligotrophy, the most well known of these being the acI lineage actinobacteria. In order to identify these unique organisms, an existing dataset of 16S rRNA sequences previously recovered and sequenced from the McMurdo Dry Valley Lakes in Antarctica will be analyzed using statistical methods to determine: (1) clades of organisms whose abundances that are significantly different from those in a temperate lake (Lake Acton, Ohio); (2) the difference in community structure inside Lake Fryxell between the moat region and the less extreme under-ice environment. The outcome of these activities will be to further describe the unique ecology of this understudied region, with a focus on the role of actinobacteria as part of this ecosystem.

BOARD 35

Immunodynamics of Chronic SIV Infection in Mesenteric Lymphoid Tissue

Jacqueline Stewart*[1], Manuja Gunasena[1], Mario Alles[1], Namal Liyanage[1]
The Ohio State University, Department of Microbial Infection and Immunity[1]

Secondary lymphoid organs play a crucial role in the immune system's ability to respond to localized infections and orchestrate an efficient immune response upon antigen re-stimulation. In the context of chronic HIV infection, the secondary lymphoid organs in the gut serve as a valuable model for understanding pathogenesis and the formation of reservoirs. In this study, we utilized a model of HIV infection in rhesus macaques chronically infected with simian immunodeficiency virus (SIV) to study immune dynamics in mesenteric lymphoid tissue. We conducted a comparative analysis of immune cell profiles and the expression of viral sensing receptors using high-dimensional flow cytometry in eight naïve and eight chronically infected animals. Initially, immune profiling in naïve animals a dominance of intermediate monocytes, CD8+ T cells, and NKG2A-Nkp44- NK cells, with no significant changes observed in the profile of T and B cells. Upon comparisons between naïve and chronic animals, classical monocytes was significantly reduced in the chronic state. Furthermore, our investigation of immune cells in mesenteric lymphoid tissue unveiled increased CD169 (Siglec-1) molecule expression by intermediate monocytes ($p=0.0030$), classical monocytes ($p=0.0062$) and dendritic cells ($p=0.0482$). Notably, the upregulation of CD169 (Siglec-1) expression on monocytes corroborated previous findings in circulating blood monocytes in HIV infection, representing a novel observation in SIV models within mesenteric lymphoid tissue. Additionally, we observed an increase in a4b7 integrin on intermediate monocytes

(p=0.0499) and and dendritic cells (p=0.0078), along with elevated CD43 expression on intermediate monocytes (p=0.0135). Examining the functional profile of these cells, we found higher frequencies of IFN-γ in CD8+ T cells (p=0.0286), increased TNF-α in CD4+ T cells (p=0.0286), and elevated CD107a in NK cells within the chronic state.
This comprehensive analysis sheds light on the intricate immune dynamics within mesenteric lymphoid tissue during chronic SIV infection, offering valuable insights into the pathogenesis of HIV and potential avenues for therapeutic intervention.

BOARD 36

Discovering Protein-Protein Interactions in the Cyclic di-GMP Pathway of Streptomyces

Jonathan Ledesma Garduno*, Jennifer Bennett
Otterbein University

Cyclic di-GMP, also called cyclic diguanylate, is a second messenger used in signal transduction pathways in a wide variety of bacteria. It serves as a regulator for various biological processes within the cell including surface adaptation, cell cycle progression, cell motility and virulence. Therefore, research involved with cyclic di-GMP is important for understanding bacterial behaviors and disease mechanisms. *Streptomyces coelicolor* is a bacterium renowned for its ability to produce a diverse array of bioactive secondary metabolites. Cyclic di-GMP plays a crucial role in regulating the developmental transitions within the *Streptomyces* cell cycle. Cyclic di-GMP controls the initiation of development in *Streptomyces* acting as a signaling molecule to transition from vegetative to reproductive stages. The bacterial two-hybrid system is a powerful genetic approach which identifies protein-protein interactions in vivo. The focus of this research is to utilize the bacterial two-hybrid system by constructing plasmids that contain Cyclic di-GMP metabolizing genes from *Streptomyces coelicolor* and testing the pairwise interactions that occur. We have also begun to determine the likelihood of protein interactions using bioinformatics. These approaches will be used to identify the interactions between proteins that are already known to play a role in the pathway and to identify unknown proteins that interact.

