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Acknowledgements

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OBASM Executive Committee

Andrew Greene
Erin Murphy
DJ Ferguson
Christine Weingart
Chet Cooper
Laura Tuhela-Reuning

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Procter & Gamble – for hosting our meeting
Neil Jordan, Vice President of Research and Development – for meeting sponsorship
Rowan Grayling – Site organizer

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

Invited Speakers

Valerie J. (Jody) Harwood
Larry Forney
Virginia Rich
Paul Hyman
Matthew Sullivan
Nathan Weyand
Haley Oliver
Ben Circello

Program Schedule for OBASM 2016

Friday, April 8

6:00 – 7:00 pm **Registration –** Main Entrance
Poster Setup and Social – Atrium

7:00 – 7:10 pm **Welcome Statement –** Auditorium
Neil Jordan
Vice President of Research and Development
Procter & Gamble

7:15 – 8:15 **ASM Branch Lecture** – Auditorium

"What's in your water: Microbial source tracking"

Valerie J (Jody) Harwood
Professor and Chair, Department of Integrative Biology
University of South Florida

8:15 – 9:00 pm **Social** – Atrium

Saturday, April 9

7:30 – 8:30 am **Registration** – Main Entrance
Poster set-up - Atrium

8:30 – 10:00 am **OBASM Keynote Lecture** – Auditorium

"The structure and function of the human vaginal microbiome"
Larry Forney
University Distinguishes Professor
Department of Biological Sciences
University of Idaho

10:00 – 10:30 am **Break –** Atrium
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 – Harvard Room

Moderator: Andrew Greene

10:00-10:30 **Virginia Rich** – Department of Microbiology, The Ohio State University
"Linking microbes to climate change in a model ecosystem"

10:30-11:30 **Paul Hyman** – Department of Biology and Toxicology, Ashland University
"**Biology and application of enterococcal bacteriophages**"

11:30-12:00 **Matthew Sullivan** – Department of Microbiology, The Ohio State University
"The global ocean virome: Re-imagining viral patterns, processes, and paradigms on the high seas"

Session 2: Medical Microbiology - Auditorium

Moderator: Erin Murphy

10:00-10:40 **Nathan Weyand** – Department of Biological Sciences, Ohio University
"Complement evasion: Recruitment of epithelial proteins protects *Neisseria gonorrhoea* from serum killing"

10:40-11:20 **Haley Oliver** – Department of Food Science, Purdue University
"Prevalence and Persistence of *Listeria monocytogenes* in retail food systems"

11:20-12:00 **Ben Circello** – The Procter & Gamble Company, Health Care Microbiology
"**Impacts of hygiene intervention on the oral microbiome**"

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

or **lunch on your own**

1:30 – 3:00 pm **Poster presentations and judging** – Atrium
Coordinator – Chet Cooper

3:00 – 5:00 pm **Podium presentations and judging** – Auditorium
Coordinator – Chet Cooper

3:00 – 3:15 pm
Establishing a connection between anaerobic virulence regulation and metabolism in *Listeria monocytogenes*

Nathan Wallace*, Ashley Zani Eric Newton, and Yvonne Sun
Miami University

3:15 – 3:30 pm
Role of Tricarboxylic Acid Cycle in *Listeria monocytogenes* Anaerobic Virulence

Ibrahim Alkhomsi*, James M. Readler, Shon Jergens, Mahmoud Alghamri, Priyanka Sharma, and Katherine Excoffon
Wright State University

3:30 – 3:45 pm
A novel mechanism of toxicity amplification: ACD toxin-produced actin oligomers poison formin controlled actin polymerization

David B. Heisler*[1,2], Elena Kudryashova[1], Blake Williams[1], Kyle Shafer[1], Dmitrios Vavylonis D[3], David Kovar[4,5], and Dmitri Kudryashov[1,2]

[1]Department of Chemistry and Biochemistry, The Ohio State University
[2]The Ohio State Biochemistry Program, The Ohio State University
[3]Department of Physics, Lehigh University
[3]Department of Molecular Genetics and Cell Biology and [5]Department of Biochemistry and Molecular Biology, The University of Chicago

3:45 – 4:00 pm

Prevalence and characterization of *Staphylococcus aureus* on public recreational beaches in Northeast Ohio

Dipendra Thapaliya*, Emily J. Hellwig, Jhalka Kadariya, Mark Dalman, Kristen Kennedy, Mackenzi DiPerna, Adrienne Orihill, Tara C. Smith

4:00 – 4:15 pm

Novel regulation of *Escherichia coli*'s ptrB mRNA involving an AUG triplet at the 5'-terminal and downstream coding sequence elements

Heather Beck*, Ian Fleming, and Gary Janssen
Miami University

4:15 – 4:30 pm

One of These Things is Not Like the Other: RyfAs in *Shigella dysenteriae*

Megan E. Fris*[1], William Broach[2], Sarah Klim[1], Tyler Sieron[3], Francis Essien[3], Ronan Carroll[1], Peter Coschigano[3], and Erin R. Murphy[3]

[1]Ohio University Department of Biological Sciences [2]University of South Florida Department of Cell Biology [3]Ohio University Heritage College of Osteopathic Medicine

4:30 – 4:45 pm

Adeno-associated virus-VEGF-165 mediated modification of adipose derived stem cells for cell therapy

Upasana Niyogi*[1], Priyanka Sharma[1], Gregory C. Gould[2], Sunishka M. Wimalawansa[2], R. Michael Johnson[2], and Katherine J.D.A. Excoffon[1,2]

[1]Department of Biological Sciences, Wright State University, Dayton, OH; [2]Department of Orthopaedic Surgery, Sports Medicine and Rehabilitation, Boonshoft School of Medicine, Wright State University, Dayton, Ohio

4:45 – 5:00 pm

Heme-Iron Starvation Enhances Stationary Phase Persistence of Nontypeable *Haemophilus influenzae* (NTHI)

Rachael L. Hardison*, Meghan O'Bryan, Sheryl S. Justice, and Kevin M. Mason
Nationwide Children's Hospital, Center for Microbial Pathogenesis

5:00 – 6:00 pm **GIS for Graduate School: Free pre- and post- acceptance advice** – Harvard Room
Valerie J. (Jody) Harwood
Professor and Chair, Department of Integrative Biology
University of South Florida

6:00 – 8:00 **Banquet and Student Awards Presentations** – Cafeteria, lower level

Abstracts of OBASM Poster Presentations
Saturday, April 9
1:30-3:00 pm

BOARD 1

The effect of capture method on microbial abundance in plumage of Eastern Yellow Robins (*Eopsaltria australis*)

