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Acknowledgements

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OBASM Executive Committee

Jennifer Bennett
Erin Murphy
D.J. Ferguson
Christine Weingart
Chet Cooper
Laura Tuhela-Reuning

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Paul Eisenstein, Dean of Arts and Sciences, Otterbein University
Jennifer Bennett – Site organizer

Kim Finer – Region 3 Branch Planning Coordinator
ASM Branch Lectureship Program
The American Society for Microbiology

Invited Speakers

Michael Ibba
Melanie Mormile
Hans Wildschutte
Zakee Sabree
Kou-San Ju
Kate Excoffon
Tim Wilson
Melody Mills

Program Schedule for OBASM 2017

Friday, March 31

6:00 – 7:00 pm **Registration –** Battelle Lobby, Battelle Fine Arts Center
Poster Setup and Social – Science Center Atrium

7:00 – 7:10 pm **Welcome Statement –** Riley Auditorium, Battelle Fine Arts Center
Paul Eisenstein
Dean of Arts and Sciences
Otterbein University

7:15 – 8:15 **ASM Branch Lecture** – Riley Auditorium, Battelle Fine Arts Center

"Translational control of antibiotic resistance (the curious story of a protein that wants to be an RNA)"

Michael Ibba
Professor and Chair, Department of Integrative Biology
Ohio State University

8:15 – 9:00 pm **Social** – Science Center Atrium

Saturday, April 1

7:30 – 8:30 am **Registration** – Battelle Lobby, Battelle Fine Arts Center
Poster set-up - Science Center Atrium

8:30 – 10:00 am **OBASM Keynote Lecture** – Riley Auditorium, Battelle Fine Arts Center

"Are There Martians in Australia? How Acid Saline Lakes Can Serve as a Mars Analog"

Melanie Mormile
Department of Biological Sciences
Missouri University of Science and Technology

10:00 – 10:30 am **Break**
Snacks and beverages

10:30 am – 12:00 pm **Symposia – Concurrent Sessions**
Session 1: Environmental and Applied Microbiology

Session 2: Pathogenic and Clinical Microbiology

Session 1: Environmental and Applied Microbiology – Riley Auditorium

Moderator: D.J. Ferguson

10:30-11:00 **Hans Wildschutte** – Department of Biological Sciences, Bowling Green State University
"A Small World Initiative Strategy to Streamline the Identification of Gene Clusters Involved in Antagonistic Activity"

11:00-11:30 **Zakee Sabree** – Department of Microbiology, The Ohio State University
"Genomic and Physiological Evidence for Beneficial Host-Microbial Symbioses"

11:30-12:00 **Kou-San Ju** – Department of Microbiology, The Ohio State University
"Systems Biology for Natural Product Discovery: From Genomes to New Antibiotics"

Session 2: Medical Microbiology - Roush Hall Room 114

Moderator: Christine Weingart

10:30-11:00 **Kate Excoffon** – Department of Biological Sciences, Wright State University
"Win the fight before it starts: preventing adenovirus infection"

11:00-11:30 **Tim Wilson** – Department of Microbiology, Miami University
"Paradoxical Roles for SLAM Family Receptors in Immunity"

11:30-12:00 **Melody Mills** – National Institutes of Health
"NIH-NIAID Microbiome Science: Bench to Bedside"

12:00 – 1:30 pm **OBASM Business Meeting**

or **lunch on your own**

1:30 – 3:20 pm **Poster presentations and judging** – Science Center Atrium
Coordinator – Chet Cooper

3:30 – 5:25 pm **Podium presentations and judging** – Roush Hall Room 114
Coordinator – Chet Cooper

3:30 – 3:45 pm
Isoform Specific Targeting of the Coxsackie and Adenovirus Receptor with the CRISPR/CAS-9 Gesicle System

James M Readler*[1], Priyanka Sharma[1], Katherine J.D.A. Excoffon[1]
[1]Wright State University

3:45 – 4:00 pm
Quantification and Characterization of the Intracytoplasmic Membranes of Methanotrophic Bacteria by Fluorescence Microscopy

Kyle Whiddon*[1] and Michael C. Konopka[1]
[1]The University of Akron

4:00 – 4:15 pm

Sulfide Generation by Dominant Colonizing *Halanaerobium* Microorganisms in Hydraulically Fractured Shales

Anne E. Booker*[1], Mikayla A. Borton[1], Rebecca Daly[1], Sue Welch[2], Carrie D. Nicora[3], Shikha Sharma[4], Paula J. Mouser[5], David Cole[2], Mary S. Lipton[3], Kelly C. Wrighton[1], and Michael J. Wilkins[1,2]
[1]Department of Microbiology, The Ohio State University
[2]School of Earth Sciences, The Ohio State University
[3]Mass Spectrometry Group, Pacific Northwest National Laboratory
[4]Department of Geosciences, West Virginia University
[5]Department of Civil, Environmental, and Geodetic Engineering, The Ohio State University

4:15 – 4:30 pm

***In vitro* reconstitution of the Glycine Betaine:Coenzyme M Methyl Transfer Pathway from *Methanolobus vulcani* B1d**

Adam J. Creighbaum* and D.J. Ferguson, Jr.
Miami University

10 minute break

4:40 – 4:55 pm

Roles of listeriolysin O, InlA, and InlB in host cell invasion by *Listeria monocytogenes*

Christopher Phelps*[1], Eusondia Arnett[1], Stephen Vadia[2], and Stephanie Seveau[1]
[1]The Ohio State University; [2]Washington University

4:55 – 5:10 pm

Functional and phylogenetic novelty in the Candidate Phyla Radiation (CPR) found within three Ohio aquifers

Danczak RE*[1], Kenah C[2], Slattery M[2], Johnston MD[3], and Wilkins MJ[1,3]
[1]The Ohio State University Department of Microbiology, [2]Ohio Environmental Protection Agency, [3]The Ohio State University School of Earth Sciences

5:10 – 5:25 pm

Establishing the role of redox equilibrium in *Listeria monocytogenes* anaerobic virulence regulation

Nathan Wallace[1], Ashley Zani[1], Eric Newton[1], Yvonne Sun[1]
[1]Univeristy of Dayton

5:30 – 6:30 pm **Careers in Microbiology Discussion Panel** – Roush 114

Jazmine Quinn – Research Technician, Battelle
Melanie Mormile – Professor, Missouri University of Science and Technology
Melody Mills – Program Officer, National Institutes of Health

6:30 – 8:30 pm **Banquet and Student Awards Presentations** – Otterbein Campus Center

Abstracts of OBASM Poster Presentations
Saturday, April 1
1:30-3:20 pm

BOARD 1

SEA Miami: Trends in Chlorophyll-a and Dissolved Oxygen Saturation in the Water Column

Marie Claire Papamarcos*[1], **Alex Lanoue*[1]**, Thatcher Creber[1],
Audrey Meyers[2] and Rachael Morgan-Kiss[1]
Miami University[1]; SEA Semester Program[2]

This project was designed to determine if and what the relationship between chlorophyll-a fluorescence and dissolved oxygen saturation is observed in the water column south of Puerto Rico. Since dissolved oxygen is produced as a byproduct of photosynthesis, it was believed that chlorophyll-a could be used a predictor of the location of photosynthetic organisms in the water column. The original hypothesis was that there was a relationship and that it was as chlorophyll-a (chl-a) increased, dissolved oxygen percent saturation would also increase. However, since the results showed that oxygen saturation would peak and begin to decline before the chlorophyll-a fluorescence started to peak, the hypothesis was rejected. The study was conducted with two probes, one for oxygen and the other for chlorophyll-a fluorescence attached to a hydrocast carousel that was lowered to a depth 600M below the surface. The oxygen probe collected both the actual saturation of oxygen in the water as well as the theoretical saturation levels using temperature, salinity and pressure readings. Using the actual and theoretical saturation, percent saturation was calculated and plotted with chl-a fluorescence against depth to find any trends. The data from all five deployment locations showed similar trends between oxygen and chl-a that were not consistent with the hypothesis. When plotted against each other, there was no visible relationship between dissolved oxygen and chl-a, which ultimately led to the rejection of the hypothesis.

BOARD 2

Bacteriophage Therapy Application in a Cystic Fibrosis Tissue Culture Model

Ryan Vagedes*[1]
[1]Denison University

Burkholderia cenocepacia is associated with high rates of mortality and morbidity in cystic fibrosis (CF) patients. Due to its resistance to antibiotics, there is an urgent need to develop alternatives to treat infections. Bacteriophage therapy is a potential antibiotic alternative. The objective of this study was to test the efficacy of phage therapy in treating *B. cenocepacia* infections in a CF tissue culture model. Human A549 lung carcinoma cells in 60% artificial sputum medium (ASMDM) were infected with *B. cenocepacia* K56-2. Subsequently, KP4 phage was either previously incorporated in the ASMDM as a prophylaxis or delivered as a treatment. After 24 hours post-treatment, trypan blue assays and standard plate counts were used to measure lung and bacterial cell concentration, respectively. Infected lung cells exposed to KP4 phage exhibited significantly lower cell death compared with no phage (ANOVA, $p<0.0001$). In the treatment and prophylaxis conditions, KP4 phage was also observed to significantly reduce *B. cenocepacia* K56-2 CFU/mL in the supernatant (t-test, $p=0.046$ and $p=0.041$, respectively) and in the lung cell layer (t-test, $p=0.039$ and $p=0.0314$, respectively) compared to the no phage condition. In an effort to understand whether phage affects *B. cenocepacia* K56-2 over time, the model was modified to observe bacterial concentration over four days. While bacterial concentrations were significantly lower one day post-treatment (t-test, $p=0.006$), they did not significantly differ two, three, and four days post-treatment. Although phage reduced *B. cenocepacia* pathogenesis by significantly lowering lung cell death and decreasing bacterial concentration one day after treatment, the effect of phage still poses unique challenges to treatment. Further research is needed to explore the pharmacology of phage therapy in the CF tissue culture model and the efficacy of a combination phage-antibiotic treatment.

BOARD 3

Novel Biocide AB569 Is Able To Kill ESKAPE Pathogens in Planktonic and Biofilm States

Cameron T. McDaniel*[1], Warunya Panmanee[1], and Daniel J. Hassett[1]
[1]University of Cincinnati, College of Medicine, Department of Molecular Genetics, Biochemistry, and Microbiology

As medicine progresses in our society, we have seen a rise of pathogens that are able to bypass our advanced treatments. Chief among these are antibiotic resistant bacteria, which have become a major problem for our healthcare system, increasing morbidity and mortality in patients and costs for healthcare providers. The most notorious of these organisms are the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp.). As resistance develops at rates similar to our ability to make new antibiotics, it is clear that we need alternative therapeutics to combat these organisms. Our efforts have focused on a novel biocide, AB569, composed of low concentrations of $NaNO_2$ and EDTA which we believed would be able to kill these organisms based on bactericidal capabilities of each of the compounds alone. To perform this study, we challenged ESKAPE pathogens grown in both planktonic and biofilm states. We challenged planktonic organisms via 96-well plate checkerboard fractional inhibitory concentration (FIC) assays with increasing concentrations of AB569. In addition, we challenged established biofilms of these species, using the same checkerboard approach and analyzing the cells via flow cytometry and a live/dead stain. Checkerboard FIC analysis has shown that the ESKAPE pathogens can be inhibited in a species dependent manner with concentrations ranging from 0.25 – 1.0 mM EDTA and 4.0 – 32.0 mM $NaNO_2$. Flow cytometry analysis reveals that higher than FIC concentrations are necessary to kill bacteria associated with their respective biofilms. Taken together, we believe this data shows that AB569 has merit as a potential therapeutic in the treatment of antibiotic resistant bacteria, and we are pursuing this property further. Further studies will involve mechanistic experiments to determine exactly how this killing is occurring.

BOARD 4

SEA Miami: Determining Factor for Copepod Pigmentation in the Waters Surrounding Puerto Rico; Ultra-Violet Radiation Versus Predation

Benjamin G. Keller*[1], Brittany Nordhaus [1],Audrey Meyers[2], and Rachael Morgan-Kiss[1]
[1]Miami University; [2]SEA Semester Program

This experiment examines the relationship between copepod pigmentation and predator avoidance in the attempt to elucidate the primary driver for diel vertical migration. It was hypothesized that there would be an abundance of blue copepods during the day in order to avoid negative effects from ultraviolet radiation, as well as blue copepods would be the most prominent color. The data was collected using a 333 micron nueston net towed for approximately one nautical mile during both day and night in the waters surrounding Puerto Rico. The data was then analyzed and one way ANOVAs were performed. An abundance of clear copepods were found in the day tows, with pink copepods being found in greater numbers in nighttime tows. Blue copepods did not have a significant presence most likely due to predator avoidance selecting for copepod pigmentation, it is also possible a lack of blue copepods in the waters surrounding Puerto Rico, or the chemical state of the carotenoid pigments could account for the results.

BOARD 5

Ginger Extract Inhibits T2 Bacteriophage Yield in E. coli

Mary Cranley*, Sarah Bergman, Nicholas Reed, Andrew Smith, and Gerald Goldstein
Ohio Wesleyan University

Extracts of ginger inhibit the growth of bacteria and viruses of animal cells. An extract of ginger was prepared with alcohol and LB broth. At concentrations of 1, 2, 4, and 6 ml of ginger extract per 100 ml of LB broth, there was very little effect on the replication of E. coli. At concentrations of 2, 4, and 6 ml of ginger extract per 100 ml of LB broth, the yield of T2 bacteriophage decreased by 22% to 28%. The longer ginger extract was in contact with the bacterial cells prior to infection, the larger was the inhibition of T2 bacteriophage yield. When added 50 minutes before infection, the yield of T2 bacteriophage was 93.75% of the control, at 100 minutes prior to infection, the yield was 87.05% of the control and at 150 minutes prior to infection, the yield was 79.91% of the control. The addition of excess glutamine partially reversed the inhibition of T2 yield by ginger extract. With 2 ml of ginger extract, the yield of T2 bacteriophage was 71.3% of the control. With ginger extract and 30 mM glutamine, the yield was 81.4% of the control and with 45 mM glutamine, the yield was 93.0% of the control. With ginger extract plus 1 mM, 3 mM, and 5 mM nucleosides, the yield was 29.85%, 106.1%, and 103.0% of the control. These results suggest that ginger extract inhibits the replication of T2 bacteriophage by inhibiting the metabolism of glutamine and the synthesis of nucleotides during T2 replication.