BOARD 37

Influence of temperature and salinity on the growth of freshwater complete ammonia oxidizers (comammox)

Hunter Coon*, Emma Marin, and Annette Bollmann
Miami University, Department of Microbiology, Oxford OH

Nitrification is a process in the global nitrogen cycle in which ammonia is oxidized in two steps: first to nitrite and then to nitrate. These steps are carried out by ammonia-oxidizing bacteria and archaea, nitrite-oxidizing bacteria, and a third group which conducts both steps, complete ammonia oxidizers (comammox). Since their initial discovery in 2015, comammox constitute a relatively understudied group of nitrifiers because the majority of currently characterized comammox strains originate from engineered ecosystems, such as wastewater treatment plants. The first freshwater comammox enrichment culture was characterized in 2023. We used comammox enrichment cultures from three different freshwater sediments to characterize the growth response of these bacteria to the environmental factors temperature and salinity. The influence of temperature was tested by growing the cultures in mineral salts medium with 500 µM ammonium at temperatures between 11°C and 38°C. The same medium with up to 100 mM added sodium chloride was used to investigate the influence of salt on the growth of comammox. The comammox enrichment cultures grew at 11°C with growth rates increasing with increasing temperature up to 30°C. No growth was observed at 38°C. In the presence of salt, the highest growth rates were observed at 0-10 mM added sodium chloride. The growth rates decreased with increasing salt concentrations and no growth was observed at 100 mM added salt. This finding is in accordance with the observation that comammox cannot be detected in marine environments. While further ecophysiological characterization of freshwater comammox is required, the present data provide insights into the impact of environmental factors on complete ammonia oxidizers.

BOARD 38

Persistence of Dysregulated NK Cell Phenotypes Following HIV Acquisition Despite Viral Suppression by Antiretroviral Therapy

Namal Liyanage, Manuja Gunasena, Mario Alles and **Joyjit Majumdar***
The Ohio State University, Department of Microbial Infection and Immunity

Antiretroviral therapy (ART) has been known to improve the immune responses and significantly reduce the morbidity and mortality associated with HIV acquisition. Despite this, those who are virally suppressed with ART continue to be at an increased risk of non-AIDS related comorbidities, due in part to a dysregulated innate immune response. Natural killer (NK) cells, a heterogenous population of innate immune cells, play a crucial role in the pathogenesis of HIV and have been associated with many of its comorbidities. However, the influence of viral loads and/or ART therapy on NK cell immune phenotypes have not been fully elucidated.
We conducted a comparative analysis of immune signatures in Peripheral Blood Mononuclear Cells (PBMCs) among three groups: HIV-positive individuals who were ART naïve (Pre-ART; n=14), the same cohort following viral suppression achieved through ART (post-ART; n=14), and a control group of uninfected individuals (n=12), utilizing high-dimensional flow cytometry. Differences between groups were calculated using one-way ANOVA followed by Dunn's multiple comparison test for pairwise comparisons. Statistical significance was obtained at $p<0.05$.
Our results revealed several activation markers of NK cells such as CD69 and HLADR to be significantly dysregulated between the three groups. Specifically, these were increasingly expressed in the pre-ART cohort and, following ART mediated viral suppression, returned to levels comparable with uninfected controls. However, the expression of CD57, a marker of maturity and senescence in NK cells, remained persistently increased following HIV acquisition and despite viral suppression. Similarly, CD27 expression remained elevated in both pre-ART and post-ART groups suggestive of persistence of functional dysregulation among NK cells ($p<0.05$ for all).
Collectively, our findings suggest that the altered phenotypes of NK cells resulting from HIV acquisition do not fully revert to levels comparable with those of uninfected individuals. This discrepancy may significantly impact the development of non-AIDS-related comorbidities, even in the presence of ART-mediated viral suppression.