Larynn Cutshaw*[1], Nadya Sotnychuk[1], and Christa Beckmann[2]
[1]Ohio Wesleyan University
[2]Deakin University, Australia

Bird plumage is an ecosystem of microbes within the avian host. Most of these microbes are soil-dwelling bacilli so birds with more soil contact tend to have higher microbial loads. To capture birds to sample their plumage microbes, we used mist nets that capture birds in flight and snap traps, a tool that restrains the bird on the ground. We questioned if capture method influenced microbial loads. We expected microbial abundance on birds captured with mist nets to be of a lower abundance than those captured with snap traps. Birds were captured at 10sites in Victoria, Australia. The abundance of *Bacillus* spp. on Eastern Yellow Robins (*Eopsaltria australis*) captured in both trap types was compared by sampling each bird with Tryptic Soy Agar contact plates at the back, tail, and venter. After statistical analyses using t-Test in SPSS, the data were significant overall ($p = 0.004$), but when comparing data from specific parts of the bird, capture method was only a significant factor on the back ($p = 0.039$; $p = 0.055$ and 0.125 on tail and venter, respectively). We assume that our methodologies have created a detection limit in our data, leaving our results inconclusive to whether or not method of capture is a factor in microbial abundance.

BOARD 2

Understanding the global prevalence of microbes

Nadya Sotnychuk* and Larynn Cutshaw
Ohio Wesleyan University

Bird plumage is an ecosystem of microbes that live on an avian host. The types of microbes in plumage, including bacteria that degrade the keratin in feathers, varies based on geography within the US but little is known about bacteria in the plumage of Australian birds. To investigate this, feathers were sampled from 298 birds of 26 species in 10 different locations within a 170 km radius of Deakin University Waurn Ponds Campus, Victoria, Australia. The locations were selected for habitat type and species diversity. Birds were sampled using contact plates of Tryptic Soy Agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, and Yeast Mold Agar. We counted various kinds of bacteria and fungi including but not limited to *Bacillus lichenformis, Bacillus cereus, Bacillus subtilis*, *Staphylococcus*, and white cottony fungi. Venter feathers were also collected from the sampled birds for extraction of *Bacillus* in the US to test for feather degradation. We isolated *Bacillus* spp. from 25 of 26 species and 86% individuals sampled. *Bacillus* was not present on the grey fantail (*Rhipidura albiscapa*) for two reasons: the first is due to a low sample size and the second is due to the foraging style of the this bird. The grey fantail is an aerial insectivore while *Bacillus* spp. are soil microbes. In contrast *Bacillus* was found on all of the white browed-scrubwrens (*Sericornis frontalis*), a forest floor dweller, sampled. This is the first report of *Bacillus* on Australian bird plumage *in vivo*. *Bacillus* spp. were isolated from the collected feathers and are currently being evaluated for feather-degrading capabilities.

BOARD 3

Group B coxsackieviruses use the apical isoform of the coxsackievirus and adenovirus receptor for entry into polarized epithelia

James Readler*, Priyanka Sharma, and Katherine Excoffon
Wright State University

Coxsackie B virus (CVB) infections of the gastrointestinal and respiratory tracts are common and are most often self-limiting, however, disseminated infections can be devastating. Once CVB has breached an epithelial lining, it can spread throughout the body, and severe complications such as encephalitis and myocarditis can occur with significant morbidity and mortality. Understanding the mechanisms by which viruses infect epithelial cells may lead to novel treatment strategies and stop disseminated infections before they begin. We have shown previously that an alternatively spliced 8-exon isoform of the Coxsackievirus and adenovirus receptor (CAREx8), which localizes at the luminal surface of polarized epithelia, mediates robust apical adenovirus infection of airway epithelial cells. We hypothesized that CAREx8 is also the primary CVB receptor on polarized epithelial cells. Initial studies, in polarized MDCK cells that stably express CAREx8 when induced with doxycycline, suggest that a difference may exist between Decay Accelerating Factor (DAF) binding and non-DAF binding CVB isolates. DAF binding isolates show increased infection with increased CAREx8 expression, but non-DAF binding isolates show no correlation in these cells. GFP fluorescent CVB infection of polarized airway epithelial cells also demonstrate that overexpression of CAREx8 in an airway cell line drastically increases the number of infected cells compared to overexpression of basolateral CAR or RFP. Future experiments will focus on the knock down or overexpression of CAREx8 on polarized airway cell lines and on primary airway epithelia.

BOARD 4

Determining the primary Cyanobacterial species responsible for nitrogenase production in the algae blooms of Sandusky Bay

Richard Anderson[1], Kasey Ashdown[1], Sydney Calez[1], Eddie Jackson[1], Maddie Kroll[1], Melissa Malek[1], Michelle Neudeck[1], Joseph Provocnik[1], Jacklyn Tobia[1], **Daniel Truitt*[1]**, Brandon Velasquez[1], Kathryn A. Durham[1],George S. Bullerjahn[2], and Robert M. McKay[2]
[1]Lorain County Community College
[2]Bowling Green State University

During the warm summer months algae growth in Lake Erie and Sandusky Bay becomes more prevalent. This threatens the water quality due to the release of toxins from cyanobacterial blooms. Previous research suggests that *Microcystis* is responsible for the blooms in Lake Erie, whereas *Planktothrix* dominates the blooms in the Sandusky Bay. A goal of this research is to determine why this species separation occurs in these two bodies of water that are intimately connected. In addition, it appears that blooms in Lake Erie are phosphorus driven while those in the Sandusky Bay are nitrogen driven. If in fact this is true then the cyanobacteria responsible must be capable of fixing dinitrogen gas into usable forms such as ammonium and nitrite to drive the Sandusky Bay blooms. A major subunit of the nitrogenase enzyme responsible for this dinitrogen conversion is encoded by the *nifH* gene. Using DNA extracted from the aquatic microorganisms of Sandusky Bay selection for the *nifH* gene was performed to determine the predominant Sandusky Bay cyanobacterial species possessing the *nifH* gene.

BOARD 5

Wide host range bacteriophages in fecal samples from Northern Ohio

Samantha Ward* and Paul Hyman
Ashland University

Bacteriophages are viruses that are capable of infecting bacteria. Each bacteriophage has a host range, or specific strains of bacteria that it can infect. While most bacteriophages are only able to infect one type of bacteria, bacteriophages with a wide host range are capable of infecting numerous strains. The objective of this research was to isolate wide host range bacteriophage for examination. In order to isolate these wide host range bacteriophages, fecal samples collected in Northern Ohio were filtered to isolate the phage from the sample and then cultured with two separate strains of *Enterococcus faecalis* bacteria to test the host range of the obtained phage. Two bacteriophages were obtained that exhibited the ability to infect both bacterial strains. One of the phage was cultured from duck feces while the other was from horse feces. Once the phages were obtained, they were passaged to ensure purity of the phages. Both were tested on multiple additional *E. faecalis* and *Enterococcus faecium* strains to determine the full extent of the host range on available bacteria strains. Both of the bacteriophages were capable of lysing (breaking open) six different strains of *E. faecalis* and one strain of *E. faecium*. Both bacteriophages were also able to reproduce on three *E. faecalis* strains and the strain of *E. faecium*, showing that these bacteriophages were in fact wider host range phages than others studied here.