BOARD 6

Expansion of the Small World Initiative Workflow Using Transposon Mutagenesis and Bioinformatics

Joseph Basalla[1], **Elizabeth Davis*[1]**, Kristen Butela[2], and Hans Wildschutte[1]
[1]Bowling Green State University, [2]Seaton Hill University

A global crisis that threatens humanity is the emergence of multi-drug resistant bacterial pathogens. Despite the need for novel antibiotics, pharmaceutical companies have dropped drug development, primarily due to low profitability; thus, efforts of antibiotic discovery are of utmost importance. The Small World Initiative (SWI) was formed in 2012, not only as a way to peak students' interest in Science, Technology, Engineering and Math (STEM) programs but also to identify novel antibiotics. At Bowling Green State University (BGSU), we developed a molecular approach for SWI to facilitate drug discovery. The current BGSU SWI workflow involves students isolating soil-derived bacteria, genetically characterizing strains using the 16S rRNA gene, identifying strains exhibiting antagonistic activity, and performing transposon mutagenesis to identify biosynthetic gene clusters involved in toxigenic compound production. Over the three semesters that SWI has been offered at BGSU, this strategy has allowed students to isolate over 200 bacterial strains, of which two have been used to identify mutants with loss of antagonistic activity. With this methodology showing success for the generation of useful genomic data, a second semester of SWI is under development to expand the workflow to include a bioinformatics approach involving whole genome analyses. This approach will include online databases and software to further characterize the inhibitory pathways identified within the sequenced genomes of strains isolated from previous semesters. Overall, we have developed an effective SWI workflow that includes a molecular approach to identify genes involved in antagonistic activity and are expanding to bioinformatic analyses involving whole genome comparisons to facilitate antibiotic discovery.

BOARD 7

Phosphodiesterase *Sco5218* in *Streptomyces coelicolor*

William King* and Jennifer Bennett
Otterbein University

Background: *Streptomyces coelicolor* is a gram-positive bacterium with a high G-C content that produces a variety of antibiotics. This soil-dwelling bacterium shows differentiation, forming a vegetative mycelium followed by a network of aerial filaments, finally producing long chains of spores. Our study focuses on phosphodiesterase-encoding genes involved in the breakdown of the signaling molecule cyclic di-GMP, which is a widespread second messenger in bacteria. Previously, our laboratory deleted EAL domain phosphodiesterase genes *rmdA* and *rmdB* which produced a delay in aerial mycelium development. Currently the genes we are examining are *Sco5218* and *Sco5219. Sco5218* is believed to contain an HD-GYP domain through bioinformatic analysis and *Sco5219* is believed to be cotranscribed. The HD-GYP domain is typically associated with cyclic di-GMP-specific phosphodiesterase activity. Each of the genes was deleted from the genome, and the resulting strains were then characterized to observe the effects.
Methods: Conjugation of Double mutants: Bacterial conjugation of *E.coli* containing the *Sco5218* deletion into *Streptomyces* mutants including *rmdA, rmdB,* and a deletion of *Sco5219* all of which also encode phosphodiesterases.
Selection of the Mutants: Using antibiotic resistant markers on observed genes we will select the proper mutants used to examine in our procedure.
Time Course: We will then witness the progression of the development of bacteria over 72 hours checking progression every 12 hours to see delays in the development of the bacterium.
Bioinformatics: Using the *Streptomyces* genome database, we will compare the genes to other orthologs in *Streptomyces* genus to see relation of the HD-GYP domain in other organisms.
Results: A mutation is apparent in the sporulation in *Streptomyces coelicolor* creating mini spores that are not fully separated like in the wild type.
Conclusions: *Sco5218* is has an active HD-GYP domain, that when removed may be compensated by another phosphodiesterase in *Streptomyces coelicolor*.

BOARD 8

Identification of a putative *metK* selenite resistance gene in *Stenotrophomonas maltophilia* OR02

Zachary A. Marinelli* and Jonathan J. Caguiat, Ph.D.
Youngstown State University

Stenotrophomonas maltophilia OR02 (S02) is a multi-metal resistant strain that was isolated from a metal contaminated site in Oak Ridge, TN. It grows in the presence of 20 mM sodium selenite and produces a red precipitate which is probably elemental selenium, and a stale garlic odor which is probably methyl-selenide. Selenite reduction may be dependent on the role of a nitrate reductase or a glutathione reductase. Then, methyl-selenide may be produced using a thiopurine methyltransferase, or an S-adenosyl-L-methionine (SAM) dependent pathway. A selenite-sensitive mutant was generated by introducing the EZ Tn5 transposome into S02. This transposon, which carried a kanamycin resistance gene, randomly incorporated itself into the S02 genome and generated thousands of kanamycin resistant transformants. Replica plating of 880 transformants yielded one selenite-sensitive S02 mutant, AX55. Liquid culture experiments showed that the minimal inhibitory concentration (MIC) for AX55 was 10 mM selenite, whereas the MIC for the S02 was 30 mM. DNA sequencing and Basic Local Alignment Search Tool (BLAST) analysis showed that the transposon interrupted the promoter region for a putative *metK* gene. The *metK* gene encodes the protein SAM synthetase, which catalyzes the formation of SAM. These results suggest that the detoxification of selenite in S02 is dependent upon SAM, and the methylation mechanism may be catalyzed by a thiopurine methyltransferase.

BOARD 9

The Effects of Nitrogen Limitation on *Planktothrix agardhii*

E. Cintron[3], S.S. Dickerson[1], **E.L. Jackson[3]***, N.M. Kern[1, 2], D.A. Leatherwood[3], D.M. Lyons[3], J.R. Wyatt[1, 2], M.J. Neudeck[1,2], K.A. Durham[2]; G.S. Bullerjahn[1]; R.M. McKay[1]
[1]Bowling Green State University, [2]Lorain County Community College,
[3]Lorain County Early College High School

Sandusky Bay has become increasingly plagued with algal blooms over the past few decades due to the surrounding agricultural land usage. Excess nutrients from fertilizers applied to these fields drain into the Sandusky River, which in turn drains into the Sandusky Bay. The shallow waters of the bay, average depth of less than 2m, are insufficient to adequately dilute the nutrients coming from the river. Although nitrogen (N) is rapidly depleted by midsummer in the bay due to sedimentary denitrification, *Planktothrix agardhii* yields a persistent bloom condition, despite the fact that this organism does not fix N (Davis et al. 2015). These blooms affect the aesthetic appearance and water quality of the Bay due high levels of chlorophyll and the production of microcystin, a cyanobacterial hepatotoxin. The Sandusky area depends on the bay as a drinking water source and must take additional actions to preserve potability during blooms. It is suspected that *Plantothrix agardhii survives N* depletion by accessing nitrogen stored in cyanophycin. This N rich polymer, composed of an aspartic acid backbone with arginine branches, is produced by the enzyme cyanophycin synthetase, encoded by the *cphA* gene. During nitrogen limitation, the enzyme cyanophycinase, encoded by the *cphB* gene, hydrolyzes cyanophycin into dimers of aspartic acid and arginine, regenerating nitrogen into central metabolism. In this study, an endemic Sandusky Bay *Planktothrix* strain was grown under N-replete and N-limited conditions to monitor expression of functions associated with N stress. Daily, the cultures were filtered and tested for chlorophyll *a* levels. RNA was extracted and RT-PCR performed to examine patterns of *cphAB* expression.

Davis TW, GS Bullerjahn, T Tuttle, RM McKay and S. Watson (2015) Env. Sci. Technol. 49: 7197-7207.

BOARD 10

Identification of unknown proteins that bind to c-di-GMP

Grant A. Snow* and Jennifer A. Bennett
Otterbein University

The ubiquitous bacterial second messenger cyclic di-GMP (c-di-GMP) is involved in several key bacterial processes that are current areas of research in medical science, such as the change from motile to sessile states, establishment of biofilms, and changes in virulence. Despite recent developments pertaining to the study of c-di-GMP and its targets, few c-di-GMP receptors have been identified. To further investigate the potential discovery of additional c-di-GMP targets, an exploratory experiment has been undertaken in which *Streptomyces scabies* cells were cultured and lysed followed by incubation of the membrane fraction and soluble proteins with a modified c-di-GMP capture compound and streptavidin coated magnetic beads to allow for isolation of proteins from the matrix. Captured proteins were then subjected to a trypsin digest and subsequently identified via mass spectrometry. Of the one hundred and thirty proteins captured, thirty-nine were present in significant amounts, including suspected c-di-GMP binding proteins and a putative c-di-GMP binding protein of particular interest. Further characterization of this novel c-di-GMP-binding protein has been initiated using a variety of bioinformatics tools and mutant construction.

BOARD 11

Antibiotic-Resistant Bacteria Discovered in Intestinal Tract of *Ambystoma mexicanum*

Erica N. Eskins*[1] and Ray A. Larsen[1]
[1]Bowling Green State University

Bacterial isolates from the intestinal microbiota of the salamander species *Ambystoma mexicanum* (a common laboratory model organism and increasingly common household pet) were surveyed for resistance to a set of antibiotics, with particular emphasis on the beta lactam antibiotic ampicillin. Fresh fecal deposits taken from the tanks of three separately-housed animals (two female, one male) were sampled on two occasions, eight days apart. Samples were plated onto general (Luria Bertani (LB)) and selective/differential (Hektoen Enteric (HE) & MacConkey (MAC)) agar and incubated at 30˚C for 24 hrs. A total of 95 isolates were characterized based on growth/phenotype on the HE & MAC plates. Antibiotic sensitivities of these isolates were tested by patching onto LB plates containing one of four different antibiotics at concentrations that inhibit the growth of laboratory *Escherichia coli* strains. Of the 95 isolates, 90% were resistant to ampicillin, 67% were resistant to chloramphenicol, 29% were resistant to kanamycin, and 2% were resistant to tetracycline. Six strains were resistant to at least three of the four antibiotics. The pattern of resistance, combined with colonial phenotypes, suggests that there are at least 79 different isolates that were collected. Ongoing studies using 16S rRNA gene sequencing will confirm the identity of the isolates. The variety of phenotypes and responses to antibiotics in this pilot study suggest the intestinal microbiota of *Ambystoma mexicanum* is sufficiently diverse to justify a broader, metagenomics survey to further evaluate species composition and variation of genes encoding resistance to beta lactam antibiotics in this habitat.

BOARD 12

Feather microbiota in Tree Swallow (*Tachycineta bicolor)* nests.

Kyle M. Davis*[1] and Edward H. Burtt, Jr.[1]
[1.]Department of Zoology, Ohio Wesleyan University, Delaware, OH 43015

The microbiology of avian plumage has been studied in adults (Burtt, J. Avian Biol. 40:349-351. 2009, Burtt and Ichida. Auk 116:364-372. 1999, Gunderson Auk 125:972-979. 2008.), but the microbiota of feathers used to line the nests of birds has not been described. I collected feathers from the nests of Tree Swallows (*Tachycineta bicolor*) from five different stages in the swallow nesting cycle beginning when the swallows first added feathers to their nest, when they laid their first eggs, when incubation started, when the first chick hatched, and when the nestlings fledged. Two feathers were collected from each nest at each stage, 96 feathers in total. The bacteria were removed from the feathers in nutrient broth and samples of the broth were cultured on selective media. The colonies of bacteria were then identified by colony morphology and the media on which they grew. Microbial diversity and abundance were greater in the later stages of the nesting cycle than in the earlier stages, as expected. The developmental and ecological importance of changes in the microbial community of the nest lining will be discussed.

BOARD 13

Optimizing Transposon Mutagenesis in *Vibrio* Strains to Identify Genes Involved in Antibiotic Production

Mahnur Khan*[1], **Chloe Murrell***[1], Abigail Joy[1], Kathryn Kauffman[2] and Hans Wildschutte[1]
[1]Bowling Green State University, [2]Massachusetts Institute of Technology

Previous studies show that interspecific competition occurs among environmental *Vibrio* strains whereby more distantly related isolates are antagonized and closely related strains are resistant to inhibition. As an extension of this work, we hypothesize that environmentally derived bacteria can inhibit *Vibrio* pathogens, and possibly, be a source of novel antibiotics. We sampled water off the coast of Nahant, Massachusetts and isolated 3,456 environmental *Vibrio* strains from 63 µm, 5 µm, and 1 µm size fractionated particles and free-living in the water column and over a three month time span. To test our hypothesis, we performed a high-throughput antagonistic plate assay and competed all 3,456 strains against a panel of five *Vibrio cholerae* and three *Vibrio parahaemolyticus* pathogenic strains that cause cholera and vibriosis, respectively, resulting in a total of 27,648 individual interactions. From all the interactions, 102 environmental strains inhibited the growth of the *Vibrio* pathogens. These data suggest that environmental *Vibrio* strains produce toxigenic compounds that directly inhibit the growth of *Vibrio* strains that cause severe gastrointestinal illnesses. To further this research, we are screening the 102 antagonistic strains for their ability to undergo conjugation and transposon mutagenesis. Candidates that are efficient in these processes will be subject to a large scale mutant hunt. Mutants that exhibit a loss-of-antagonism phenotype will be subject to whole genome sequencing and linker-mediated PCR to identify gene clusters involved in antibiotic production.

BOARD 14

Detection of *Iflaviridae* in Diseased Honeybee Colonies in Delaware County of Ohio

C. Scott Payne*[1] and Suren S. Ambegaokar[1]
[1]Ohio Wesleyan University

The Stratford Ecological Center (SEC) in Delaware, Ohio, contains an apiary with several colonies of the honeybee, *Apis mellifera*. Several of these colonies are “diseased,” with bees that have impairments in general movement, produce little honey, and that die more quickly than bees in “healthy” colonies. Impairments such as poorly developed wings and abnormalities in the premature stages of life are oftentimes caused by viral infections. These diseased colonies may lead to full colony collapse disorder (CCD), a global epidemic of which there is still no preventative or cure, and of which there may be several different causes. Deformed Wing Virus (DWV) is a +ssRNA virus (*Iflaviridae*) that has been associated with many (but not all) cases of CCD, in particular when the colony is also infected with the parasitic genus of mite, *Varroa*, which is thought to be a vector for DWV. Our aim was to determine if DWV was present in bees from diseased colonies at the SEC in Delaware, and if *Varroa* was the vector in that colony. Using RT-PCR and confirmed with sequencing data, we identified *Iflaviridae* sequences in the diseased bees and *Varroa;* however, sequencing data suggests different Iflavirus species in the bees than in the mites. We also tested via PCR for the presence of the microsporidian parasite, *Nosema*, whose species (*N. apis* and *N. cerenae*) are also known to parasitize *A. mellifera*; however we could not detect either species in a diseased colony. Ongoing studies aim to quantify the levels of the immune genes Serpin, Abaecin, and Defensin-1 in diseased bees using qPCR. Together, these findings lead us to believe that there may be multiple vectors for Iflaviruses in diseased bee colonies, and that the lack of *Nosema* may delay the onset of CCD.