BOARD 39

A Novel Role of the Histidine Kinase in *Streptomyces coelicolor* Cell Division

Feng-Thea Lee*, Alexandra Sherman, and Jennifer A. Bennett
Otterbein University

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. If these genes are silenced or deleted in *Streptomyces*, these bacteria will retain their ability for growth. When *ftsQ* is deleted in *Streptomyces*, it causes a loss of septum formation in the aerial hyphae, and therefore a loss in spore formation, which can be visualized under the microscope.
Research performed in this project has resulted in the discovery and characterization of three new *ftsQ*-null suppressor strains, which were demonstrated to partially compensate for the division loss in the *ftsQ*-null mutant. All three strains contain a mutation within the gene *sqnA* (suppressor of *ftsQ*-null). This gene encodes a histidine kinase, located next to a gene encoding a response regulator. These proteins potentially function together as a two-component regulatory system, which has previously been implicated to play a role in bacterial stress response. SCO4155's closest orthilog is a two-component system in *Mycobacterium tuberculosis*, a bacterium known for its highly adaptive qualities in host organisms, its resistance to antibiotic treatments, and its resiliency in response to environmental stress. The three mutant backgrounds were compared with the wild-type using Phyre2's transmembrane prediction software, and the tertiary protein structure visualized with JMOL. There were significant differences, including several additional alpha helixes.
Future research will include the deletion of genes of interest to construct new strains for phenotyping using the Lambda REDIRECT recombinase system in both the wild-type and *ftsQ*-null suppressor strains. Novel

information produced from this study will further elucidate the cell division process by potentially identifying a new role in division for these genes of interest.

BOARD 40

Inhibition of MRSA Using Laser-Exposed Gold-Iron Oxide Nanoparticles

Jordan L. Jones*, William G. Sturrus, and Chester R. Cooper, Jr.
Youngstown State University

Staphylococcus aureus is recognized as a pathogenic organism that causes a wide range of infections. The pathogenicity of *S. aureus* stems from multiple virulence factors including toxin production, biofilm formation, and mechanisms of antibiotic resistance. Strains of *S. aureus* that are resistant to methicillin, termed MRSA, are a particularly significant source of morbidity and mortality. Many MRSA strains are also multidrug resistant making treatment of infections problematic. Hence, a search for novel chemotherapeutic interventions to treat MRSA infections is of critical importance.

The use of nanotechnology to treat bacterial infections has emerged over the last twenty years. Gold-nanoparticles have been studied for their antibacterial properties against Gram-positive species, including *S. aureus*. The present investigation examined the viability of MRSA strains when exposed to laser irradiated, gold-coated iron-oxide nanoparticles (GNPs). MRSA strain ATCC 33591 was suspended in phosphate buffer with or without GNPs, then subjected to 10 minutes of laser (532 nm) exposure. Laser treatment alone or non-laser treated suspensions containing GNPs demonstrated little to no reduction in viability. In contrast, laser-treated *S. aureus* suspensions containing approximately 21,000 GNPs per cell exhibited an average decrease in viability of 93%, whereas laser-treated suspensions containing approximately 11,000 GNPs per cell showed an average 84% decrease in viability. Similar results were obtained with a second MRSA strain (ATCC 43300) and a non-MRSA strain (ATCC 25923). These results suggest that this stratagem presents a potentially novel approach to treating *S. aureus* infections, particularly those due to antibiotic-resistant strains.

BOARD 41

Evolution of a Small Protein from a Signal Peptide to Acquire a New Function Regulating Manganese Homeostasis in *Escherichia coli*

Zachary Wright[1], Mackenzie Seymour[1], Kalista Paszczak[1], Katherine Senn[1], Samuel Stilp[1], Nickolas Jansen[1], Kylee Moore[1], Tyler Lewis[1], **Lauren Waters*[1]**
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Manganese (Mn) is an essential micronutrient that aids survival of pathogenic and symbiotic bacteria in eukaryotic hosts. Mn is also important for free-living bacteria to thrive in stressful environments, such as during oxidative stress. However, like all metals, Mn is toxic in excess. Intracellular Mn levels are tightly controlled by a suite of Mn importers, exporters, and transcription factors to maintain optimal levels under different environmental conditions.