BOARD 6

An RNA thermometer mediates temperature-dependent regulation of *Shigella* periplasmic heme-binding protein ShuT

Yahan Wei*[1] and Erin R. Murphy[2]
[1]Ohio University, Department of Biological Sciences
[2]Ohio University, Heritage College of Osteopathic Medicine

An RNA thermometer is a sequence of transcript that incorporates the ribosomal binding site into a temperature responsive hairpin structure and prevents translation at non-permissive environmental temperatures. Currently, RNA thermometers have been characterized in regulating an increasing number of virulence-associated bacterial genes, including *shuA* from *Shigella dysentariae*, which is involved in the utilization of heme, a host-associated iron source. Similar to other bacterial pathogens, survival of *Shigella* in the host is dependent on its ability to acquire essential nutrients, such as iron. In the human body, as an innate immune defense, the concentration of available iron is maintained at an exceedingly low level by sequestration of the element within host compounds. One such compound is heme, which contains approximately 95% of the iron in the human body. Depending on the genes within the *Shigella* heme utilization (*shu*) locus, *Shigella dysenteriae* has the potential to use heme as the sole iron source during infection. Our research indicates that besides *shuA*, another *shu* gene, *shuT*, encoding the periplasmic heme-binding protein, is also regulated by an RNA thermometer in response to changes in environmental temperature. A temperature-dependent *cis*-acting regulatory element is predicted in the 5'-UTR of *shuT* by *in silico* analysis, whose regulatory function was confirmed by Real-time PCR and Western Blot analysis. Subsequently, the predicted secondary structure of the *shuT* RNA thermometer and mechanisms of the inhibitory hairpin responding to temperature changes were characterized by the RNA structure probing assay *in vitro*.

BOARD 7

The effects of propionate and oxygen on the intracellular growth of the foodborne pathogen *Listeria monocytogenes*

Elizabeth Abrams*, Kristine Perez, Nathan Wallace, and Yvonne Sun
University of Dayton

Listeria monocytogenes causes illnesses in immunocompromised individuals by colonizing the intestine. During infections, *Listeria* adapts to the intestinal environment, which is low in oxygen but rich in fermentation acids. It is unclear how these acids influence *Listeria* pathogenesis under anaerobic conditions. We investigated the effects of anaerobic exposure to propionate on *Listeria*.

To test the effect of propionate, we used a macrophage cell line as our model host cells and monitored intracellular growth of *Listeria* after exposure to different propionate levels under aerobic and anaerobic conditions. Results showed that while anaerobically grown *Listeria* was compromised during late stages of intracellular growth compared to aerobically grown bacteria, supplementation of propionate at 15mM did not significantly impact intracellular growth.

Survival and escape from the acidifying phagosomes is critical during *Listeria* intracellular growth. To test the effects of propionate on *Listeria* survival in acidic conditions, we conducted survival assays with aerobically and anaerobically grown *Listeria* after 1 hour exposure to pH 4, 5, 6, or 7 buffers. Data showed that *Listeria* did not survive in the pH 4 buffer. For anaerobically grown *Listeria*, survival at pH 5 was significantly reduced compared to survival at pH 6 and 7. Propionate supplementations did not cause a significant change in survival.

Together, our data suggest that anaerobic exposure, not propionate at 15mM, plays an important role in *Listeria* pathogenesis. We will continue our investigations with higher concentrations of propionate. Our research will help elucidate the behavior of *Listeria* during the intestinal phase of infections.

BOARD 8

Effects of mutations in *Escherichia coli* ExbD C-terminus domain on its efficiency to function

Kelly Cholewa* and Ray Larsen
Department of Biological Sciences, Bowling Green State University

The TonB Energy Transduction Complex (TonB, ExbB, and ExbD) couples the proton motive force to acquire and transport iron in gram-negative bacteria. ExbD is thought to assist TonB in refolding after it transfers energy. This study examined the ability of three site-directed mutagenesis constructs of ExbD proteins to properly function in an *E. coli ΔexbD* strain. By using a colicin sensitivity assay, we determined that the changes made to each construct did not have an effect of the overall function of ExbD.

BOARD 9

Preliminary detection of antibiotic resistance in coliform bacteria

Austin Cunningham*, Mike Dercoli*, Jamielynn Doyle*, and Isaac Pearce*
Youngstown State University

Coliform bacteria and water quality in shallow water and creeks near urban communities vary depending on factors such as runoff and temperature fluctuation. Yellow Creek in Poland, Ohio is subject to these factors and receives storm water runoff from a nearby point source, which could lead to increased antibiotic resistance in present coliform bacteria. This study seeks to evaluate the effect of pollution on water quality and antibiotic resistance in present coliform bacteria. Three sites were chosen for water quality analysis and antibiotic resistance assays located upstream, downstream, and at the point source. Dissolved oxygen was measured using an Orion probe. Turbidity, pH, temperature, and conductivity were determined with a YSI Sonde. Nitrate, Sulfate, and Chloride levels were also determined using Ion Chromatography. Biochemical oxygen demand and coliform levels were measured using standard methods. Antibiotic resistance was determined by spread plates tested for seven antibiotics; Ciprofloxacin, Tetracycline, Colistin, Sulfamethoxazole, Ampicillin, Ceftriaxone, and Piperacillin. Vancomycin was used as a negative control. Preliminary results indicate increasing average chloride concentrations were elevated downstream from the point source (14.5 ppm to 18.6 ppm respectively). Average coliform counts do not currently show pronounced differences between sites (12 colonies per 100 mL). Antibiotic susceptibility was observed for Colistin and Tetracycline. These findings were found under winter conditions without much inflow from the water runoff point source. Spring values are expected to be different.