BOARD 15

Driving lipopolysaccharide out of its membrane via an unusual ABC transporter

Blake Bertani*[1] and Natividad Ruiz[1]
[1]Department of Microbiology, Ohio State University

With antibiotic resistance on the rise, the need for new antimicrobials is growing. Gram-negative organisms, are particularly problematic in this regard due to their intrinsic resistance to many antibiotics. This resistance is mediated, in part, by a potent permeability barrier generated by lipolysaccharide (LPS) at the surface of the cell. This protective feature makes LPS biogenesis an attractive target for antimicrobial development, as inhibiting this process could sensitise Gram-negative organisms to existing anitbiotics. Additionally, LPS is essential in many organisms, and thus sufficient inhibition of LPS biogenesis could also mediate direct killing. The synthesis of LPS is well characterized. However, how this molecule, once synthesized, traverses the cell envelope to assemble at the cell surface is not entirely understood. This process is mediated by a seven-protein complex, termed the **Lpt** complex (**L**ipo**p**olysaccharide **t**ransport), which forms a proteinacous bridge spanning all compartments of the cell. This complex is powered by an unusual ATP-binding cassette (ABC) transporter, $LptB_2FG$. LptB is the ATPase of the system, but the functions of the transmembrane subunits LptF/G are not well characterized. We now report evidence suggesting that LptG interacts directly with LPS during transport. Specifically, we have identified a positively charged region in LptG critical for function. Amino acid substitutions in this region confer defects in LPS biogenesis, particularly when adding more negative charge to this region of LptG. Moreover, these defects can be suppressed by activation of the BasSR system, a two-component system which controls chemical modification of LPS. This suppression by activation of BasSR requires the enzymes EptA and ArnT, which modify negatively-charged phosphates on LPS with positively charged moieties. This suppression analysis suggests a charge-charge interaction between this newly identified region of LptG and LPS. We propose that LptFG directly extract LPS from the inner membrane and load it onto the Lpt machinery.

BOARD 16

Understanding the Effects of Propionic Acid on *Listeria monocytogenes* Growth, Survival, and Virulence

Elizabeth A. Abrams*[1], Eric Newton[1], Kaitlin Beemiller[1], Nathan Wallace[1], Erica Rinehart[1], and Yvonne Sun[1]
[1]University of Dayton

Listeria monocytogenes is a bacterial pathogen that causes foodborne gastrointestinal illnesses. In the absence of a strong immune system, *Listeria* can cause fatal infection by breaking the intestinal wall and spreading to other organs. Therefore, prevention of fatal infections relies on blocking *Listeria* from adhering to the intestinal wall. My work focused on understanding how propionate, a natural intestinal acid in healthy individuals, affects *Listeria's* ability to grow and cause disease. We first conducted growth curves in BHI and found that propionate supplementations with concentrations up to 25mM resulted in a small decrease in in vitro growth under both aerobic and anaerobic conditions. In contrast, using hemolytic assays, we found that propionate supplementation resulted in a significant increase in listeriolysin O (LLO) supernatant activity after anaerobic growth, but a significant decrease in LLO supernatant activity after aerobic growth. To further determine the effect of propionate on *Listeria* pathogenesis, we infected RAW264.7 macrophages with *Listeria* grown aerobically or anaerobically, with or without propionate supplementations. Our results showed that compared to aerobically grown *Listeria*, anaerobically grown *Listeria* exhibited significantly higher intracellular CFUs during early infection time points, but lower intracellular CFUs during later time points. Supplementation of propionate during *Listeria* in vitro growth did not impact intracellular growth. Finally, we tested the hemolytic ability and intracellular growth of environmental *Listeria* isolates and saw results mimicking those of our lab strain. Together, our results suggest that *Listeria* is capable of growth with high levels of propionate but likely adapts to propionate differently depending on the presence or absence of oxygen. Further research is being conducted to test for protective effects of propionate on mammalian cells by treating the cells with propionate prior to infection. We hope to fully understand the extent of influence propionate has on host-pathogen interactions.

BOARD 17

Analysis of a Novel Mutation Located in the *ccr5* gene with Potential Effects on HIV Infectivity

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To infect a human T cell, HIV must first bind to the CD4 receptor protein, along with one of two co-receptors, CCR5 or CXCR4. In 1995, a missense point mutation in the *ccr5* gene, which encodes for the CCR5 protein, was found in a member of an African-American family. The family consisted of five children who were exposed to the mother's HIV during natural childbirth. Only the second born child did not contract HIV. The mutation, named TG5, was found in the second born child's *ccr5* gene. The mutation occurs at the 314th amino acid and causes a change from lysine to arginine. To determine if the mutation has any effect on the infectivity of HIV, it must first be isolated. Original samples of the mutation were ligated into a pcDNA vector. The sample was digested and ligated into a transitional vector, Invitrogen's pCR®4-TOPO® Vector, and transformed. The sample was confirmed in the TOPO Vector using PCR techniques with primers selecting for a segment of CCR5 which would otherwise not be present in TOPO. Afterward, the sample was ligated into the Clontech pLNCX2 retroviral vector. A lipofection was first used to transfect PT67 packaging cells with pLNCX2-TG5. The packaging cells produced retroviral particles which were purified and used to perform a transduction into H9 cells that express the CCR5 protein. Using neomycin selection, cells expressing the TG5 mutation will be isolated. Mutated cells will be tested for HIV infectivity as well as examined to see the effect on the structure of their CCR5 protein. If the mutation has an effect on HIV infectivity, it could potentially be used in future gene therapies.

BOARD 18

Prevalence and molecular characterization of *S. aureus* and MRSA in kids play grounds: How safe are our kids?

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Staphylococcus aureus is a major public health concern due to the emergence of virulent and drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). Although numerous studies have been conducted to assess the environmental contamination of *S. aureus* in health-care and household settings, little is known about the prevalence and epidemiology of *S. aureus* and MRSA in environmental surfaces of children's playgrounds. This study aimed to investigate the prevalence and molecular epidemiology of *S. aureus* and MRSA at playgrounds in northeast Ohio (NEO).

A total of 280environmental samples were collected from 10 playgrounds in NEO in July, 2016. We selected sites based on playground size and availability of equipment located in both small and large cities. Samples were analyzed using established microbiology methods, and resulting *S. aureus* isolates were typed by *spa* typing. PCR was used to detect the presence of the Panton-Valentine Leukocidin (PVL) and *mec*A genes. Antibiotic susceptibility was tested via the Vitek-2 System.

The overall prevalence of *S. aureus* and MRSA was 28.6% (80/280) and 5% (14/280) respectively. A total of 29 *spa* types were detected from 55 tested *S. aureus* isolates. Overall, t189 was the most common *spa* type, accounting for 21.8% (12/55) of the tested isolates. Four isolates were t002, a common hospital-associated strain, and two isolates (3.6%) were t008, a common community-associated strain. Sixteen percent of the isolates were resistant to oxacillin, and were presumptive MRSA. Multi-drug resistance was observed in 26.4% of the isolates. Further molecular work is in progress.

The results of this study indicate that environmental surfaces of playgrounds in NEO were contaminated with *S. aureus* and MRSA. These data reinforce the need for implementing effective prevention strategies to mitigate the risk imposed to children by environmental contamination of MRSA.

BOARD 19

Cloning and Sequencing of a *lacZ* gene from *Enterobacter* sp. YSU

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Enterobacter sp. YSU (YSU) is a multi-metal resistant bacterium which was isolated from a metal contaminate site in Oak Ride, TN. It also grows on minimal salts medium containing lactose and form blue colonies LB medium supplemented with IPTG and X-gal, which indicates β-galactosidase activity. To see if it contained a *lacZ* gene, universal primers for *lacZ* and the polymerase chain reaction (PCR) were used to copy a *lacZ* gene segment. The resulting PCR product was cloned into a plasmid vector. Sequence and Basic Local Alignment Search Tool (BLAST) analysis showed that the fragment was homologous to a *lacZ* gene. The PCR fragment was labeled with biotin and used to probe a Southern blot of *Eco*R I digested YSU genomic DNA. It hybridized to 7 kb fragment. Additional *Eco*R I digested genomic DNA from YSU was separated by agarose gel electrophoresis and a 7 kb band was purified from the gel slab. The purified DNA was ligated it into a plasmid vector and transformed it into *E. coli*. Of the 196 transformants, 106 formed blue colonies and 90 formed white colonies on IPTG and X-Gal plates. Plasmid isolation, *Eco*R I digestion and gel electrophoresis from the blue colonies showed that the plasmids contained 7 kb inserts. Sequencing of the 7 kb fragment and BLAST analysis revealed that it was similar to a *lacZ* gene from *Enterobacter cloacae* CAV1669. We plan to delete the lacZ gene in YSU and use it as a reporter gene to study gene expression of metal resistance genes in this strain.

BOARD 20

RyfA and RyfB sRNA pairs are important for regulation of *Shigella dysenteriae* pathogenesis

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Each year, 161 million infections and 1.1 million deaths are attributed to diarrheal disease caused by *Shigella*. Currently, no vaccines are available to treat infections with *Shigella dysenteriae* and antibiotics are considered contraindicated. By understanding the molecular components regulating pathogenesis of *S. dysenteriae* new drug targets may be created. All species of *Shigella* (and many species of *E. coli*) contain a set of sRNAs termed *ryfA* and *ryfB* which are located in tandem to one another, encoded divergently from each other, and whose location of the chromosome do not overlap. *S. dysenteria*e contains two sets of this sRNA pair called *ryfA1/ryfB1* and *ryfA2/ryfB2*, distinguishing *S. dysenteriae* from the other three species of *Shigella*. Bacteria which contain a *ryfA1/ryfB1*-like set of genes are associated with gastrointestinal pathogenesis, while bacteria which contain a *ryfA2/ryfB2* set of genes are either non-pathogenic or associated with urogenital infections. In *S. dysenteriae,* overproduction of RyfA1 inhibits the ability of the bacteria to spread from cell to cell within a monolayer of human epithelial cells. Additionally, overproduction of RyfB1 has been demonstrated to decrease RyfA1 levels likely through complimentary base-pairing. This regulation between RyfA1 and RyfB1 may account for fine-tuning pathogenesis of *S. dysenteriae* within the gut. Specifically, RyfA1 is able to downregulate *ompC* (outer membrane protein C). It has been demonstrated that an *ompC* knockout cannot spread from cell-to-cell in plaque assay. In addition, it has been speculated that the small porin size of OmpC gives an advantage to bacteria living in the gut by preventing biosalts and other toxins into the bacteria. The adapted control RyfA1/RyfB1 may provide over OmpC (whether indirect or direct) may be advantageous to gut pathogens and provide a target for drug development.

BOARD 21

Curcumin Enhances T2 Bacteriophage Yield in *E. coli*

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It has been demonstrated that turmeric and curcumin, a chemical present in turmeric, have anti-bacterial activity and anti-viral activity for viruses of humans. We investigated the effect of curcumin on the replication of T2 bacteriophage in *E. coli*. Concentrations of purified curcumin from 5 µg/ml to 40 µg/ml have no effect on the replication of *E. coli* bacteria cultured in Luria-Bertani broth. Increasing concentrations of curcumin enhance the replication of T2 bacteriophage in *E. coli* cells. *E. coli* cells treated with purified curcumin at a concentration of 40 µg/ml enhance T2 bacteriophage replication to 313% of the control culture. The earlier curcumin was added to the *E. coli* cells before infection, the greater was the enhancement of the T2 bacteriophage yield, suggesting that curcumin stimulates the metabolism of bacteriophage replication and not the assembly of bacteriophage particles. The addition of glucose to *E. coli* cultures infected with T2 bacteriophage caused an increase in the yield of the T2 bacteriophage. At 0.25% glucose, the T2 bacteriophage yield was 158.4% of the control. Adding increasing amounts of glucose to *E. coli* cultures treated with curcumin and then infected with T2 bacteriophage caused a decrease in the bacteriophage yield. At a concentration of 0.3% glucose, the T2 bacteriophage yield decreased from 566% of the control to 130% of the control. These results suggest that curcumin stimulation of T2 bacteriophage yield is the result of the stimulation of adenylate cyclase enzyme activity and cAMP production by *E. coli* cells.

BOARD 22

The identification of gene clusters involved in antagonistic activity against cystic fibrosis derived pathogenic *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a human pathogen that infects the lungs of cystic fibrosis (CF) patients and now an urgent concern is its emerging resistance to most antibiotics. Within the CF-lung, *P. aeruginosa* exhibit a high fitness evident of its long term persistence, yet its abundance in some ecological environments is less compared to other groups of pseudomonads. Thus, we hypothesize that in the absence of selection in a human host, ecological interactions among environmental pseudomonads result in a decreased fitness of *P. aeruginosa* in the environment. A trait that is likely to contribute to such fitness effects is the ability of pseudomonads that dominate soil and water habitats to inhibit pathogenic *P. aeruginosa* in one-to-one competitions by the production of inhibitory factors. We used antagonistic interactions to identify inhibition of growth not only among environmental *Pseudomonas* but also against CF-derived *P. aeruginosa*. We isolated >2,000 strains from soil and freshwater habitats and determined the population-level structure with ~330 of these isolates using the *gyrB* housekeeping gene. Our antagonistic results showed that pseudomonads displayed the ability to inhibit both environmental and clinical strains. We performed transposon mutagenesis with seven wildtype strains to identify mutants with a loss-of-killing phenotype. We identified mutants for each strain with loss-of-activity and through subsequent whole genome sequencing and linker-mediated PCR identified gene loci involved in the production of antagonistic factors such as nonribosomal peptides, bacteriocins, pyoverdine and phenazines that inhibit CF pathogens. Using this methodology we have optimized molecular techniques to identify gene clusters with the goal of identifying novel antibiotics.

BOARD 23

Obtaining a CCR5 Knockout Using the CRISPR/Cas9 System

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Some survivors of the Black Plague, caused by *Yersenia pestis*, have a selective advantage in that they lack a functional *ccr5* gene. A 32 base pair deletion mutation, delta 32, confers resistance to *Yersenia pestis* and HIV infection. Timothy Ray Brown, the only known person to be cured of HIV, received a bone marrow transplant from a donor who was homozygous for the *ccr5* delta 32 mutation (Hütter, G., Nowak, D., et al NEJM 12 Feb. 2009). A possible cure for HIV might be the down modulation of CCR5. It has been hypothesized that the amino-terminus of the CCR5 delta 32 protein is capable of exerting a negative regulatory effect on wild type CCR5 as well as CXCR4, an additional secondary co-receptor (Agrawal, L., Lu, X., et al J Virol, Mar. 2004). This study was designed to determine the effect of the complete removal of the *ccr5* gene in human cells. Gene editing was performed using the CRISPR/Cas9 system to eliminate the expression of the CCR5 protein by removing a section of the sequence from both copies of the *ccr5* gene. The human T cell line H9 was co-transfected with plasmids containing guide RNA sequences that have homology to the amino-terminus of the *ccr5* gene along with a plasmid containing the CRISPR/Cas9 gene. Puromycin toxicity was determined by serial diluting puromycin into culture medium and counting cells. Stable transformants were obtained by puromycin selection. The presence of the gene in transfected cells was confirmed by PCR of the puromycin gene. The successful ablation of CCR5 will be confirmed and used to test expression of both CCR5 and CXCR4.