In addition, the enigmatic small protein MntS (42 amino acids) helps regulate Mn homeostasis and intracellular usage in *Escherichia coli*. Small proteins (< 50 amino acids) are ubiquitous across bacterial and eukaryotic genomes, and often function by binding to and regulating larger proteins during stress responses. We show that MntS binds and inhibits the Mn exporter MntP, establishing a new mechanism of regulation for Mn transporters beyond the known transcriptional and post-transcriptional control. We also demonstrate that MntS binds to itself in the presence of Mn, providing a possible mechanism of downregulating MntS activity to terminate its inhibition of MntP Mn export.

Intriguingly, MntS bears homology to the signal peptide (SP) region of SitA, the periplasmic metal-binding subunit of a Mn importer. Remarkably, related SitA SPs can recapitulate MntS activities, showing that they have a second function beyond protein secretion. These SitA SPs are only the second known “functional signal peptides” in bacteria, and the first known in gram-negatives. Conserved gene neighborhoods support that MntS evolved from an ancestral SitA, acquiring a life of its own with a distinct function in Mn homeostasis. Overall, we establish that small proteins can emerge and develop novel functionalities from gene remnants.

BOARD 42

A Heme binding AnkB protein of *Pseudomonas aeruginosa* facilitates peroxide stress induced KatB catalase activity

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Pseudomonas aeruginosa (PA) is a ubiquitous Gram-negative bacterium and an opportunistic multidrug resistant pathogen that has the tendency to form antibiotic resistant biofilms and frequently infects the lungs of CF and COPD patients, as well as causes other infections. PA infection generates a robust respiratory burst (RB) response in phagocytes that generates many bactericidal agents, where hydrogen peroxide (H_2O_2) is the leading DNA-damaging and proton motive force perturbing oxidant. The major PA defense to counter damaged caused by H_2O_2 is induction of a two gene operon, encoding two proteins strategically deployed to the periplasm: (1) a group 1 heme *b*-type catalase (KatB) that converts H_2O_2 into H_2O and O_2 and (2) a putative ankyrin repeat protein (AnkB) of unknown function and structure. Here using a combinatorial approach of knockout mutant studies, biophysical, and X-ray structural studies of AnkB and its interaction with KatB protein, we demonstrate that AnkB is a novel heme binding protein that is required to transfer heme to inactive monomeric KatB to facilitate the formation of catalytically active KatB tetramers. Further, the AnkB structure revealed that it is composed of four ankyrin repeats with a cysteine disulfide bond between the second and third repeats. We identified seven key residues in AnkB that are important for heme binding and that affect KatB activity. This mechanism is likely an important feature of the *in vivo* confrontation between PA and the H_2O_2 component of the RB mediated by phagocytic cells during PA infection. Our study provides key insights in the physiology of multi-drug resistant, biofilm-forming PA that is exposed to phagocyte-derived H_2O_2.

BOARD 43

Neisseria persistent oral colonization causes dynamic alterations in the local and systemic immune landscape

Mario Alles[*1], Manuja Gunasena[1], Tauqir Zia[2], Adonis D'Mello[3], Saroj Bhattarai[2], Will Mulhern[1], Luke Terry[1], Trenton Scherger[1], Saranga Wijeratne[4], Sachleen Singh[5], Asela J. Wijeratne[5], Dhanuja Kasturiratna[6], Hervé Tettelin[3], Nathan Weyand[2] and Namal P.M. Liyanage[1]
[1]The Ohio State University; [2]Ohio University; [3]University of Maryland School of Medicine; [4]Nationwide Children's Hospital; [5]Arkansas State University; [6]Northern Kentucky University