BOARD 10

Distal gut microbiota structural, functional, and metabolite profiles differ between healthy adolescents from Egypt and USA

Vijay Shankar, Mostafa Gouda, Jessica Moncivaiz, **Alex Gordon***, Nicholas Reo, Laila Hussein, and Oleg Paliy
Wright State University

Cultural traditions, diet, and lifestyles of ethnic groups living in different geographic locations can serve as a source of variability in human gut microbiota. To examine the differences in gut microbiome of geographically distinct populations, we have carried out phylogenetic, functional, and metabolite analyses of the distal gut microbiota of healthy adolescents from United States and Egypt using high-throughput sequencing and proton nuclear magnetic resonance. Ordination techniques separated Egyptian samples from US samples based on both distal gut microbiota and fecal metabolite profiles. Gut microbiomes from the Egyptian group were enriched in members of the genera Prevotella and Megasphaera. In contrast, significantly higher levels of Bacteroides and Blautia were observed in US samples. Clustering of all samples based on fecal microbiota composition resulted in two distinct sample groups, which matched previously described microbiota enterotypes. Clusters were enriched in either Prevotella or Bacteroides. All of the Egyptian samples belonged to the Prevotella cluster and most of the US samples belonged to the Bacteroides cluster. Quantification of metabolites revealed higher levels of short-chain fatty acids in the Egyptian samples and higher levels of amino and bile acids in the US samples. Comparison of community functional capacity through metagenomic sequencing showed concordant differences in gene presence within pathways involved in production of these metabolites. The distal gut microbiomes of US and Egyptian adolescents can be distinguished based on key differences in the structural, functional, and metabolite profiles. These differences are consistent with the dietary patterns in these the two populations.

BOARD 11

Identification of Novel Biofilm-Specific RNA's (sRNAs) in *Staphylococcus aureus*

Caleb Burke* and Ronan Carroll
Ohio University

To identify RNAs that contribute to biofilm formation the transcriptome of an *S. aureus* biofilm was determined by RNAseq, revealing 20 new sRNAs that are up-regulated, or unique during biofilm growth.

BOARD 12

CCR5 Knockout Using the CRISPR/Cas9 System

Kayla S. Zamborsky*, Darla M. Balawender, Michelle J Neudeck, Lisa E. Parrish-Thompson, Jennifer L. Taylor, and Harry W. Kestler
Lorain County Community College

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 person known to have been cured of HIV, received a bone marrow transplant from a donor who was homozygous for the ccr5 delta 32 mutation. 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. Transformed cells were biologically cloned by dilution and some were identified as potential ccr5 knockouts by PCR using ccr5 primers. The successful ablation of CCR5 will be confirmed and used to test expression of both CCR5 and CXCR4.

BOARD 13

Salt stress acclimation leads to attenuation of state transition response in *Chlamydomonas* spp.

Isha Kalra* and Rachael Morgan-Kiss
Miami University, Oxford, Ohio

The ability or efficiency to utilize light energy defines the productivity of all photosynthetic organisms. These organisms not only have to increase their light harvesting capacity in low light environment, but also protect their photosynthetic apparatus under high light conditions. In plants and green algae, including the model green alga, *Chlamydomonas reinhardtii*, state transition is major mechanism to counter short-term, high-light pressure. State transitions accompany electron transfer from photosystem II to photosystem I by phosphorylation of LHCII proteins. However, the Antarctic psychrophilic *Chlamydomonas* sp. UWO241, is a natural state transition variant and is able to survive without the presence of this major mechanism. Instead of phosphorylating LHCII proteins, UWO241 phosphorylates PSI-cytochrome b6f supercomplex to enhance cyclic electron flow. This phosphorylation of a different protein is predicted to be associated with the organism's adaptation to high salt in its natural environment. To understand if salt stress adaptation is responsible for inhibition of state transition response, we used three *Chlamydomonas* spp., the model mesophile *C. reinhardtii*, Antarctic pychrophille C. sp. UWO241 and a recent Antarctic isolate C. sp. ICE-MDV. The three species are adapted to different environmental conditions. Using 77K fluorescence spectra, PAM fluorescence and P700+ absorbance, we were

able to elucidate that high salt acclimation leads to attenuation of state transition response in salt sensitive species *C. reinhardtii* and C. sp. ICE-MDV. Also, acclimation to high salt increases the cyclic electron flow around PSI. Thus, high salt environment plays a major role in inhibition of state transition.

BOARD 14

The effect of dietary probiotics on human subject GI bacteria, GI Archaea, and stress response

Dan Lisko, **Rachel Centofanti***, Aaron Stevens, and Carl Johnston
Youngstown State University

The gastrointestinal (GI) tract is a complex microbial ecosystem which affects host physiological function. Research has indicated that consuming probiotic bacteria can benefit host health and influence microbial community composition. This study focused on measuring the effect of probiotic pill consumption on the GI microbial and archaeal communities and determining if consumption of probiotics affects the stress response in healthy human test subjects. The presence of microbes and Archaea was found using polymerase chain reaction (PCR). The products from PCR were used for Illumina next-generation sequencing (NGS) technology, which was used to determine the composition of the GI microbial and archaeal communities. Quantitative polymerase chain reaction (qPCR) was used to find mean relative abundance. Average daily stress was measured with salivary cortisol sampling. Perceived stress was determined using a questionnaire. High ropes (fear of height) exposure showed measured stress decreased over time in the treatment group. Daily measured stress decreased for the treatment group after 15 days of treatment, but increased 30 days after treatment stopped. High ropes perceived stress had no change over time, but daily perceived stress increased at day 15 for control and test subjects. PCR product was observed for *Lactobacillus, Bifidobacteria*, and Archaea. NGS results indicate the presence of *Lactobacillus*, *Bifidobacteria* (~325 taxanomic units), and Archaea (~20 taxonomic units, all of which fell under the genus *Methanobacter*).

BOARD 15

Identification of novel genes via random transposon mutagenesis in *Streptomyces coelicolor*

Garrett Kandell* and Jennifer Bennett
Otterbein University, Department of Biology and Earth Science, Biochemistry and Molecular Biology Program

Streptomyces coelicolor is a bacterium with a high GC content that is predominately found in soil and decomposing vegetation. The linear chromosome is the largest bacterial genome to be sequenced to date. The genus synthesizes over two-thirds of available antibiotics. Transposon mutagenesis was employed to identify novel developmental genes involved in the complex life cycle. First, a vegetative mycelium forms, consisting primarily of hyphal elongation and DNA replication without cellular division. Aerial hyphae growth follows and then sporogenic cells are developed. There are many developmental genes still believed to be undiscovered and are thought to play key roles in this complex life cycle. Random transposon mutagenesis of S. coelicolor was performed to compare the wild-type strain against mutants that exhibited potential developmental defects through visual screens followed by phase-contrast microscopy. The transposon insertion site was identified by chromosomal DNA isolation, restriction enzyme digestion, ligation, and inverse PCR followed by sequencing. This short sequence of mutant strain NBS96 was then subjected to a nucleotide BLAST and the transposon was found in an intergenic region upstream of the uncharacterized gene SCO2255. SCO2255 codes for a putative membrane protein and the mutant displays a developmental defect at the macroscopic and microscopic levels. Many novel developmental genes are yet to be explored in this important bacterium and this research provides multiple genes of interest for further study.