BOARD 24

Escherichia coli evolution under alkaline stress

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Strains of *Escherichia coli* K-12 W3110*,* two from each of four populations, were obtained following daily dilution 1:100 in buffered medium up to pH 9.3 over 2,187 generations. The strains were sequenced using breseq, a computational mutation prediction pipeline. We tested the growth of mutant strains in comparison to the ancestor strain, as a measure of relative fitness. Growth curves of all sequenced strains were run from pH 4.6 to pH 9.4. Under alkaline stress, the mutant strains remained in stationary phase while the ancestor strain entered death phase. Under acidic conditions, the mutant strains sustained a robust stationary phase, although they were not able to reach the optical density of the ancestor strain. Log-phase growth was similar for all strains, suggesting that the high pH-evolved mutations contribute primarily to long-term survival rather than exponential growth. We observed a deletion of the *torI* gene in several of the evolved strains. The *torI* gene, an inhibitor of the *torCAD* operon, is known for its role in the reduction of trimethylamine N-oxide (TMAO) to trimethylamine (TMA) and its subsequent pH effects (Bordi *et al.* 2003). The frequency of the *torI* deletion indicated a potentially significant role of *torI* in decreasing alkaline survival; we therefore inferred that the *torCAD* genes may contribute to alkaline survival. We constructed knockout strains for the *torI, torA,* and *torR* genes and examined the growth curves of each strain in media buffered at high pH. The Δ*torI* knockout (deletion of *torCAD* repressor) conferred a surprising growth advantage at high pH. The Δ*torA* gene does not appear to impact alkaline growth, but Δ*torR* (deletion of *torCAD* activator) decreased growth. We are dissecting the mechanism by which the Tor system affects high pH stress.

BOARD 25

Studying antimicrobial resistance in *Neisseria*

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Neisseria gonorrhoeae, the causative agent of gonorrhoea is emerging as a global threat by showing increased resistance to antibiotics in current use. *N. gonorrhoeae* can colonise the pharynx. Since *Neisseria* species are naturally competent, *N. gonorrhoeae* frequently acquires the antimicrobial resistance determinants from commensal species that cohabit the pharynx. Pharyngeal gonorrhea is often asymptomatic and difficult to treat. However, the strict tropism of human pathogenic *Neisseria* make them difficult to study *in vivo.* Recently, an animal model using macaques was developed to study the colonization and persistence of *Neisseria* species in the pharyngeal niche (Weyand et al. 2013, PNAS, 110 (8): 3059-3064). During this study, animals were treated with Enrofloxacin to clear the pharynx of pre-existing *Neisseria*. However, some animals still harbored their *Neisseria* flora. We are currently characterizing isolates from this study as a possible treatment failure for fluoroquinolone-dependent clearing of the pharynx. Sequence analysis has identified point mutations in *gyrA* and *parC* genes. Transformation of *gyrA* and *parC* genes from Enrofloxacin resistance strains into sensitive strains significantly elevated the MIC to Enrofloxacin. Future work aims to confirm that point mutations in *gyrA* and *parC* genes are responsible for the observed Enrofloxacin resistance in *Neisseria*. Moreover, these mutations are being introduced into *Neisseria musculi*, a species from mice, so we can study their biological fitness and persistence *in vivo*.

BOARD 26

Effect of the Length of Antisense RNA and Bacterial Enzyme Production

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Antisense RNA molecules in bacteria cause inhibition of translation of specific mRNA molecules and rapid destruction of the resulting double stranded RNAs by specific double stranded RNase enzymes. Antisense RNA therapy has been used successfully in the field of oncology to inhibit the synthesis of cell growth factors, growth factor receptors and proteins responsible for the invasive potential of tumor cells. Some antisense RNA molecules are short in length, 100 nucleotides, while other antisense RNA molecules are much larger. Some antisense RNA molecules are complementary to the 5' end of the target mRNA molecule, the 3' end of the target mRNA molecule, or the entire length of the mRNA molecule. We investigated the effectiveness of different lengths of antisense RNA molecules targeted against the 3' end of the mRNA molecules for E. coli beta-galactosidase and Proteus vulgaris urease enzymes. Each of the antisense RNA molecules was complementary to the Shine-Dalgarno sequence, the initiation codon for translation, and various lengths of their respective mRNAs. The shortest antisense RNA molecule for beta-galactosidase, 76 RNA nucleotides in length, and for urease, 51 RNA nucleotides in length, inhibited synthesis of their respective enzymes 61% and 45%. The longest antisense RNA molecule for beta-galactosidase, 210 RNA nucleotides, and for urease, 226 RNA nucleotides, inhibited synthesis of their respective enzymes 0% and 20%. These results suggest that for these specific enzymes in these specific bacteria, the shorter the length the antisense RNA molecule was, the greater was the inhibition of enzyme synthesis.

BOARD 27

Microbial Communities in Wastewater Treatment Plants

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Wastewater treatment plants are crucial in the global water cycle. Humans use water for drinking and other anthropogenic purposes, generating chemically and biologically contaminated wastewater. This wastewater is treated to reduce the amount of contaminants before being re-introduced to natural reservoirs such as rivers and lakes. The microbial communities present in wastewater treatment plants are vital as the microorganisms break down organic contaminants and remove nitrogen and phosphorus. The goal of this study was to determine the composition of the microbial community and the effect of location and seasonality.
Samples from the aeration tank of seven wastewater treatment plants in Cincinnati, Ohio were collected in summer 2015 and winter 2016. The DNA was isolated and 16S rRNA was sequenced using a barcoding approach and Illumina MiSeq. The composition of microbial communities in the wastewater treatment plant samples were determined and analyzed based on sample location and season.
The dominant bacteria in the wastewater treatment plants belonged to class *Alpha-* and *Betaproteobacteria*, and the phyla *Actinobacteria* and *Bacteroidetes*. Many of the same bacteria have been found in other wastewater treatment plants around the world. The comparison of the microbial communities (beta diversity) showed that location within the Cincinnati sewer district had a clear influence on the microbial communities, whereas the seasons had only a small influence.

BOARD 28

Investigating the role of the *Staphylococcus aureus* small RNA Teg41 via antibiotic susceptibility profiling

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In *Staphylococcus aureus* regulatory or non-coding RNAs are collectively known as small RNAs (sRNAs). Hundreds of sRNAs have been identified, however in most cases very little is known about their function. As more sRNAs are studied in detail their importance to the bacteria cell is becoming evident, and they are increasingly appreciated as being integral to gene regulation processes. To investigate the role(s) of previously unstudied sRNAs in *S. aureus* we obtained an sRNA transposon mutant library from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). This collection of 121 sRNA mutants was screened for phenotypic differences and during this process it was discovered that a mutant in the sRNA Teg41 has a defect in its ability to lyse red blood cells, suggesting that Teg41 may play a role in the virulence of *S. aureus*. The goal of this project is to characterize the Teg41 mutant to determine the role of Teg41 in the bacterial cell. Antibiotics target many different pathways in bacterial cells and if these pathways are altered the bacterial cell can become more susceptible (or resistant) to specific classes of antibiotics. In order to test if there are any differences between the wild type *S. aureus* and the Teg41 mutant strain we determined the minimum inhibitory concentrations (MIC) for each strain using a variety of antibiotics. Differences in MIC will offer an insight into the role and function of Teg41, allowing the cellular role of the sRNA to be uncovered. Determining the role of Teg41 in *S. aureus* may help guide future studies to design therapeutics and/or treatments to combat *S. aureus* infection.

BOARD 29

Elucidating how the LptB motor drives Lipopolysaccharide transport

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Bacterial interactions with the environment are primarily mediated by the cell surface. To tightly control what enters the cell, many bacteria, the Gram-negatives, coat the cell surface with a highly impermeable lipid called lipopolysaccharide (LPS). This layer of LPS comprises the outer leaflet of the outer membrane and prevents toxic compounds, like antibiotics, from entering. After it is synthesized at the cytoplasmic membrane, LPS needs to be transported across the periplasm and the outer membrane to be assembled at the cell surface. This process is best studied in *Escherichia coli* which requires seven essential *lpt* (LPS transport) genes to encode the machinery. Here we explore the function of an unusual ATP-binding cassette (ABC) transporter, $LptB_2FG$, that provides energy for LPS transport. The dimer of LptB utilizes the conserved features of ABC motor domains to hydrolyze ATP and cause conformational movements. These conformational changes are transmitted to transmembrane proteins LptFG to drive LPS transport. Utilizing structure-function and suppressor analyses, we have identified unique features of the ATPase LptB required for LPS transport. While LptB shares motifs with other members of the ABC transporter family for ATP binding and hydrolysis, it has a unique motif at its C-terminus that is essential for function. We demonstrate that the C-terminus of LptB functions with ATP-binding motifs in LptB and transmembrane regions of LptFG to coordinate ATP hydrolysis with LPS transport.

BOARD 30

Determining HIV Infectivity By Overexpressing CXCR4 and CCR5 Proteins

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The Human Immunodeficiency Virus, HIV, is an immunodeficiency disease that later progresses to Acquired Immunodeficiency Syndrome, AIDS. Before HIV can progress to AIDS, it must interact with two receptors on a human T-cell, a primary receptor, CD4, and one co-receptor, CCR5 or CXCR4. Agrawal et al.[3] determined CCR5-delta32 might affect the CXCR4 expression as well as full length CCR5. The goal of this study is to conclude the exact effects the CCR5-delta32 mutation exerts on CXCR4, and how it prevents HIV from infecting the human T-cell.

The *cxcr4* gene used was obtained from an H9 T-cell line, and will be amplified using a PCR with primers containing HindIII and NotI sites. C*xcr4* and the pCR4-TOPO vector were cut with the enzymes HindIII and NotI, then ligated together. Once it successfully formed TOPO-CXCR4, a digestion was performed with the previous enzymes to extract *cxcr4* then incorporate it with the target vector, pLNCX2. The purpose of cloning into pCR4-TOPO was to create sticky ends on *cxcr4. Cxcr4* cut fragment was then ligated with HindIII and NotI into our pre-digested pLNCX2. Addition of enzymes Bgl and SphI deactivated the TOPO vector. With a successful ligation of pLNCX2-CXCR4, a bacterial transformation was performed and grown in competent *E. coli* cells. The infected cells were grown on ampicillin plates, to which pLNCX2 is resistant, and kanamycin, which Topo is resistant to, thus determining if it contains *cxcr4*.

The future plans of this research include transfecting the pLNCX2-*cxcr4* clones into PT67, a packaging cell line. These cells will produce retroviral particles containing *cxcr4* and will be used to stably transfect various cells. In addition, several CCR5 alleles will transiently transfect CEMX174 and U937, primary cells. The infectivity of these cells will then be evaluated, determining whether CCR5 mutations play a role in down-modulating CXCR4.

[3] L. Agrawal *et al*., J. Virol. 78;5, 2277-2287 (2004).

BOARD 31

The *Staphylococcus aureus* small RNA Teg41 is required for human erythrocyte lysis

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Staphylococcus aureus is a Gram-positive bacterial pathogen responsible for a wide variety of infections in humans, resulting in approximately 13,000 deaths per year in the United States. Many species of bacteria, including *S. aureus*, have genetic regulatory elements known as small RNAs (sRNAs), noncoding RNAs that have a variety of functions. sRNAs may target bacterial proteins and alter their function, or they may bind to complementary mRNA to effect gene expression. In recent years, the increasing prevalence of RNA sequencing (RNAseq) has led to numerous studies identifying and characterizing sRNAs, and many have been found to have large-scale effects on virulence. A well-studied example in *S. aureus* is RNAIII, an sRNA and global regulator of toxin production. Previous studies in our lab have annotated all known sRNAs in *S. aureus*, and have screened a transposon mutant library of *S. aureus* sRNA mutants for possible phenotypes. From this screen, we identified a hemolysis defect in a Teg41- mutant strain. Teg41 is a sRNA approximately 200 nucleotides in length, originally identified by RNAseq. Its function and phenotypes are unknown. We have shown that a Teg41- mutant strain has decreased hemolysis of human erythrocytes compared to the wild type strain. This may indicate that Teg41 has a regulatory effect on *S. aureus* virulence genes, specifically those involved in hemolysis. Complementation of the Teg41- mutant, by providing the gene *in trans*, restored hemolysis, demonstrating that the hemolytic defect is specifically due to Teg41. The goal of this study is to determine the role and mechanism of action of Teg41 in *S. aureus*. We are investigating the direct targets of Teg41 by pull-down assay, defining the transcript by rapid amplification of cDNA ends (RACE) and Northern blotting, and investigating the role of Teg41 in *S. aureus* pathogenesis.

BOARD 32

Analysis of the secreted protein profile of a *Staphylococcus aureus* Teg41 mutant strain

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Ohio University

Small regulatory RNAs (sRNAs) are emerging as an important class of regulatory molecules in bacteria. Although not as well studied as regulatory proteins we recently demonstrated that sRNAs greatly outnumber protein regulators in the important human pathogen *Staphylococcus aureus*. To begin to characterize the role of sRNAs in *S. aureus* we performed a hemolysis screen using an sRNA transposon mutant library. In this screen a strain carrying an insertion in the sRNA Teg41 was shown to have decreased ability to lyse erythrocytes. The reduction in hemolysis was abrogated when Teg41 was introduced on a plasmid into the Teg41 mutant strain, demonstrating that the reduction in hemolytic activity is directly dependent on the presence of Teg41. To investigate which secreted toxin(s) is responsible for the hemolytic defect in the Teg41 mutant, the *S. aureus* secreted protein profile was examined by TCA precipitation, SDS-PAGE analysis, and silver staining. Results indicate that the global secretion profile of the Teg41 mutant is comparable to the wild type strain, suggesting that the decrease in hemolysis is not due to a global affect on secretion, rather it is due to that activity of one (or a small number of) specific toxin(s). Western blots analysis was subsequently performed to identify the specific toxin(s) responsible for the hemolysis effect.