Commensal bacteria shape host physiology and immunity. *Neisseria gonorrhoeae* (NG), a human pathogen, also causes asymptomatic colonization. Up until now, animal studies on NG for the purpose of vaccine-design have been unsuccessful. *Neisseria musculi* (NM), a commensal *Neisseria* species of wild mice shares many genes involved in host-bacteria interaction with NG and is a surrogate to study NG colonization.
Eight A/J mice were orally inoculated with a NM bacterial suspension while controls (n=8) were mock-inoculated with phosphate-buffered saline. Four from each group were euthanized at day 5 and the rest at day 33 following inoculation. Immune signatures from blood, lung and spleen, and the palate transcriptome were compared between mice orally inoculated with NM and control mice using Wilcoxon rank-sum test ($p<0.05$). Associations between bacterial colony counts and immune signatures were explored using Pearson correlation coefficient. Oropharyngeal colonization of NM was established through serial enumeration of NM colonies from oral swabs. Transcriptomics from palate tissue revealed enriched mucosal immunity and inflammation in colonized tissue. Day 5 data suggested reduced colonization resistance (reduced monocytes), while data at day 33 showed enriched adaptive (memory B cells) and immunoregulatory cells (Tregs) in colonized tissue. Immunophenotypes in the adjacent respiratory tissue were similar to above. At day 5 of colonization, systemic immune response (blood and spleen) was pro-inflammatory. Splenic NK cells at day 5 expressed higher IL22, suggesting reinforcement of mucosal immunity during early colonization. At day 33 of colonization, the systemic immune response displayed reduced enrichment of immune signatures compared to that of day 5 of colonization suggesting immune homeostasis. Positive correlations between NM palate colony counts and enriched immune subsets suggest the impact of mucosal colonization on immunophenotypes ($p<0.05$ for all).

This data is crucial for defining transcriptional and immunological endpoints of *Neisseria* mucosal colonization, aiding in developing vaccine candidates against NG.

Abstracts of OBASM Podium Presentations

Saturday, April 6
10:30 am – 12:15 pm

10:30 – 10:45 am

Increased KLRG1 expression on NK cells following BCG immunization may compromise their antiviral responses against HIV.

Manuja Gunasena*[1,2], Mario Alles [1], Will Mulhern[1], Richard Robinson[1] and Namal P.M Liyanage[1,2]
[1]Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, [2]Department of Veterinary Bioscience, College of Veterinary Medicine, The Ohio State University

Bacillus Calmette-Guérin (BCG) vaccine is the only approved vaccine against tuberculosis (TB), which is typically administered shortly after birth to protect children against BCG elicits non-specific immunological responses in addition to its known TB-specific effects. NK cells are specifically stimulated to perform memory-like functions, which improves the immune system's reactivity to different infections. These reactions could affect the host in both negative and positive ways, illustrating the varied consequences of BCG that go beyond TB prevention.
This study looks into how the BCG vaccination affects NK cell responses and how it changes a person's susceptibility to HIV infection. The splenic NK cell responses of four unvaccinated mice (n = 4) and four BCG-vaccinated mice (n = 4) were compared using the C57BL/6 animal model. The study examined the immune-dependent alterations in mice's cells by subjecting isolated lymphocytes to HIV Gag peptide and Mycobacterium bovis lysate stimulation for six hours in vitro, and then monitoring the results using multicolor flow cytometry. The obtained data indicated a significant ($p<0.05$) upregulation of Killer cell lectin-like receptor subfamily G member 1 (KLRG1) expression in the splenic NK cells of mice that received the BCG vaccination. When exposed to mycobacterial antigens, these KLRG1-expressing cells did not exhibit any functional reduction; nevertheless, when exposed to HIV Gag antigens, there was a notable decrease in functionality. Particularly, NK cells with KLRG1+ (potentially BCG-trained) showed a significant reduction in the production of IFNγ, TNFα, IL1β, and Granzyme B along with a decreased polyfunctionality ($p<0.05$). HIV peptide exposure-related negative associations between NK polyfunctionality and KLRG1+ NK cells imply that these BCG-trained NK cells are less effective at combating HIV infection. This suggests that past BCG vaccination may increase vulnerability to HIV acquisition, especially in areas with high rates of HIV exposure.