BOARD 16

Comparative metagenomic analysis of microbial communities based on 16S rDNA from anaerobic sediment enrichments with quaternary amines

Dinesh Hariraju*[1], Brandon Briggs[2], Tomislav Ticak[3], Margarette Bayron Arcelay[4] and Donald J. Ferguson Jr.[1, 5]
[1]Department of Microbiology, Miami University, Oxford, OH 45056; [2]University of Alaska Anchorage, Anchorage, AK 99508; [3]University of Idaho, Moscow, ID 83844; [4]University of Puerto Rico: Mayaguez; [5]Miami University, Hamilton, OH 45011

Two novel strains of methylotrophic methanogens were recently isolated in our lab using anaerobic enrichments with glycine betaine and tetramethylammonium (QMA) from an anoxic sediment sample collected from the Southwest Branch Back River in Virginia, a brackish water environment. Following this study, two more freshwater anoxic sediment samples collected from Acton Lake in Ohio and Pigeon River in Tennessee were also enriched anaerobically with the following substrates: choline, glycine betaine, trimethylamine (TMA), and QMA. The enrichments were carried out in a defined brackish medium or freshwater medium and grown at 37^{o}C. After each week, the enrichment cultures were transferred into fresh culture tubes with 4% culture volume for four transfers and samples were saved from each transfer for DNA extraction. DNA isolated from the sediment and the enrichments was sequenced with 16S rDNA primers according to the protocol of the earth microbiome project. Microbial community analyses were performed using online tools provided by the Visualization and Analysis of Microbial Population Structure (VAMPS) project. Four genera of archaea and 31 genera of bacteria represented at least 1% of OTUs in the enrichments. Archaea were dominant compared to bacteria based on the percentage of OTUs. Among the archaea, *Methanococcus*, *Methanococcoides*, and *Methanolobus* were the dominant genera in brackish sediment, whereas *Methanomethylovorans* was the dominant genus in the freshwater sediments. Among the bacteria, *Desulfovibrio* was the dominant genus in the enrichments, except Pigeon River. Here we present a comparison of microbial communities of brackish water and freshwater sediment enrichments selected with quaternary amines.

BOARD 17

The effects of homemade mouthwashes on the bacterial concentration in the mouth

Kelsey Ash*, Nathaniel Beres, and Justin Pruneski
Heidelberg University

The daily removal of dental plaque is a major factor in preventing caries, gingivitis, and periodontitis. Chlorhexidine is the most common ingredient in mouthwash and is used as a gold standard to compare the effects of new products. The problem with Chlorhexidine is that with its use there are potential side effects of pigmentation, taste alteration, and formation of a calculus limit. Due to debates surrounding the chemicals in store bought mouthwash, a search for safer alternatives have been on the increase. This research project tested the effectiveness of homemade mouthwashes compared to store bought mouthwashes.

Several homemade mouthwashes were made using items commonly found in a grocery store including: garlic, lemon, coconut oil, cinnamon, peppermint oil, baking soda, aloe vera, onion, and mint leaves. Four species of bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* were treated using the disk diffusion method to test each homemade mouthwash and individual components *in vitro*. To test effectiveness *in vivo*, volunteers were asked to use a homemade mouthwash while maintaining standard dental hygiene practices, such as brushing and using dental floss. Participants' mouths were swabbed immediately following the mouthwash along with 1.5-2.5 hours after use in order to monitor the changes in oral bacteria over multiple testing days. Surveys were used to determine participant opinions of each mouthwash. These experiments provided evidence that homemade mouthwashes were not as effective as store bought mouthwash in combatting oral bacteria.

BOARD 18

Antiviral properties of the curry spice, curcumin

Jemil Ahmed* and Suren Ambegaokar
Ohio Welseyan University

Curcumin is a polyphenolic extract from the spice turmeric. Previous research on curcumin indicates a variety of pharmacological benefits, including anti-inflammatory, antioxidant and antiviral activities. Our research uses Vero cells (a cell line derived from African monkey Kidney cells) infected with vesicular stomatitis virus(VSV), a rhabdovirus. VSV can infect and cause severe illnesses in many important livestock, such as horses, cattle and sheep. Our preliminary data suggests that infection was reduced significantly in cells treated with curcumin. VSV infection induces oxidative stress to promote apoptosis to cause cell lysis, thus allowing the release of newly replicated virus particles. Future work will investigate if the antiviral effect of curcumin is mediated by reducing oxidative stress. This work will better inform our understanding of VSV pathology, and the potential use of curcumin as an antiviral therapeutic.

BOARD 19

Isolation of phages against *Staphylococcus aureus* and phage therapy of wound infections in *Rattus norvegicus*

Bradley Palocko* and Justin Pruneski
Heidelberg University

Antibiotic resistance is an important and growing global concern. Methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, and drug resistant *Streptococcus pneumoniae* are just a few examples of severe bacterial infections that now have dramatically diminished response to traditional antibiotics. Many alternative treatments are being researched to overcome the limitations of antibiotics, including phage therapy. Phage therapy is a form of antibacterial treatment that uses bacteriophages, viruses that infect and lyse bacterial cells with great specificity, to combat bacterial infections. Phage therapy is a promising treatment method because of its high degree of specificity, limited side effects, and its ability to overcome bacterial resistance through coevolution. In this study bacteriophages specific to a lab strain of *S. aureus* were isolated from samples of raw sewage, amplified, quantified, and tested in vitro against a variety of *S. aureus* isolates. A phage therapy trial was conducted using twenty common laboratory rats, *R. norvegicus*, which were exposed to *S. aureus* bacteria via inoculation of an open wound. Phage-treated rats were compared to control rats, receiving the antibiotic oxytetracycline. Results from the phage therapy trial were inconclusive, likely as a result of a minimal wound that healed rapidly and limited virulence of the bacterial strain. In this study, a naturally isolated phage sample was shown to be effective and specific in combating *S. aureus in vitro*, but additional in vivo testing would be required to determine its usefulness for phage therapy.