BOARD 33

Identifying *Listeria monocytogenes*' route to infecting the placenta and the host's response

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The human placenta is an effective barrier for protecting fetus from infection by pathogens. Pregnant women are more susceptible to infection by certain pathogens because of their particular immune status. *Listeria monocytogenes* is one such pathogen that is innocuous to healthy persons but can become deadly when encountered by persons with weakened or altered immune systems, such as the elderly, immunocompromised individuals, and pregnant women. Because infection of the placenta can lead to spontaneous abortion or lifelong morbidities in surviving infants, the placental syncytiotrophoblast, at the interface between maternal and fetal tissues, serves as a barrier to protect the fetus against pathogens. Indeed, the placenta is very resistant to infection, and yet *L. monocytogenes* still manages to cross this fetal-maternal interface. Not every encounter with *L. monocytogenes* however leads to an infection. This has interested us in the question of what makes the placenta so resistant to infection and, moreover, how can *L. monocytogenes* overcome the placental innate defenses. We plan to answer these questions by evaluating the antimicrobial mechanisms of the syncytiotrophoblast. Since the syncytiotrophoblast results from the fusion of underlying cytotrophoblastic cells, we plan to use an immortal cell line to explore whether or not cell fusion itself is accompanied with an increase in the antimicrobial response. By using immunofluorescence microscopy, we can measure the efficiency of bacterial invasion and intracellular survival between fused and non-fused cytotrophoblasts. This analysis could serve as a first approach to establish the syncytiotrophoblast mechanisms of resistance to infection.

BOARD 34

Antibiotic Resistance Mechanisms in *Rahnella* Bacteria

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Antibiotic resistance is a growing problem in the field of healthcare. Antibiotics are becoming less effective as species of bacteria adapt and share resistance mechanisms. If transmission of mechanisms can be better understood at the molecular level, inhibitors could be developed to lessen the likelihood of antibiotic resistance. In this study, *Rahnella* spp were isolated from environmental sources on MacConkey plates containing 100 µg ml^{-1} ampicillin, and confirmed by 16S rRNA gene sequencing. Whole genomic DNA was extracted from isolates and initial amplifications were performed by the polymerase chain reaction (PCR) using primers specific for TEM, SHV and CTX β-lactamase genes. Products were generated only with CTX primers, and these were weak. New primers were designed based on the sequence of a β-lactamase identified in a *Rahnella* genome. These primers provided strong amplification. The products of these amplifications were sequenced, with the predicted protein products showing high sequence similarities to the previously identified *Rahnella* β-lactamase and less homology to CTX β-lactamases. Individual sequences were compared and found to cluster into two distinct groups, with each being distinct from the known *Rahnella* β-lactamase. Additional *Rahnella* isolates have been collected and further analysis is ongoing.

BOARD 35

Characterization of Cyclic di-GMP Regulation in *Streptomyces*

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Steptomyces is predominately a soil dwelling bacterium with a high Guanine-Cytosine content. The genus produces over two-thirds of the world's antibiotics. *Steptomyces* has a large number of known genes involved in its complex lifecycle, but many have yet to be explored. Through bioinformatics, a gene that possibly regulates a second messenger known as bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) was discovered in *Streptomyces coelicolor*. C-di-GMP regulates a variety of processes in bacteria, including motility, biofilm formation, lifecycle completion and antibiotic production. Specific domains like the GGDEF domain which codes for diguanylate cyclase (DGC) activity and the EAL domain which codes for phosphodiesterase (PDE) activity are known to play a role in c-di-GMP regulation. DGCs produce c-di-GMP while the PDE enzymes break down c-di-GMP. The *Streptomyces coelicolor* gene of interest was found to contain PAS, GGDEF, and EAL domains. An ortholog found in *Streptomyces scabies*, was found to contain these same domains organized in a similar manner to that of the *S. coelicolor* protein. Through bioinformatics analyses, it was predicted that the EAL domains of both orthologs would lack PDE activity. Mutants were constructed for both species via the REDIRECT lambda red recombination system. The *S. coelicolor* mutant displayed rapid aerial mycelium growth. The *S. scabies* mutant displayed a visual delay in aerial mycelium and a microscopic delay in sporulation on agar plates. The differences in phenotype were not predicted and further investigation is being conducted. Understanding the functions of these genes will lead to better knowledge of the regulation of c-di-GMP. This in turn will help elucidate c-di-GMP-regulated processes, such as antibiotic production in *Streptomyces* and other bacteria.

BOARD 36

Identification and Analysis of *Staphylococcus aureus* Small RNAs (sRNAs) that Contribute to biofilm formation

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In *Staphylococcus aureus* very little is known about the regulation, function, or mechanism of action of most small regulatory RNAs (sRNAs). sRNA genes are typically absent from genome annotation files and, as a consequence, their existence is often overlooked, particularly in global transcriptomic studies. Recently we performed a study that catalogued and annotated sRNA genes on the genome of the *S. aureus* community-associated, methicillin resistant (MRSA) strain USA300. The newly created USA300 Genbank genome file is a valuable resource that facilitates improved detection and analysis of sRNAs in *S. aureus.* In this study, we performed an RNAseq of *S. aureus* USA300 growing as a biofilm in order to (i) identify novel sRNAs that are uniquely expressed in a biofilm, and (ii) to determine global transcription changes in known sRNAs during biofilm growth. This analysis led to the discovery of 19 previously unidentified sRNAs which we termed biofilm specific RNA (bsr1-19). In addition we identified known sRNAs that are significantly up-regulated and down-regulated during growth as a biofilm. Finally, to test the contribution of sRNAs to biofilm growth we identified and knocked out the genes for 19 sRNAs that are up-regulated during biofilm growth and assessed the ability of each mutant to form a biofilm.

BOARD 37

Modulation of MAGI-1 PDZ1 Binding to CAR^{Ex8} via Small Peptide Interference

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Adenovirus is a common pathogen that typically causes cold-like symptoms in the majority of the population. However, rates of morbidity and mortality due to adenovirus infection are very high in cohorts such as immunosuppressed patients, members of the armed services, and patients with severe infections such as acute respiratory distress syndrome. The coxsackievirus and adenovirus receptor (CAR) is the primary receptor for most serotypes of adenovirus. While the CAR^{Ex7} isoform localizes on the basolateral side of epithelial cells, the CAR^{Ex8} isoform is present on the apical side where it can facilitate adenovirus entry into the airway epithelium. MAGI-1, a cellular scaffolding protein, has been shown to regulate CAR^{Ex8} expression via two of its PSD-95/Dlg/ZO-1 (PDZ) domains, PDZ1 and PDZ3. It has been demonstrated that the PDZ3 domain of MAGI-1 promotes the degradation of CAR^{Ex8}, while MAGI-1 PDZ1 protects CAR^{Ex8} from degradation. Our lab has found that blocking the interaction between MAGI-1 PDZ1 and CAR^{Ex8} can decrease adenovirus infection and pathogenesis. Preliminary data of three peptides targeting the PDZ domains of MAGI-1 show high affinity binding via fluorescence resonance energy transfer (FRET). We hypothesize that MAGI-1 PDZ1 binding peptides can be optimized to bind with higher specificity and decrease adenovirus infection more efficiently than first generation peptides. To test this, we are using a combination of fully-flexible protein docking and peptide design algorithms to create new small peptide candidates for PDZ domain binding. These peptides will be further tested experimentally via a fluorescent high throughput screening assay to evaluate their effect on CAR^{Ex8} expression. By optimizing peptide binding specificity, we can increase our ability to modulate adenovirus susceptibility and improve future therapeutic potential.

BOARD 38

Virucidal action of tea extract on enterococcal phages

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A variety of plant extracts have been shown to have virucidal activity toward bacteriophages (phages) and other viruses in the laboratory. While the medical applications of simple extracts are limited, they can be a useful tool to inactivate viruses during experimental procedures especially as they often have no effect on the host cell. Tea extracts have been found to be a virucide for bacteriophages when mixed with ferrous sulfate (TEAF). This has been shown for bacteriophages infecting *Escherichia coli, Bacillus cereus,* and *Staphylococcus aureus.* We have tested this method using three bacteriophages isolated in our laboratory that infect *Enterococcus faecalis*. Phage AUEF3 only infects *E. faecalis* while AUEF4 and AUEF5 have broader host ranges, infecting both *E. faecalis* and *Enterococcus faecium.*

Tea extract was prepared by boiling black tea (National Cup Orange Pekoe) for 10 minutes. The extract was mixed with 4.3 mM ferrous sulfate (3 mM final concentration) just before use. When mixed with samples of each bacteriophage, we found inactivation occurred very quickly. At three minutes of treatment, the effective titer of the phage stocks was reduced 1000-fold or more. The three phages did vary somewhat in sensitivity to the TEAF. We also found that, consistent with other groups, the virucidal activity can be eliminated by the addition of Tween 80. Overall, we conclude that TEAF can be effective at inactivating our enterococcal phages. TEAF will be a useful tool in experiments that require the infection process to be time limited.

BOARD 39

Differential recruitment of cellular DDR factors to adenovirus DNA replication centers is ATM-dependent

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The DNA damage response (DDR) is a critical process for maintaining genome integrity and preventing the development of cancer cells. Adenovirus (Ad) infection delivers exogenous linear double strand (ds) DNA genomes to the nucleus of the host cell, which may mimic ds DNA breaks (DSBs) resulting in DDR activation. The Mre11-Rad50-Nbs1 (MRN) sensor complex is one of the first proteins recruited to DSBs and Ad genomes, and is important for activating the Ataxia-Telangiectasia Mutated (ATM) kinase. Ad type 5 (Ad5) produces regulatory proteins from early region 4 (E4) that prevent DDR activation. Infection with E4 mutants that lack these viral regulatory proteins activates ATM autophosphorylation (pATM), which colocalizes with viral DNA replication centers and inhibits viral DNA replication. We investigated the mechanism through which pATM inhibits E4 mutant DNA replication. Analysis of Ad5 mutants suggests that localization of pATM to viral DNA replication centers is important for its inhibitory affect. 53BP1 and RNF8 both require ATM dependent chromatin modification for their recruitment to ionizing radiation (IR)-induced DSBs. We used immunofluorescence staining to compare recruitment of DDR factors to viral DNA replication centers in ATM+ and ATM- cells. We find that in the absence of ATM or when ATM is not localized at viral replication centers DDR proteins 53BP1 and RNF8 depend on ATM. ATM is known to affect the organization of chromatin in the vicinity of DNA breaks induced by IR, and could interfere with viral DNA replication by altering viral chromatin. Micrococcal nuclease digestion of Ad5 DNA followed by Southern blotting revealed differences in chromatin organization at the ends of viral DNA. The genome of Ad5 and mutants contained similar banding patterns that did not match the nucleosome ladder. These findings suggest that chromatin organization of the terminal repeat region does not impact viral DNA replication.

BOARD 40

SEA Miami:
Chlorophyll-A at 1% PAR

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Chlorophyll a is a major photosynthetic pigment found in plants, algae and cyanobacteria. Deep Chlorophyll maximum (DCM) defines the subsurface region with the water column that contains the maximum concentration of Chlorophyll-A. (Cullen, JJ. 1982). The depth of the DCM can vary across different marine habitats. For example, the DCM in polar waters t may be present at multiple depths in the water column, while in tropical waters the DCM often correlates with the location of the thermocline. Photosynthetically active radiation (PAR) describes the amount of light available for photosynthesis and is within the spectral range between 400 to 700 nanometers. While marine algae need sufficient PAR levels to drive photosynthesis, surface waters of the ocean where PAR is maximal are often limiting for nutrients. Moreover, PAR levels beyond those needed for photosynthesis can lead to cellular damage through oxidative stress. As part of a SEA Semester voyage, our project measured the location of the DCM in relation to PAR availability in the marine plankton communities residing in the Caribbean Ocean around Puerto Rico. We hypothesized that the DCM would occur deeper in the water column where there is sufficient nutrients and low PAR levels, specifically at the depth of 1% PAR.

BOARD 41

Characterizing the effects of the delta32 mutation found from *Yersinia pestis* survivor descendants on CCR5 expression and HIV infectability

Jeremy Leighty*, Gary Dodson, Keira Magdos, Alysana Martinez, Tin Phan, and Harry Kestler

Human Immunodeficiency Virus (HIV) is a virus that can cripple the patient's immune system rendering it unable to fight it or other infections. HIV must first bind to the primary receptor on the human T-cell, CD4, and one of two secondary receptors, CCR5 or CXCR4 to infect an individual cell. After the HIV glycoprotein 120 successfully binds to the receptors, it uses glycoprotein 41 to pierce and infect the cell. There is a mutation known to affect HIV infectivity. Delta 32 is a deletion mutation of 32 base pairs of the CCR5 human gene sequence. It is theorized that this mutation truncates the CCR5 receptor from the surface of the human T-Cell making HIV. This mutation confers resistance to HIV infectivity as well as *Yersinia pestis* when the individual is homozygous. Twenty percent of European caucasians are heterozygous for CCR5 while only one percent of the same population are homozygous.

We have identified an individual (subject EN2) who is heterozygous for CCR5 delta32. They are a descendent of survivors of a *Yersinia pestis* outbreak, more commonly known as the Black Plague. One of EN2's ancestors was a Swedish woman who was described as the lone survivor of a plague outbreak in her village. The woman found a man from a neighboring village by walking through the forest and blowing a whistle. The man was also the lone survivor in his village. Presumably both of them were CCR5 delta32 homozygous and this couple, from the mid 1500s, was responsible for the repopulation of the region. This whistle is currently in a museum in Sweden, as evidence of the story.

We have performed a PCR amplification from our subject EN2 using primers that circumscribe the CCR5 gene. We were able to obtain both wild type and delta32 allele from this individual. This product was then cloned into pCR4-TOPO vector and will then be excised and cloned into pLNCX2 retroviral expression vector. The retroviral products will be used to create stable and transient transductants into H9 Lymphoid cells and will be tested for HIV infectability and CCR5 expression.

BOARD 42

Method for Detection of Relative Cytoplasmic Reduction Potential in *E. coli* Using RedoxSensor Green™ Dye and Fluorescence Microscopy Following Antibiotic Treatment

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Production of reactive oxygen species (ROS) and a breakdown of the oxidative phosphorylation pathway have been tied to the process of antibiotic induced bacterial cell death. Using RedoxSensor Green™ (RSG) dye in *Escherichia coli* we have developed a method to monitor relative cytoplasmic reductive potential as it relates to antibiotic induced death. *E. coli* were grown at a controlled temperature while being monitored by fluorescence microscopy for changes in RSG and propidium iodide (PI) signal. Following treatment with ampicillin, RSG fluorescence experienced a strong signal increase preceding cellular death. The signal increase observed coincides with a previously described cell membrane bulging phenomenon that is consistent with ampicillin's mechanism of action targeting cell wall integrity. Using our method, we have monitored the time to cell death, time to RSG fluorescence increase and total heightened fluorescence time in *E. coli* exposed to a lethal dose of ampicillin. A distribution of killing times and heightened fluorescence times was observed, consistent with a continuum of antibiotic susceptibility for wild type *E. coli*.