10:45 – 11:00 am

Single-cell RNA sequencing of the fungal pathogen, *Pneumocystis carinii,* for determination of its life cycle

Aaron W. Albee*[1,2], Steven G. Sayson[1,2], Alan Ashbaugh[1,2], Aleksey Porollo[3], Melanie T. Cushion[1,2]
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Pneumocystis species are fungal pathogens that are obligated to live in host mammalian lungs, causing pneumonia in immunocompromised individuals. We developed a method to create viable single-cell *Pneumocystis carinii* (Pc) suspensions for RNA sequencing. Using a gradient separation technique, Pc populations of trophic and ascus stages were obtained from infected rats' bronchial alveolar lavage fluid. The samples were sequenced using the 10X Chromium X single cell 3' v3.1. Cluster analysis was performed on gene differential expressions to identify unique developmental stages. This data can be used to develop biomarkers and improve targeted treatment strategies by identifying specific stages of development.

11:00 – 11:15 am

ZFP36L1 Suppresses Virus Replication Independent of Poly(A) Tail Deadenylation

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ZFP36L1, a CCCH-type zinc figure protein (ZFP) is recognized for its involvement in RNA stability and its decay via poly A tail deadenylation [removal of the poly(A) tail from mRNA]. This ability allows ZFP36L1 to exhibit antiviral activity against a variety of viruses, including flaviviruses, retroviruses, and alphaviruses. Our preliminary investigation demonstrated the antiviral role of ZFP36L1 against the human coronavirus OC43 (HCoV-OC43) and murine norovirus 1 (MNV1). The overexpression of ZFP36L1 reduced the HCoV-OC43 production, whereas its knockdown significantly enhanced the virus production. We aimed to understand how ZFP36L1 influences viral replication. To gain further insight into the role of poly-A tail deadenylation in the suppression of HCoV-OC43 by ZFP36L1, we knocked down CNOT1, a key regulator of mRNA decay, in ZFP36L1 overexpressed cells. Results showed that the virus titer reduction was not rescued in CNOT1 knockdown cells compared to the overexpressed ZFP36L1 cells. These results indicated that the reduction in HCoV-OC43 replication was independent of poly-A tail deadenylation.
We further employed computational analysis, utilizing RNA-protein interaction prediction software and docking simulations, which indicated a significant interaction between ZFP36L1 and the viral nucleocapsid. Further investigations into ZFP36L1's effect on nucleocapsid expression revealed that ZFP36L1 overexpression led to a decrease in nucleocapsid expression suggesting a direct interaction between ZFP36L1 and viral components that may underlie its antiviral activity. We have also performed RNA immunoprecipitation experiments which have shown the interaction between ZFP36L1 and the nucleocapsid. This interaction will further be validated using luciferase assays. Overall, our study showed an additional pathway by which ZFP36L1 suppresses the virus replication.

11:15 – 11:30 pm - Break

11:30 – 11:45 am

Metabolic functional redundancies may dampen microbiome functional 'service outages' resulting from diet-induced species losses.

Kayla Cross[*1], Noelle Beckman[2], Benjamin Jahnes[1], Zakee Sabree[1]
[1]Ohio State University; [2]Utah State University

The gut microbiota of many animals is species-rich and diverse, playing a crucial role in diet processing and nutrient provision. Changes in diet can alter the composition and diversity of the gut bacterial community, but the extent to which this affects the metabolic functional capabilities of the microbiome is not well understood. Perturbations leading to the loss of microbiome taxa and their associated metabolic functions may result in a decline in microbiome-level services. However, if metabolic functions are conserved across multiple taxa (i.e. functional redundancy), including distantly related lineages, the impact of taxon/function losses may be mitigated. We fed two nutritionally imbalanced diets to omnivorous *Periplaneta americana*, the American cockroach, over eight weeks to perturb their typically species-rich gut microbiomes. This resulted in a dramatic loss of up to 25% of family-level taxa based on 16S rRNA gene amplicon sequencing. However, inference of microbiome metabolic capabilities after feeding on imbalanced diets revealed changes only up to 15% of pre-treatment metabolic functionality. Our findings suggest that the nonlinear relationship between taxon and functional losses is due to conserved metabolic functions between microbiome members, where remaining lineages are potentially capable of preventing microbiome 'service outages' due to functional redundancy. Despite diet-induced taxonomic variability, many metabolic features of the gut microbiome remain intact, likely due to being performed or produced by several constituent species.