BOARD 20

Prevalence and Molecular Epidemiology of *Staphylococcus aureus* among Bhutanese Refugees in Nepal and in Northeast Ohio

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Every year the United States resettles thousands of refugees from around the world. Although studies have shown that human migration is one of the risk factors for the spread of drug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), there has not been any study conducted regarding the prevalence of MRSA among refugee populations in the U.S. This study aimed to assess the prevalence and molecular characteristics of *S. aureus* among Bhutanese refugees living in Nepal and in Northeast Ohio (NEO). One hundred adult Bhutanese refugees from each location were enrolled between August 2015 and January 2016. The participants were interviewed to collect the demographic information and exposures to potential risk factors for carriage. Nasal and throat swabs were collected from each individual and processed within 24 hours according to the study protocol. All *S. aureus* isolates were typed by *spa* typing and multi-locus sequence typing (MLST). The presence of the Panton-Valentine Leukocidin (PVL) and *mec*A genes were detected via PCR. The Vitek-2 System was used to test the antibiotic susceptibility. Of the 100 participants enrolled in Nepal, the median age was 35 years (mean 36.75; standard deviation, 13.28; range, 57). Twenty-nine percent and 71% of the participants were male and female, respectively. The overall prevalence of *S. aureus* was 45% (45/100). The prevalence of MRSA was 2% (2/100). The overall prevalence of PVL genes among *S. aureus* isolates was 25% (13/52). A total of 31 *spa* types were detected from 52 *S. aureus* isolates. The most common *spa* type was t345(9.6%). One isolate was t008 (USA300), a common community-associated strain. One isolate was found to be t002, a common healthcare-aasociated strain (USA100). Twenty-one isolates (40.4%) were multi-drug resistant. Analyses of data from NEO are ongoing. The findings of this study indicate that Bhutanese refugees living in Nepal had high prevalence of *S. aureus* and high prevalence of multi-drug resistant *S. aureus.*
Key words: *Staphylococcus aureus,* MRSA, Northeast Ohio, Bhutanese Refugee, Nepal

BOARD 21

The motility and chemotaxis of *Bacillus* spp. isolated from songbird plumage

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Ohio Welseyan University

The plumage of birds is an ecosystem that harbors a diverse community of microbes. Among these are species of *Bacillus*, some of which utilize feathers as a source of carbon by secreting keratinases that degrade β-keratin. *Bacillus* are motile, chemotactic bacteria. Thus, amino acids from damaged parts of feathers could act as chemoattractants for plumage-dwelling Bacillus spp. Uropygial oil is used by birds in preening, and is thought to protect plumage from bacteria although not all uropygial oil has antibacterial properties. In this study, the inhibitory effect of increased viscosity on *Bacillus* chemotaxis was quantified to investigate the possibility that preen oil functions as a vicious physical barrier against the chemotaxis of feather-degrading *Bacillus*. Preliminary motility assessments were conducted for *Bacillus* cultures, then growth curves were performed in coordination with motility estimates. From these data, *Bacillus* 4201TV was chosen for chemotaxis assays. The chemotaxis of *Bacillus* 4201TV was quantified via capillary assays in modified Palleroni chambers. Proline, valine, and asparagine were tested at concentrations of 250 μM and 750 μM. *Bacillus* 4201TV exhibited a positive chemotactic response towards proline, valine, and asparagine at both concentrations, with the greatest response ratios at 750 μM. To determine the effect of increased viscosity, dual quantitative chemotaxis assays were performed using 750 μM proline or asparagine. An increase in viscosity was achieved by preparing the amino acid solutions in 0.05% agar, and was found to reduce the chemotactic response of *Bacillus* 4201TV toward by 41% and 97%, with p-values of 0.0403 and 0.309. These data suggest that uropygial oil may function

as a physical barrier to inhibit the chemotactic movement of feather-degrading *Bacillus* spp. towards areas of feather damage, thus protecting bird plumage.

BOARD 22

Supplementation of propionate inhibits the anaerobic growth of the foodborne pathogen *Listeria monocytogenes*

Eric E. Newton*, Nathan Wallace, Ashley Zani, Elizabeth Abrams, Mario Mutillo, Kristine Perez, and Yvonne Sun
University of Dayton

Listeria monocytogenes is an infectious bacterium that is known to cause severe diseases in people who are pregnant, elderly, or generally immunocompromised through consumption of contaminated food products. To help develop preventative strategies to protect these high-risk individuals, our lab focuses on the approach of enhancing the chemical barrier naturally existing in the intestinal tract to block *L. monocytogenes* from interacting with the human intestinal epithelium and causing fatal infections. The chemical environment inside the human intestinal lumen is rich with fermentation acids produced by the endogenous microbes. I tested the efficacy of propionic acid, one of the three major fermentation acids naturally abundant in the human gastrointestinal tract, against the in vitro growth of *L. monocytogenes*. To determine the effect of propionic acid on growth, I supplemented *L. monocytogenes* cultures with sodium propionate and grew the culture either aerobically with agitation or anaerobically with a 2.5% hydrogen in nitrogen atmosphere. I monitored growth by measuring culture optical density every hour and calculated bacterial doubling time during the exponential phase of the growth. I observed that under aerobic conditions, propionate supplementations did not cause a significant impact on bacterial doubling time. However, under anaerobic conditions, propionate supplementation at 25mM led to a significantly increased doubling time, a result indicating an inhibitory effect of propionate on growth. These results demonstrate an inhibitory effect of a naturally occurring fermentation acid in the human intestines and therefore highlighted the potential values for propionic acid as a preventative chemical agent against *L. monocytogenes* infections.

BOARD 23

A Novel Mutation in CCR5 Obtained From an African American Family

Ocatvia Whitfield, **Kennedy Figueroa***, Brandon Holcomb, Austyn Lilly, and Nya Washington
Lorain County Community College

The CCR5 and CXCR4 receptors facilitate inflammatory responses. Additionally, both receptors act as secondary receptors for HIV infection. A novel mutation in the ccr5 gene, which codes for the CCR5 receptor, was found in a child of an African American family. Before the birth of the first child, the mother of this family contracted HIV and unknowingly exposed her five children to HIV through natural childbirth. Out of the five children, only the second born did not acquire the infection. After testing this individual, we discovered a point mutation in the cytoplasmic domain of the ccr5 gene. This mutation is referred to as TG5 changes the lysine codon into an arginine codon. The allele containing the TG5 mutation was isolated by the PCR-TOPO subcloning vector. The TOPO vector was used to transfer the TG5 mutation into the pLNCX2 retroviral plasmid vector. Next, the pLNCX2-TG5 clones will be used to transfect the packaging cell line PT67. The PT67 cell line then will be assembled viral particles containing the TG5 gene. These retroviral particles will be isolated and introduced into H9 and CEMx174 cell lines through retroviral transduction. We plan to test the expression of the TG5 gene in H9, CEMx174, and primary cells. We will assess its effect on HIV infectability. In addition, we will examine the whether TG5 can down-modulate the expression of wild-type CCR5 and or CXCR4 receptors.