BOARD 43

Examination of a putative methyltransferase from the quaternary amine degrading methanogen *Methanococcoides methylutens* strain Q3c

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Methanogenesis is an anaerobic biological process resulting in the production of methane. Methane is both a strong greenhouse agent and potential renewable energy source, can be carried out by some members of the domain Archaea. A recently isolated methanogenic archaeal strain, *Methanococcoides methylutens* Q3c, has been shown to demethylate two quaternary amines, choline and tetramethylamine (QMA). Based on sequence homology, we have hypothesized that strain Q3c uses a three component methylotrophic pathway to demethylate these quaternary amines. In this pathway, a single methyl group is transferred from the substrate to a corrinoid binding protein, which is then demethylated by a second methyltransferase in order to methylate coenzyme M. The third protein, MtbA, in this three component pathway is a methyltransferase. Based on sequence homology, six potential MtbA homologues have been identified in Q3c. Based on previous research we predict that the second methyltransferases in novel methylotrophic pathways for QMA and choline will be among these homologues. We intend to express and purify each of these homologues for further testing to examine their roles. We have now expressed, purified, and demonstrated the methyltransferase ability of one of these proteins, MM0619. This was done using traditional cloning methods to insert the MM0619 gene into the vector, pASK-IBA43+. Then, the protein was expressed in *Escherichia coli* BL21-DE3 and affinity purified. The methyltransferase activity of the enzyme was assayed spectrophotometrically to show conversion of methylcob(III)alamin to cob(II)alamin with concomitant methylation of coenzyme M. Our current work is directed toward expressing, purifying, and determining the methyltransferase function of the remaining MtbA homologues. These enzymes will be used in later attempts to reconstitute the in vitro pathways of QMA and choline dependent methanogenesis.

BOARD 44

Platelet Activating Factor Receptor Expression Correlates with Decreased Adenovirus Infection

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Treatment for respiratory viruses is rooted in the understanding of the factors that increase and decrease the susceptibility of the patient to the pathogen. For most human adenoviruses, the primary receptor is the Coxsackievirus and adenovirus Receptor (CAR), a protein that regulates cell-cell adhesion of polarized epithelia. CAR is characterized into two transmembrane-containing isoforms, CAR^{Ex7} and CAR^{Ex8}, which differ by 13 amino acids at the extreme C-termini. CAR^{Ex7} is the more abundant isoform of CAR. CAR^{Ex7} resides at the basolateral membrane of cells where it can regulate cell-cell adhesion, but is hidden from airborne viruses. CAR^{Ex8}, while less abundant, resides at the apical surface of polarized epithelia where it can facilitate apical viral binding and infection of polarized epithelia. Platelet activating factor (PAF) is a lipid mediator that activates platelet aggregation and degranulation, inflammation, and IL-8 expression. Our group has previously found that interleukin 8 (IL-8), a proinflammatory cytokine and neutrophil chemoattractant, increases protein expression of CAR^{Ex8} via activation of AKT/S6K and inhibition of GSK3β. We hypothesized that PAF may induce CAR^{Ex8} expression and increase adenovirus infection. Nasopharyngeal carcinoma cells that either were normal or deficient for the PAF receptor were infected with recombinant adenovirus encoding *LacZ* in a dose dependent manner and analyzed with a β-galactosidase assay to determine viral infection. CAR^{Ex8} expression was measured by Western blot and quantified relative to actin and total CAR. Contrary to our hypothesis, the cells expressing the PAF receptor had less CAR^{Ex8} and significantly lower adenovirus infection than cells without the receptor. Future studies will investigate how CAR^{Ex8} expression and adenovirus infection correlate with PAF receptor expression and activity after polarization into an epithelium.

BOARD 45

Using genetic manipulations to select and screen *Mycoplasma iowae* transformants that have motility defects

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Mycoplasma iowae, an opportunistic bacterial pathogen of turkeys and chickens can cause complications such as feathering and bone deformities in young poults. This opportunistic pathogen can also cause infertility, low hatchability and respiratory tract diseases. Although the *M. iowae* genome has been sequenced, identifying genes and processes associated with virulence is in its earliest stages. In several mycoplasma species, including *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, and *Mycoplasma gallisepticum*, adherence and gliding motility, which are mediated by a polar attachment organelle, are strongly implicated in virulence. *M. iowae* also has an attachment organelle, but its role in virulence is unknown. To identify *M. iowae* genes associated with motility, we generated random Tn*4001* insertion mutants and screened them for reduced motility. Electrocompetent *M. iowae* serovar K strain DK-CPA cells were transformed with pTF20, which contains a modified Tn*4001* and a tetracycline resistance gene. Transformants were selected based on reduced incidence of satellite colonies and filter cloned three times to generate pure cultures. Motility videos were generated to visualize transformants of interest and rate and regularity of movement were computed. Transformant 3aA moved more slowly, while 1aA, 2aA and 8aA move less frequently, and less persistently than wild-type *M. iowae* cells. Adherence properties of the transformants were assessed through hemadsorption (HA). The transformants similar to *M. iowae* were identified to be HA^+. We have been able to successfully transform *M. iowae* cells, select transformants, and pre-screen them for reduced motility by observing a colony phenotype on specialized media, and the irregularity in motility has been confirmed by microcinematography. We plan to continue to use this strategy to identifying gene(s) responsible for movement and to test the roles of motility in virulence.

BOARD 46

Reduction of *Salmonella* burden in poultry using new generation small molecules

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Poultry industry represents an important part of the United States economy and poultry products are a major source of animal protein in American eating habits. However, poultry products are the main source of human foodborne outbreaks associated with nontyphoidal *Salmonella*. Current *Salmonella* control methods in poultry have limited effects necessitating development of novel control strategies. Advanced chemical technologies have made accessible a wide range of small molecules (SMs) with encouraging properties for antimicrobial treatment. Here we used a library of new generation SMs to identify *Salmonella* growth inhibitors. Growth inhibitors were identified by challenging *S.* Typhimurium with 200 µM of SM. Dose-response and activity spectrum were determined using multiple *Salmonella* serotypes. SMs toxicity was tested on epithelial and macrophage cell lines. Finally, the effect of these SMs on *Salmonella* and gut microbiome was evaluated in one-week-old broilers. Out of the 4182 SMs screened, we identified four SMs with a lethal effect against ten *Salmonella* serotypes and a low cytotoxicity on eukaryotic models at 200 µM. A decrease in *Salmonella* population in ceca and systemic tissues was detected when *Salmonella* infected broilers treated for 5 days with 100 µg of SM per treatment. Metagenomic analysis of the chicken cecal microbiota displayed minimal changes in the cecal microbiota post SM treatment. However, reduction in *Salmonella* coincided with the increased population of certain short chain fatty acid producing commensal bacteria and increased body weight gain, which also supports the use of non-antibiotic microbial therapeutics to control *Salmonella* in poultry.

BOARD 47

Identification and Characterization of RNA Thermometers in *Staphylococcus aureus*

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Staphylococcus aureus causes many community acquired and nosocomial infections in humans. This bacterium is a commensal organism (part of the normal microflora), but it can also infect the body at various sites. Diseases caused by S. aureus differ greatly, ranging from skin lesions to potentially life-threatening invasive infections including sepsis. Such infections can lead to serious complications including impaired cardiac function and decreased blood pressure that cause low oxygen delivery, organ failure, and death. Given the severity of infection, along with the alarming rate of antibiotic resistance seen, the development of anti-*Staphylococcus* therapeutics is of utmost importance; a goal dependent upon a full understanding of the complex interactions between *S. aureus* and the human host. Depending on the site and /or stage of the infection *S. aureus* experiences distinct environmental temperatures, a cue that in other pathogenic bacterial species influences the expression of virulence-associated genes. One mechanism by which bacterial regulate gene expression in response to environmental temperature is by the activity of RNA thermometers (RNATs), a regulatory elements located in the 5′ untranslated region (UTR) of a regulated gene. The basic principle underlying the activity of an RNAT is that the Shine-Dalgarno (SD) sequence is sequestered within a hairpin structure at low temperature. Increasing temperature destabilizes the inhibitory structure, allowing the ribosome-binding site to become accessible and facilitating the initiation of translation. While characterized in a variety of bacterial pathogens, the role of RNATs in controlling gene expression in *S. aureus* has not been investigated. The goal of this study is to identify and experimentally characterize RNA thermometers in *S. aureus*.

BOARD 48

Development of novel cell-penetrating PDZ domains to control adenovirus infection

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Developing methods to regulate the protein levels and localization of the Coxsackievirus and Adenovirus Receptor (CAR) within the cell would be useful to both prevent opportunistic adenovirus infections, and increase the chance of infection for the purposes of adenovirus-based gene therapy. One isoform of CAR, encoded by all 8 exons of the *CXADR* gene, CAR^{Ex8} , is tightly regulated by the PDZ1 and PDZ3 domains found within the cellular scaffolding protein, Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 1 (MAGI-1). Whereas the PDZ3 domain is able to sequester CAREx8 within the cell to decrease levels of infection, the PDZ1 domain prevents this effect and allows CAREx8 to exist on the apical surface of polarized epithelial cells, where it can bind to adenovirus and increase levels of infection. We hypothesized that decoy PDZ1 or 3 domains would allow the up- or downregulation of CAR^{Ex8}, respectively. PDZ domains, connected to the HIV cell-permeable TAT sequence and a myc-tag for detection, were cloned into a prokaryotic expression vector. Domains were purified from Rosetta *E. coli*. MDCK epithelial cells were treated with purified TAT-myc-PDZ1 or TAT-myc-PDZ3 domains. Western blots were used to detect TAT-myc-PDZ domain entry into to the cell and CAR^{Ex8} levels. Entry was achieved, and a clear relationship was shown between increasing amounts of applied PDZ1 and PDZ3 domains and CAR^{Ex8} levels. Altogether, conjugation to the cell-permeating TAT peptide is an effective way to deliver PDZ1 and PDZ3 domains into cells for CAR^{Ex8} regulation. Future work will confirm the effect of PDZ domain activity on adenovirus infection.

BOARD 49

Microbial Communities in Wild Beer Enrichments

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Spontaneous fermentation is used to brew sour beers. This method captures airborne bacteria and yeasts to generate wild enrichment cultures which ferment the wort. During fermentation bacteria secrete various organic compounds, particularly lactic acid, which influence the sour flavor of the beer. This is inefficient as 85% of wild cultures have either undesirable flavors, aromas, and/or the growth of mold, causing many beer enrichments to be discarded after months of fermentation. The goal of this study is to analyze the composition of the microbial community of wild beer enrichments and how its structure influences the flavor and aroma of sour beer. Samples of wild beer enrichment cultures (three months) and barrel beer (three or more years) were collected to investigate the composition of the microbial community. The DNA was isolated and 16S rRNA was sequenced using a barcoding approach and Illumina amplicon sequencing. The composition of the microbial community in the samples was analyzed and compared to the quality control variables: aroma, flavor and/or mold growth. In addition, the presence of key indicator organisms and on their impact on flavor and aroma of the sour beer was determined.
# Authors contributed equally.

BOARD 50

Proteomics-Driven Insights into Catabolic Demethylation of the Proatherogenic Quaternary Amine gamma-butyrobetaine by *Eubacterium limosum*

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Recent work has found a novel relationship between the occurrence of cardiovascular disease (CVD) and gut microbial quaternary amine (QA) catabolism. Dietary QAs such as L-carnitine, choline, glycine betaine and gamma-butyrobetaine are cleaved by gut microbial enzymes that release trimethylamine (TMA), whose subsequent oxidation to trimethylamine-N-oxide triggers atherosclerosis. Another type of gut microbial QA degradation demethylates QAs without liberating proatherogenic TMA. For example, we have shown that a gut isolate of the acetogen *Eubacterium limosum* demethylates each of the proatherogenic QAs. Acetogens typically use 3-component methyltransferase systems to demethylate substrates and methylate tetrahydrofolate for acetogenesis, however such QA dependent systems had been unknown. We previously identified a glycine betaine dependent 3-component system in the non-acetogenic *Desulfitobacterium hafniense*. Only recently, we identified a *E. limosum* carnitine dependent system. In both cases, the QA dependent methyltransferase components were members of the MttB superfamily of proteins. Here we undertook proteomic analysis of *E. limosum* grown on gamma-butyrobetaine. Identified high-abundance proteins included members of the acetogenesis and butryogenesis pathways. Homologs of each member of a 3-component methyltransferase system were significantly elevated in abundance compared to lactate grown cells. These included an MttB family member, a corrinoid-binding protein, and a corrinoid:tetrahydrofolate methyltransferase. We also identified a homolog of the reductive activation protein required to rescue oxidized corrinoid proteins. The activating, THF methylating, and corrinoid-binding proteins have been found in proteomic analysis of *E. limosum* grown on other QAs. The MttB homolog is unique to gamma-butyrobetaine grown cells, suggesting this may be a butyrobetaine dependent corrinoid dependent methyltransferase. The current proteomic results, plus those from cells grown on carnitine and proline betaine, lead to a model in which only the substrate:corrinoid methyltransferase is unique for the 3-component methyltransferase systems for each substrate. Biochemical analysis of the members of the putative gamma-butyrobetaine methyltransferase system is now underway.

BOARD 51

Dependence of Bacterial Cell Respiration on Membrane Fluidity

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Electron Transport Chain (ETC) is used to generate the energy through the multiple redox reactions during cellular respiration where oxidation of sugar molecules generates ATP via proton pumping across a membrane from cytoplasm to periplasm. Normally, electrons are transported through the membrane soluble electron carrier ubiquinone. It is proposed that the ETC activity of the cell is related to the membrane fluidity because of the diffusion of ubiquinone. To analyze this phenomenon, membrane fluidity and cell respiration measurements will be performed on the same single cells. Fluorescence Recovery After Photobleaching (FRAP) technique is used to determine the fluidity of membrane components. Cell respiration measurements in bacteria are carried out by measuring the consumption of oxygen by *Escherichia coli*. Redox sensitive dyes are used to look at ETC activity.