11:45 – 12:00 pm

Microfungal distribution & Interactions in Antarctic Lake Bonney across seasonal and spatial scales

Eckhardt Karsten*[1], *Emily Reynebeau[2], Cristina Takacs-Vesbach[2], Rachael Morgan-Kiss[1]*
[1]Miami University, [2]University of New Mexico

Fungi are recognized as critical players in terrestrial ecosystems, though their role in aquatic ecosystems has been historically understudied. Improved molecular techniques have allowed for advancements to be made in understanding aquatic fungal ecology and the larger impact they have on protists and microbial food webs. As saprotrophs and parasites of algae, fungi can have major consequences on the carbon cycling of an aquatic system, especially those dependent on autochthonous carbon such as ice-covered lakes. The McMurdo Dry Valleys of Antarctica (MDVs) represent one of the driest and coldest deserts in the world, housing a collection of perennially ice-covered lakes. These lakes are natural laboratories for exploring microbial interactions, though research has been largely limited to the austral summer due to the difficulty of accessing the region in winter. The deployment of a suite of Autonomous Lake Profile Samplers (ALPS) into Lake Bonney has enabled the collection of physicochemical & biological samples about every three weeks over an entire year. The ALPS project provides an opportunity to explore seasonal changes in microbial community structure and potential interactions. By exploring diversity metrics and generating association networks, fungal diversity and function can be assessed across depth and season which allows for a clearer view on the microbial loop within polar lakes. Preliminary 18S rRNA gene amplicon data suggests that pathogenic fungi are significant network members across all seasons, increasing in connectivity with the onset of polar night.

12:00 – 12:15 pm

Identification of RNA:RNA Interaction Partners in *Staphylococcus aureus*

Julia Tennant*[1], Paul Briaud[1], Rachel Zapf[1], David Lalaouna[2], Ronan Carroll[1]
[1]Department of Biological Sciences, Ohio University, Athens, OH; [2]University of Strasbourg, IBMC-CNRS, Strasbourg, France

Staphylococcus aureus is a Gram-positive opportunistic pathogen that causes a range of infections, from skin and soft tissue infections to endocarditis and sepsis. Methicillin-resistant *S. aureus* (MRSA) infections are of concern in many U.S hospitals due to the difficulty of treating antibiotic resistant infections. The complex regulatory network present in *S. aureus* allows for the pathogen to rapidly adapt to the environment, being able to shift from a commensal lifestyle on the skin to producing virulence factors and disease within a host. Many of these virulence factors are controlled by small regulatory RNAs (sRNAs). Our lab previously identified a novel small RNA, Teg41, that has pleiotropic effects on several *S. aureus* virulence factors. Disrupting the gene encoding Teg41 leads to attenuation of virulence in both localized and systemic murine infection models. RNAseq analysis, performed at three distinct timepoints, showed that expression of up to 200 genes was impacted when the Teg41 gene was disrupted. Importantly, seven genes showed altered expression at all three timepoints tested, including immunoglobin G binding protein (*spa),* an ABC transporter (*SAUSA300_2453)*, D-lactate dehydrogenase (*ddh)*, and the a-phenol soluble modulins (*PSMa).* To determine if any of these Teg41-regulated transcripts are direct targets for binding by Teg41, we performed a MS2-affintiy purification coupled with RNA sequencing (MAPs) analysis. MAPs allows the isolation of sRNAs and their interacting partners without relying on RNA chaperones like Hfq (which is nonfunctional in *S. aureus*). Our MAPs analysis identified several direct interaction partners of Teg41, including *spa* and *SAUSA300_2453* both of which were identified by RNAseq as being Teg41-regulated. These results suggest that *spa* and *SAUSA300_2453* are direct targets of Teg41-mediated regulation, which contribute to the overall understanding of sRNA mediated regulation and regulatory networks that facilitate the switch in *S. aureus* from colonization to infection.

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. and Jann M. 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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