BOARD 24

Effect of iron on the photochemistry and photosynthetic apparatus of three *Chlamydomonas* spp.

Gregory Cook* and Rachael Morgan-Kiss
Miami University

Most of the earth is composed of permanently cold habitats where a host of photosynthetic psychrophilic organisms thrive by using unique mechanisms to grow and reproduce under permanent environmental stresses. Even though there have been extensive studies on model plants and algae, adaptive strategies employed by non-model organisms are generally poorly understood. The *Chlamydomonas* spp., isolated from a perennially ice-covered lake in the McMurdo Dry Valley of Antarctica, provide novel examples of adaptation due to being exposed to a myriad of abiotic stresses. In particular, iron deficiency is tied to limited photosynthetic capability in these organisms. Interestingly, the photosynthetic apparatus of *Chlamydomonas* sp. UWO241 resembles that of the model mesophile *Chlamydomonas reinhardtii* under iron deficiency, illustrated by a down-regulated photosystem I (PSI). To establish if this phenomenon is present in other organisms from this ice-covered lake, a second psychrophile, *Chlamydomonas* sp. ICE-MDV, was chosen and characterized under iron stress conditions. Utilizing various photochemical techniques, a comparative study of these three algal species was conducted in order to elucidate the role of iron in energy allocation between the components of the photosynthetic apparatus, as well as other fluorescence induction parameters. These experiments revealed that not only were the psychrophiles more sensitive to iron deficiency, but that UWO241 was unable to re-establish PSI under iron excess conditions. Alternatively, ICE-MDV responded to iron excess conditions with a dramatic increase in PSI fluorescence, which is comparable with *C. reinhardtii*. Thus, iron deficiency is not responsible for down-regulated PSI in UWO241.

BOARD 25

Using environmental bacteria as a source of antibiotics to inhibit cystic fibrosis-derived pathogenic *P. aeruginosa*

Payel Chatterjee*, Elizabeth Davis, Sarah James, John LiPuma, and Hans Wildschutte
Bowling Green State University

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 antibiotics. In the human CF-lung, *P. aeruginosa* exhibit a high fitness evident of its abundance and long term persistence. In contrast, from our repeated sampling efforts, we very rarely detect *P. aeruginosa* strains in the environment suggesting that such isolates have a decreased fitness in natural habitats. Thus, we hypothesize that in the absence of selection in a human host, ecological interactions among wild *Pseudomonas* 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 natural *Pseudomonas* to inhibit pathogenic *P. aeruginosa* by the production of antimicrobial factors. We have 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 have determined the population-level structure with ~320 of these isolates using the gyrB housekeeping gene. Our antagonistic results against both natural *Pseudomonas* and CF-derived *P. aeruginosa* show strong inhibition suggesting that environmental isolates produce compounds that inhibit pathogenic *P. aeruginosa*. We have developed a genetic system to identify the gene regions encoding antimicrobial compounds through a loss of antagonistic phenotype with subsequent biochemical and genetic characterization. Using this methodology we aim to identify novel compounds that inhibit human pathogens.

BOARD 26

Using macaques to study the persistence of antimicrobial resistance in *Neisseria*

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The effective treatement of gonorrhea is threatened by growing antimicrobial resistance (AMR) in *Neisseria gonorrhoeae,* also called the gonococcus. Monotherapy treatments have been abandoned. The CDC now recommends dual drug therapies that are also threatened by increased gonococcal AMR. *N. gonorrhoeae* frequently acquires AMR from nonpathogenic commensal *Neisseria* species that inhabit the pharynx. The pharynx is a location where commensal and gonococcal strains can coexist and share AMR determinants. Pharyngeal gonorrhea is frequently asymptomatic and therefore difficult to eradicate. Increased knowledge of the pharmacodynamics of antibiotic therapy on neisserial persistence in the pharynx is crucial to the development of effective treatments.

Previously we have used rhesus macaques to study neisserial colonization of the pharynx, and *in vivo* horizontal gene transfer of AMR. Surprisingly, we found several animals harbored *Neisseria* resistant to enrofloxacin, a fluoroquinolone frequently used in veterinary medicine. Regardless, enrofloxacin, was used to to remove each animal's pre-existing *Neisseria* flora before inoculating with defined strains. Enrofloxacin treatment of rhesus macaques temporarily blocked recovery of enroflaxacin-resistant *Neisseria* from pharyngeal swabs. Once enrofloxacin treatment was stopped recovery by culture resumed within twelve days only if an animal's pre-existing flora was resistant to enrofloxacin. Genome sequencing of three enrofloxacin-resistant strains identified putative mutations in *gyrA* and *parC*. Our results demonstrate that the macaque model can be used to study how sensitive and resistance strains respond to antimicrobial therapy. This knowledge will help us understand neisserial persistence in the pharynx, an important reservoir for disease transmission and the emergence of AMR.

BOARD 27

RNAi as a potential mechanism of antiviral immunity in neurons

Yuxiao Tan* and Suren Ambegaokar
Ohio Wesleyan University

Innate immunity has been demonstrated as a way to protect against bacterial and fungal infections through Toll signaling and immune deficiency (Imd) pathways. RNA interference (RNAi) was more recently demonstrated as a mechanism to protect against viral infection; however it is uncertain which cell types can employ RNAi as an immune mechanism against viruses, and to which viruses RNAi is an effective form of immunity. To test if neurons can use RNAi to counter virus infection, we are using Drosophila melanogaster (fruit fly) lines that have specific loss-of-function mutations in genes that control RNAi activity – Dicer-2 and Argonaute-2 – and the neurotropic Sigma virus (SV). Mutations in Dicer-2 or Argonaute-2 are predicted to increase neuronal susceptibility to infection, and thus higher viral loads in vivo. This study will better define innate immune properties of neurons and the nervous system, which may lead to improved therapies for neuronal diseases and infections.