BOARD 52

Nucleotide utilization by the archaeal corrinoid protein reductive activase RamA

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The methanogenic Archaeon *Methanosarcina barkeri* is capable of reducing the methyl groups from different methylamines to methane, a potent greenhouse gas. Metabolism of each methylamine requires a substrate-specific methyltransferase that transfers a methyl group to its cognate corrinoid protein. The corrinoid protein is demethylated by a second methyltransferase, which then methylates coenzyme M, forming the precursor to methane. Such three-component methyltransferase systems are broadly analogous to tetrahydrofolate methylating systems found in many anaerobic Bacteria. The methyltransfer reactions are dependent on the cobalt ion in the corrinoid protein alternating between the reduced Co(I) and the methyl-Co(III) states. Adventitious oxidation of Co(I) corrinoid to the Co(II) state inactivates the protein and requires the RamA protein to reduce the Co(II) protein to the low potential Co(I) state in an ATP-dependent reaction. RamA contains two C-terminal [4Fe-4S] clusters, and requires ATP to carry out the reduction. Bacterial homologs differ from RamA in that they have one N-terminal [2Fe-2S] cluster, and they have been reported to hydrolyze one ATP per electron transferred. Here, we further examine ATP usage by the archaeal enzyme. We found RamA can use either ATP or ADP to drive electron movement. The Km of RamA with ADP is within physiological concentrations, with a Vmax similar that with ATP. Furthermore, in contrast to the data reported for the bacterial activases, we found that two ATP are hydrolyzed per electron transferred to Co(II) corrinoid protein. This data suggests distinct differences may exist between the bacterial and archaeal enzymes. The use of both ATP and ADP as substrates is highly unusual for an ATP-utilizing protein. Additionally, the use of two ATP per electron transferred is similar to the findings for the HgdC protein which is also a RamA homolog that reductively activates a metabolic enzyme by one electron transfer.

BOARD 53

SEA Miami: Factors Affecting Zooplankton Bio-density and Diversity in the Coastal and Open Waters around Puerto Rico

Keiko Wilkins*[1], Trevor Holm[1], Koby Schneider[1], Audrey Meyers[2], and Rachael Morgan-Kiss[1]
[1]Miami University; [2]SEA Semester Program

Zooplankton are important because they are grazers of primary production, prey for organisms and are important in aquatic carbon and nutrient cycles. It has been well documented within lakes that zooplankton are horizontally distributed in non-random patterns. Major impacts on zooplankton distribution include predation, competition and Diel Vertical Migration (DVM). In marine habitats, there is a tradeoff for zooplankton between phytoplankton availability and predator avoidance. Coastal waters generally have higher levels of macroalgae compare with open waters which represent both a habitat for zooplankton predators and higher phytoplankton biomass growth. In open waters macroalgae is less abundance which means lower predator abundance. The hypothesis of this study was that distribution of zooplankton in the water column will be driven by predator abundance and will therefore be higher in the open ocean waters compared with coastal locations. To test this hypothesis, zooplankton, chlorophyll-a concentration and Sargassum (macro algae) surface samples were collected both day and night off the coast of Puerto Rico. Chlorophyll-a levels varied regardless of distance from shore and time of day. In contrast, zooplankton bio-density showed trends which were dependent upon sampling time. Zooplankton bio density was significantly higher in the nighttime samples relative to the daytime samples. In addition, zooplankton bio-density exhibited a negative correlation with increasing distance from shore, but only in the daytime samples. These results indicate that zooplankton abundance in surface waters are affected by multiple environmental factors.

BOARD 54

SEA Miami: Phytoplankton Biodiversity: A Comparison of Coastal and Open Ocean Waters

Martha L. Carter[1*], Sarah Hiner[1]
Audrey Meyers[2] and Rachael Morgan-Kiss[1]
[1]Miami University; [2]SEA Semester Program

Phytoplankton are abundant, photosynthetic primary producers, and the basis of all freshwater and marine aquatic food webs. Phytoplankton have an important role in cycling nutrients, especially carbon (eg: inorganic carbon fixation) and nitrogen (eg: nitrogen fixation by cyanobacteria). Phytoplankton also play a major role in regulating atmospheric levels of CO_2, a greenhouse gas, as they fix ~50% of the inorganic carbon on earth. Understanding the phytoplankton community gives insight into the status of the atmosphere and the marine ecosystem, so the composition of the phytoplankton community is important to continue to research as the world faces climate change and anthropogenic activities, such as nutrient run-off from over-fertilization and improperly treated sewage. The purpose of this study was to monitor the impact of nutrient levels on phytoplankton abundance and diversity in marine surface waters. Phytoplankton nets were drifted in the photic zone at various locations (coastal vs. open ocean) around Puerto Rico and Vieques, the microorganisms that it captured were morphologically identified and counted, and classified as either a diatom or a dinoflagellate, the two major classes of phytoplankton. In addition, phosphate levels were monitored as a proxy for nutrient load. Diatoms were the dominant phytoplankton across all samples. The results show no significant correlation between diatom distribution or diversity with phosphate concentration, indicating that other environmental factors must influence phytoplankton community composition.

BOARD 55

Prevalence and molecular characterization of *Staphylococcus aureus* and MRSA in environmental samples from Iowa

Saima Wase[3], Mohit Daga[3], Dipendra Thapaliya[1,2,3], Megan K Quick[1,2], Sarah Farina[1,2], Ashley O'Brien[1,2], and Tara C Smith[1,2,3]

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Staphylococcus aureus is a normal part of the human microbiota, resulting in a wide spectrum of infections, from superficial skin infections to life-threatening infective endocarditis. Recent decades have seen an increasing trend of community-associated methicillin-resistant *S. aureus* (CA-MRSA) infection, which has imposed major economic and disease burdens on the U.S. As environment plays an important role in *S. aures* transmission, we investigated the prevalence and molecular characteristics of *S. aureus* and MRSA in environmental samples collected from two regions in Iowa (Iowa City and Keokuk County, Iowa).

A total of 276 environmental samples were collected from high hand-touch areas within and surrounding grocery stores in Iowa between 2011 and 2012. Samples were analyzed using established microbiology methods, and resulting *S. aureus* isolates were typed by *spa* typing and multi-locus sequence typing (MLST). Polymerase chain reaction was used to detect the presence of the Panton-Valentine Leukocidin (PVL) and *mec*A genes. Antibiotic susceptibility was tested via the Vitek-2 System.

The overall prevalence of *S. aureus* and MRSA was 13.4% (37/276) and 2.5% (7/276) respectively. The prevalence of *mec*A and PVL genes among *S. aureus* isolates were 18.9% (7/37) and 5.4% (2/37) respectively. Seven isolates (18.9%) were resistant to oxacillin, and 7 isolates (18.9%) were multi-drug resistant. A total of 14 *spa* types were detected from 37 *S. aureus* isolates. Overall, t726 was the most common *spa* type accounting for 48.6% of isolates (18/37). Five isolates were t002, a common hospital-associated strain, and two isolates (5.4%) were t008, a common community-associated strain. Other *spa* types included t012, t024, t12002, t189, t1964, t2029, t273, t359, t4816, t5099, and t693.

The results of this study indicate that high hand-touch areas within and surrounding grocery stores in Iowa were contaminated with *S. aureus* and MRSA. These data reinforce the need to consider the environment as a potential reservoir for *S. aureus* and MRSA.

BOARD 56

Investigating the biological effect of juglone on the *Staphylococcus aureus* chaperone PrsA

Nikki M. Meyer*, Richard E. Wiemels, and Ronan K. Carroll
Department of Biological Sciences, Ohio University

Staphylococcus aureus is a highly adaptable human pathogen. With antibiotic options running low, there is an urgent need for new approaches to combat infection. The ability of *S. aureus* to cause disease is primarily mediated by secreted and cell wall-associated proteins therefore one potential therapeutic approach would be to target and inhibit the ability of the bacteria to secrete proteins. This would result in a decrease in virulence without killing the bacterial cell. Previous work in our lab has investigated the role of the cell wall-associated lipoprotein PrsA and its contribution to protein secretion in *S. aureus*. PrsA is a member of the parvulin family of peptidyl-prolyl *cis/trans* isomerases (PPIases), and is required for the proper refolding of proteins after they have been secreted from the bacterial cell. In this study we investigated juglone, (a molecule previously shown to inhibit parvulin-type PPIases) to determine (i) if it inhibits *S. aureus* PrsA, and (ii) if inhibition of PrsA *in vivo* can result in a corresponding decrease in activity of secreted proteins. We have previously demonstrated that activity of the *S. aureus* secreted phosphatidylinositol-specific phospholipase C (PI-PLC), a well-characterized

virulence factor, is significantly decreased in a *prsA* mutant. Therefore we determined the activity of secreted PI-PLC with and without the addition of sub-inhibitory concentrations of juglone to the growth medium. Supernatants collected from cultures grown with juglone have decreased PI-PLC activity when compared to the wild-type. Results from these experiments suggest that juglone may be inhibiting *S. aureus* PrsA and this results in a decrease in secreted PI-PLC activity. The findings suggest that juglone could be used as a potential PrsA inhibitor for limitation of secreted virulence factor activity and infection progression. Finally, an *in vitro* PPIase activity assay was performed to further investigate the potential inhibitory effect of juglone on PrsA.

BOARD 57

The role of PpiB: a peptidyl-prolyl *cis/trans* isomerase (PPIase) in *Staphylococcus aureus*

Rebecca A. Keogh*, Richard E. Weimels, and Ronan K. Carroll
Department of Biological Sciences, Ohio University

Staphylococcus aureus is a Gram-positive bacterial pathogen causing diseases ranging in severity from minor skin and soft tissue infections to severe septicemia, endocarditis and necrotizing fasciitis. The emergence of antibiotic resistant *S. aureus* strains is of great medical concern and new antimicrobial treatments are urgently needed. The ability of *S. aureus* to cause disease can largely be attributed to the vast number of secreted and cell wall associated virulence factors it produces. These virulence factors are produced inside the cell and must be successfully translocated and refolded outside of the cell to be functional. The majority of proteins secreted by *S. aureus* utilize the general secretory (Sec) pathway in which they are translocated in a denatured state. Following translocation proteins must refold to become fully functional. One family of enzymes that assist in this protein secretion/folding process are peptidyl-prolyl *cis/trans* isomerases or PPIases. PPIases catalyze the *cis/trans* isomerization of proline peptide bonds, which is the rate-limiting step in the refolding of many proteins. The *S. aureus* PPIase PpiB is critical for the efficient refolding of a number of secreted virulence factors including Staphylococcal nuclease (Nuc). In the absence of PpiB there is reduced activity of Nuc, although similar quantities of the protein are produced. The reduction in activity is due to decreased Nuc refolding in the absence of PpiB. To further investigate the role of PpiB, and in particular, its contribution to virulence, a PpiB-PPIase null strain of *S. aureus* was constructed. Site-directed mutagenesis of the predicted PpiB active site residues resulted in the construction of a substituted form of PpiB in which PPIase activity is abolished. Determining the virulence potential of this substituted strain, along with the wild type and a *ppiB* mutant, will allow us to determine the contribution of PpiB PPIase activity to *S. aureus* infection.

BOARD 58

Prevalence and antibiotic susceptibility of coagulase-negative Staphylococci in Bhutanese refugees in Nepal and Ohio

Jhalka Kadariya[1], Dipendra Thapaliya[1], Sabana Bhatta[1], Ram Lal Mahatara[2], Nabin Dhakal[3] and Tara C. Smith[1*]
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Coagulase negative staphylococci (CoNS) are a major cause of nosocomial bloodstream infections, accounting for 73% of all neonatal bacteremia in the United States. Although several studies have documented CoNS carriage, most have focused on clinical settings. This study aimed to examine the epidemiology and antibiotic resistance of CoNS isolates from Bhutanese refugees living in camps in eastern Nepal and in Northeast Ohio (NEO), United States.

One hundred adult Bhutanese refugees from each location were enrolled, and were interviewed to collect demographic and environmental exposure information. Nasal and throat swabs were collected from each

individual and processed within 24 hours. Coagulase negative isolates were designated as CoNS based on colony morphology and were tested with the VITEK-2 system for species-level identification and antibiotic susceptibility.

Twenty participants (20%) from NEO and 43 participants (43%) from Nepal were colonized with CoNS. The overall prevalence of CoNS was significantly higher among participants in Nepal compared to NEO (p=<0.001). Additionally, we saw more diversity in CoNS species in Bhutanese refugees within Nepal than in NEO. In both populations, *S. epidermidis* (82.8% in NEO and 32.7% in Nepal) was most common. NEO participants carried 4 species of CoNS, while participants within Nepal carried 11 different species. The prevalence of methicillin-resistant CoNS was 6.1% (4/65) in NEO and 34% (15/44) in Nepal. Eight (12.3%) isolates from NEO and 23 (52%) isolates from Nepal were multi-drug resistant.

The findings of this study indicate that Bhutanese refugees living in Nepal had high prevalence of CoNS and high prevalence of multi-drug resistant strains. In addition, this study provided an opportunity to understand the variation in species and antibiotic resistance in two geographical locations (Nepal and Ohio, USA).

BOARD 59

Investigating the function of *in vivo*-induced small RNAs (sRNAs) in *Staphyloccocus aureus*

Richard E. Wiemels* and Ronan K. Carroll
Ohio University

Staphylococcus aureus is a versatile pathogen capable of causing infection in virtually every niche in the human body. Its versatility is due, in part, to a complex regulatory network that ensures appropriate expression of virulence genes at the optimal time and location during infection. Recently, small RNAs (sRNAs) have emerged as an important class of virulence regulators in *S. aureus*. Relatively few of these molecules have been studied in detail despite the fact that that they outnumber protein regulators in the cell. In order to identify sRNAs that may play a role in infection, we performed RNAseq to identify sRNAs that are upregulated during growth in human serum. This analysis revealed three promising candidates for future study, tsr25, rsaOG, and Teg23 which are upregulated 583-fold, 376-fold, and 11-fold respectively in human serum compared to growth in laboratory media. Furthermore, analysis of recently published RNAseq datasets preformed during *S. aureus* murine infection has revealed that tsr25, rsaOG, and Teg23 are upregulated 70-fold, 51-fold, and 172-fold respectively in kidney infection compared to laboratory media. These results strongly suggest that tsr25, rsaOG, and Teg23 play an important role during *S. aureus* infection. To investigate the role of these sRNAs we have constructed mutant strains, strains in which each sRNA is overexpressed, and strains in which an antisense copy of each sRNA is expressed (i.e. “knock-down” strains). Preliminary data using the rsaOG knock-down construct shows a decrease in biofilm formation in this strain. In addition to characterizing the mutant, overexpression, and knock-down strains, we are investigating the direct targets of each sRNA using a biotin pulldown assay. Identifying the direct binding targets of each sRNA will allow us to determine the role and mechanism of action of each molecule in the bacterial cell.

Abstracts of OBASM Podium Presentations

Saturday, April 1
3:30 – 5:25 pm

3:30 – 3:45 pm

Isoform Specific Targeting of the Coxsackie and Adenovirus Receptor with the CRISPR/CAS-9 Gesicle System

James M Readler*[1], Priyanka Sharma[1], Katherine J.D.A. Excoffon[1]
[1]Wright State University

The Coxsackievirus and adenovirus Receptor (CAR) is the primary receptor for the majority of adenovirus serotypes and Coxsackie B viruses. CAR is a cell-cell adhesion molecule in the immunoglobulin superfamily that functions to maintain epithelial junctions and promote leukocyte transepithelial migration. CAR, encoded by the *CXADR* gene, has two transmembrane isoforms that localize very differently in epithelial cells. The high abundance seven exon-encoded isoform (CAR^{Ex7}) localizes below the tight junctions of polarized epithelial cells whereas the eight exon-encoded isoform (CAR^{Ex8}) localizes to the apical surface of polarized epithelial cells. We have previously shown that this difference in localization results in the abundance of CAR^{Ex8} being a major determinant of apical adenovirus infection of polarized epithelial cells. Studies on CAR^{Ex8} have been limited by potentially confounding effects exerted by CAR^{Ex7}. To address this, we are using CRISPR/Cas-9 technology (TAKARA Gesicle System) to produce isoform specific CAR^{Ex8} knockout airway epithelial cells. Multiple sgRNA sequences, with no anticipated off-target effects, that target regions flanking the 42 nucleotides composing the 8th exon of the *CXADR* gene were identified. *In-vitro* reactions with recombinant Cas-9 protein containing these sgRNA sequences show efficient cutting at the expected site in airway epithelial cells. These sgRNA sequences were then cloned into the TAKARA gesicle production plasmid and subsequently transfected into a gesicle production 293T cell line. Gesicles were isolated from the media of these cells and the presence of Cas-9 was confirmed by WB. These gesicle populations, containing sgRNAs that cut 5' and 3' of the *CXADR* exon 8 region, were applied to Calu-3 and KB cells. Future experiments will involve the isolation and expansion of single cells, and then validating the degree of knockout by PCR. CAR^{Ex8} knockout epithelial cells are expected to be powerful tools for probing CAR isoform-specific contributions to virus infections.

3:45 – 4:00 pm

Quantification and Characterization of the Intracytoplasmic Membranes of Methanotrophic Bacteria by Fluorescence Microscopy

Kyle Whiddon*[1] and Michael C. Konopka[1]
[1]The University of Akron

Methanotrophic bacteria have the ability to utilize methane as their sole carbon and energy source. This unique metabolic property makes them an attractive candidate to synthesize potentially useful chemicals, such as biofuels and bioplastics, from a starting material which is both a relatively cheap waste material, and a potent greenhouse gas. To take advantage of this process it is necessary to have an understanding of the intracytoplasmic membrane (ICM) systems in which methane oxidation takes place. These ICM structures have commonly been visualized by transmission electron microscopy (TEM). This technique has significant drawbacks for studying ICM including: long sample preparation, lack of variability in the z direction, and above all the fact that dynamics cannot be observed in TEM samples. We have developed a method using fluorescence microscopy to overcome these drawbacks, allowing us to rapidly collect dynamic data at the single-cell level. By employing styryl lipophilic dyes we are able to view ICM structures in live cells. These structures can then be quantified under differing conditions or over time to analyze membrane dynamics. Using this technique we are able to replicate a well documented copper response in ICM formation. We also combine this membrane staining with other dyes of varying properties to extract more information about ICMs, such as

redox state and plasma membrane connectivity; or with fluorescent protein fusions to observe metabolic compartmentalization in relation to ICMs.

4:00 – 4:15 pm

Sulfide Generation by Dominant Colonizing *Halanaerobium* Microorganisms in Hydraulically Fractured Shales

Anne E. Booker*[1], Mikayla A. Borton[1], Rebecca Daly[1], Sue Welch[2], Carrie D. Nicora[3], Shikha Sharma[4], Paula J. Mouser[5], David Cole[2], Mary S. Lipton[3], Kelly C. Wrighton[1], and Michael J. Wilkins[1,2]

[1]Department of Microbiology, The Ohio State University
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[5]Department of Civil, Environmental, and Geodetic Engineering, The Ohio State University

Black shale formations underlay much of the continental United States, and through hydraulic fracturing, represent a significant natural gas resource. Despite the use of biocides, *Halanaerobium* species become the dominant microbial community member (in some instance up to 99%) in produced fluids from many fractured shales, regardless of their geographic location. This accumulation of biomass in pipelines and reservoirs is considered detrimental by industry, due to the potential for souring (H_2S production), microbially-induced corrosion, and pore clogging by cells and biogenic gases. Sulfide is a particular problem in both conventional and unconventional reservoirs; toxicity associated with this compound poses health risks to workers, while biogenic sulfide causes corrosion of steel infrastructure pipes. Here we investigate *Halanaerobium* biogenic sulfide production from a strain isolated from hydraulically fractured Marcellus Shale using coupled 'omics' technologies, geochemical field observations, and laboratory growth experiments. Produced fluid geochemical and isotopic data collected over 120 days, tracked the reduction of thiosulfate, the presence of sulfide, and increased $\delta^{34}S$ values, all of which are indicative of microbially-mediated reductive sulfur transformations. *Halanaerobium* is a gram negative, obligate anaerobic fermenter, and genomic analysis identified the presence of three rhodanese-like thiosulfate:cyanide sulfur-transferases and an anaerobic sulfite reductase capable of converting thiosulfate to sulfide. Proteomics verified these proteins were in higher abundance when thiosulfate was present in the growth media. While the growth rate of *Halanaerobium* is not enhanced by the presence of thiosulfate, a sulfide-trapping assay could only detect sulfide accumulation when thiosulfate was present. With these observations, we hypothesize that *Halanaerobium* uses thiosulfate to remove excess reductant during fermentation. During this process, rhodanese-like enzymes convert thiosulfate to sulfite, which is subsequently converted to sulfide via anaerobic sulfite reductase. These findings emphasize the detrimental effects thiosulfate-reducing microorganisms may play in hydraulically fractured shales, which would go undetected using current industry-wide corrosion diagnostics.

4:15 – 4:30 pm

In vitro reconstitution of the Glycine Betaine:Coenzyme M Methyl Transfer Pathway from *Methanolobus vulcani* B1d

Adam J. Creighbaum* and D.J. Ferguson, Jr.
Miami University

Methanogenesis from quaternary amines (QAs) is relatively uncharacterized in the spectrum of one-carbon metabolism. Previous studies showed select strains in the genus *Methanococcoides* were capable of growth on tetramethylammonium (QMA) glycine betaine (GB), and choline but the physiological pathways showing breakdown of the substrates were not elucidated. We recently described the first strain in the genus *Methanolobus* that is able to catabolize GB to produce dimethylglycine and methane. We hypothesized that this

process is performed through a corrinoid-dependent GB:CoM methyl transfer pathway. Using the model for trimethylamine (TMA) dependent methanogenesis, which involves the pyrrolysine (pyl)-containing enzyme (MttB), we demonstrated the function of a non-pyl MttB homolog, MtgB. This functionality is critical as the majority of the MttB superfamily lacks the 22nd genetically encoded amino acid, pyrrolysine, that is proposed to be essential in TMA metabolism; thus leaving a gap in knowledge for non-pyl MttBs. Our draft genome sequence of *M. vulcani* B1d revealed genes encoding predicted methyltransferase enzymes (MtgB: MV1727 and methylcorrinoid:CoM methyltransferases: MV0221, 0257, and 2112), a corrinoid binding protein (MtgC or MV1728) and corrinoid reductive activation enzymes (RAM: MV0274, 2040, 2107). We produced our proteins of interest in either *Escherichia coli* or *Methanosarcina acetivorans*. Recombinant MV1727 catalyzed GB dependent methylation of free cob(I)alamin and recombinant MV0221/MV0257 catalyzed methylcobalamin dependent methylation of CoM. These two enzymes combined with recombinant MV1728, and MV2107 *in vitro* successfully reconstituted the GB:CoM methyl transfer pathway from this organism. To our knowledge, this is the first demonstration of the reconstitution of this pathway *in vitro* using purified enzymes. In addition to discovery of a novel methanogenic pathway, we have also gained further insight into the largely uncharacterized MttB superfamily.

10 minute break

4:40 – 4:55 pm

Roles of listeriolysin O, InlA, and InlB in host cell invasion by *Listeria monocytogenes*

Christopher Phelps*[1], Eusondia Arnett[1], Stephen Vadia[2], and Stephanie Seveau[1]
[1]The Ohio State University; [2]Washington University

Listeria monocytogenes (*Lm*) is used as a model organism to study intracellular parasitism. *Lm* expresses two major invasins, InlA and InlB, which specifically interact with host cell receptors to induce bacterial internalization. Additionally, we discovered a novel *Lm* invasion mechanism activated upon plasma membrane perforation by the secreted pore-forming toxin listeriolysin O (LLO). Previous data showed that LLO is sufficient to induce *Lm* internalization into the human hepatocyte cell line HepG2 and the cervical epithelial cell line HeLa. The goals of this study are to establish if the LLO-dependent internalization pathway is active in the multiple cell types *Lm* invades and to determine its importance relative to the InlA- and InlB-dependent internalization pathways. Using automated wide-field fluorescence microscopy, we measured the efficiencies of *Lm* association with and internalization into host cells. Wild type *Lm* and isogenic deletion mutants deficient for LLO, InlA or InlB (single, double, and triple deletion mutants) were constructed and incubated for 30 min at 37°C with human hepatocytes, cytotrophoblasts, and umbilical vein endothelial cells (HUVECs) before fixation and processing for microscopy analysis. Our data show that LLO is critical for *Lm* internalization, but not association, in all tested human hepatocyte cell lines (HepG2, Hep3B, PLC5, and Huh7). InlA is critical for *Lm* association and internalization in some, but not all, hepatocytes. In contrast, no role for InlB was detected in hepatocytes. In the BeWo cytotrophoblastic cell line, InlA contributes to *Lm* association and had a minor effect on internalization, whereas LLO and InlB play no detectable role in these processes. In HUVECs, the absence of each invasin does not affect *Lm* association nor internalization; however, collectively, the three invasins affect *Lm* association. In conclusion, this work demonstrates that when LLO is utilized by *Lm* during hepatocyte invasion, it is a major contributing factor to the process.

4:55 – 5:10 pm

Functional and phylogenetic novelty in the Candidate Phyla Radiation (CPR) found within three Ohio aquifers

Danczak RE*[1], Kenah C[2], Slattery M[2], Johnston MD[3], and Wilkins MJ[1,3]
[1]The Ohio State University Department of Microbiology, [2]Ohio Environmental Protection Agency, [3]The Ohio State University School of Earth Sciences

The Candidate Phyla Radiation (CPR) represents a significant portion of the bacterial tree of life, despite only recently being described. Members of this superphylum are generally inferred to be obligate anaerobes, have reduced genome size with limited metabolic potential and are detected in a broad range of different environments. To examine CPR distribution across subsurface environments, groundwater was collected quarterly over a two-year period from three geochemically and geographically distinct aquifers in southern Ohio, and was analyzed using 16S rRNA gene sequencing and shotgun metagenomic sequencing. Amplicon sequencing results suggested that CPR members constituted between 1-20% of the groundwater microbial community while read-mapped ribosomal protein S3 relative abundances suggested between 5-40%. In order to closely examine the metabolic capabilities of CPR members in these communities, metagenomic assemblies were binned using MetaBAT. From 9 separate metagenomic samples, a total of 64 high quality CPR bins (>70% completion and <10% misbin; 88 bins with >50% completion) were obtained. Of the 64 high quality bins, ~45 bins were phylogenetically placed into previously existing and novel taxonomies using a concatenated ribosomal tree. Glycoside hydrolase (GH) profiles of CPR genomes identified distinct functional niches for different taxa within this superphylum, with GH families involved in cellulose degradation being the most abundant. Additionally, a previously undescribed role in nitrogen cycling was identified in 4 separate genomes through the identification of genes encoding a nitrite reductase enzyme. Overall, these data suggest that CPR members in Ohio aquifers likely play a critical role in the processing of complex carbon substrates, resulting in the generation of fermentation products that help drive respiratory metabolisms in the subsurface.

5:10 – 5:25 pm

Establishing the role of redox equilibrium in *Listeria monocytogenes* anaerobic virulence regulation

Nathan Wallace[1], Ashley Zani[1], Eric Newton[1], Yvonne Sun[1]
[1]Univeristy of Dayton

Listeria monocytogenes (Listeria) is a Gram-positive facultative anaerobe and causative agent of listeriosis in humans. As a saprophyte and enteric pathogen *Listeria* must adapt and respond to varying levels of oxygen. Yet, it remains unclear what effect suboxic conditions have on *Listeria* pathogenesis. As a facultative pathogen *Listeria* has the ability to modulate its metabolism in accordance to its environment in order to maintain redox equilibrium. So far in *Listeria* we have made 2 interesting observations when grown anaerobically: (1) the ratio of NAD^+: NADH is much higher than in aerobically grown cultures and (2) there is no catalase production, an enzyme that degrades hydrogen peroxide into oxygen and water. These observations together indicate that during anaerobic growth *Listeria* may be experiencing oxidative stress. We have hypothesized that this increase in oxidative stress may be a signal that leads to modulation of virulence related genes. To substantiate this claim we had to first demonstrate that there is increased production of reactive oxygen species (ROS) under anaerobic conditions. Using fluorescence intensity as a readout we were able to determine that anaerobically grown *Listeria* are exposed to more oxidative stress in the form of various ROS. One of the ROS that we quantified was hydrogen peroxide, since this is a common form of oxidative stress experienced by living cells we wanted to understand what role it had on virulence (secretion of listerolysin O (LLO)) and metabolism (production of acetoin). Using hemolytic assays and a modified Vogues-Proskauer assay we were able to determine that the supplementation of hydrogen peroxide in overnight cultures led to a decrease in aerobic production of LLO and a decrease in the production of acetoin (both phenotypes typically observed anaerobically). These results support a role of redox equilibrium in *Listeria* anaerobic virulence regulation.

Description of Awards for Presentations

N. Paul Hudson Award for Research Excellence

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

Donald C. Cox Award for Research Excellence

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

J. Robie Vestal Award for Research Excellence

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

Ohio Branch ASM Award for Research Excellence

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

Allan A. Ichida Undergraduate Research Award

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

The Ohio Branch ASM Award for Pre-college Research Excellence

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

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