BOARD 28

CCR5 and CXCR4 Molecular Interactions

Virginia Ford*, Lyndsey Darmos, Riley Figueroa, ShyAnne Nobel, and Jared Repas
Lorain County Community College

Human Immunodeficiency Virus (HIV) requires two receptors to enter human T-Cells: a primary receptor (CD4), and one of two co-receptors (CCR5 or CXCR4). Agrawal et al.1 performed experiments which suggested CCR5delta32 may have an effect on CXCR4 expression in Human T-Cells. Our study aims to determine what effects various CCR5 mutations will have on CXCR4.
The cxcr4 gene sequence used in this study was extracted from a human T-Cell line, H9. These cells were amplified through PCR for the cxcr4 gene. After purification, the cxcr4 gene was inserted into pCR®4-TOPO®-vector (TOPO-vector) which is resistant to both ampicillin and kanamycin. The TOPO-CXCR4 was digested using HindIII and NotI enzymes. The separated TOPO-vector was digested with BglI to inactivate ampicillin resistance. Concurrently, a pLNCX2-vector (confers ampicillin resistance) was digested with the same enzymes. The digested pLNCX2-vector and CXCR4 fragment were ligated together, transformed into E. coli, and grown on ampicillin plates. These cultures were tested for kanamycin resistance (TOPO-vector is kanamycin resistant; pLNCX2-vector isn't). The cultures that were only resistant to ampicillin were continued.
Our goals are to transfect pLNCX2-CXCR4 clones into the retroviral packaging cell line PT67. PT67 cells will produce retroviral particles that will infect CEMX174 and U937 cells. We will determine whether the additional copies of cxcr4 genes change receptor density for CXCR4 proteins and CCR5 proteins on the transfected cells. Transfected cells will also be sent out to a lab capable of determining HIV infectivity.
1 L. Agrawal et al., J. Virol. 78;5, 2277-2287 (2004).

BOARD 29

Species in multibacterial environments may use sequential adherence in response to modification of receptors by other species

Margaret A. Grau*[1], Anirudh K. Singh[2], Shireen A. Woodiga[2], and Samantha J. King[2,3]
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Host cells are decorated with glycans, which can both be receptors for bacterial adherence and be modified by bacterial glycosidases. We recently demonstrated that adherence is more dynamic than previously thought, in that bacteria can bind sequentially to carbohydrates within a glycan structure. *Streptococcus oralis*, an oral colonizer and cause of endocarditis, binds platelets via sialic acid, but also expresses sialidase which cleaves terminal sialic acid. Following removal of sialic acid, *S. oralis* also binds an underlying carbohydrate, β-1,4 galactose. In multibacterial environments, glycosidases of one species affect an environment shared with others. This led to the hypothesis that some bacterial species use sequential adherence as a result of other bacteria in the same niche removing carbohydrate receptors. This is being tested using *Streptococcus gordonii*, an oral colonizer which binds sialic acid and does not produce sialidase. Other bacteria in the oral cavity, including *S. oralis*, produce sialidase, and other published evidence suggests that *S. gordonii* exists in an environment lacking terminal sialic acid. Adherence assays to oral epithelial cells indicated that at least some *S. gordonii* bound an additional receptor in the absence of terminal sialic acid. Further studies demonstrated that this adherence is significantly reduced when β-1,4 linked galactose is removed by β-galactosidase. This demonstrates that *S. gordonii* uses sequential adherence in the presence of exogenous sialidase. Current studies are determining if sequential adherence could occur in vivo by performing assays in the presence of *S. oralis*. This study demonstrates the impact of the multibacterial environment on adherence.

BOARD 30

Activity of a non-pyrrolysine methyltransferase suggests a novel pathway of methanogenesis on the quaternary amine glycine betaine

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Miami University Department of Microbiology, University of Idaho Department of Biological Sciences, Middle Tennessee State University Department of Biology, Miami University Department of Microbiology, Miami University Department of Microbiology

Methane is a potential fuel source as well as a powerful greenhouse gas and thus contributes to global climate change. Biological production of methane by methanogenic archaea can occur through a small number of known pathways. One general mechanism is the methylotrophic methanogenesis pathway in which methylated compounds such as methylamines and methanol are demethylated. Until recently, quaternary amine (QA) compounds (glycine betaine, carnitine, choline, QMA) were not thought to be direct substrates for methanogens and were thought to only be broken down through cleavage by fermentative bacteria. Recently, our lab and others have shown that some methanogens can utilize QAs directly, however these specific pathways have not yet been elucidated. Work within our lab demonstrated that the bacterium *Desulfitobacterium hafniense* Y51 was capable of demethylating glycine betaine using a methyltransferase called MtgB. MtgB uses glycine betaine to methylate a corrinoid binding protein, MtgC, which then acts a substrate for a second methyltransferase MtgA that methylates tetrahydrofolate. A methanogen we isolated based on its ability to utilize glycine betaine as a methanogenic substrate, *Methanolobus vulcani* B1d, encodes enzymes highly similar to MtgB and MtgC. We cloned and expressed the putative MtgB, MV1727, and demonstrated *in vitro* its ability to catalyze glycine betaine demethylation using free cobalamin as a substrate. We predict that MV1727 is catalyzing the initial step of a novel methylotrophic pathway of methanogenesis from glycine betaine. We are currently working to identify the remaining enzymes of the pathway to reconstitute the pathway *in vitro*.

BOARD 31

The effect of ccr5delta32 on the expression of wild-type CCR5 and CXCR4

Jacob Haller*, Norah Ali, Gary Dodson, Jeremy Leighty, Keira Magdos, Kaitlin Smith, and India Worthy
Lorain County Community College

Human Immunodeficiency Virus (HIV) must use CD4 and one of two secondary receptors, CCR5 or CXCR4 to enter human cells. Some individuals who were exposed to HIV, but not infected were shown to be heterozygous for a deletion of 32 base pairs (delta32) in CCR5. Timothy Ray Brown was functionally cured of HIV by a bone marrow transplant from a homozygous ccr5delta32 donor. It is hypothesized that this mutation disrupts transport of CCR5 to the surface of the cell, preventing HIV infectivity. It has been hypothesized that the truncated CCR5 delta32 protein (208 amino acids) may be able to exert a negative regulatory effect on both wild-type CCR5 and CXCR4.

Fifty-eight individuals were screened for ccr5delta32 allele by PCR. Five CCR5 heterozygous individuals, and no homozygous individuals were identified. One of the heterozygous samples, called AF2, was used as a source to subclone both the wild-type and ccr5delta32 alleles. A PCR was performed on our DNA sample AF2. This PCR was then purified to remove any excess primers. The purified sample was ligated into a linear pCR 4-TOPO cloning vector. The sample was then digested out of the TOPO vector and will be ligated into a eukaryotic expression vector known as pLNCX2. Both Wild-Type and delta32 alleles will be used in transient and stable transfections.

The pLNCX2 cells will be transfected into a PT67 a retroviral packaging cell line. Retroviral particles will be infected into H9 lymphoid cells. These cells will be tested for HIV infectability and CCR5/CXCR4 expression.

BOARD 32

An intracellular PPIase is required for secretion of *Staphylococcus aureus* nuclease

Richard E. Wiemels*, Stephanie M. Cech, Andy Weiss, Anastacia Parks, Lindsey N. Shaw, and Ronan K. Carroll
Ohio University

Peptidyl-prolyl cis-trans isomerases (PPIases) are enzymes that assist in the folding of proteins. They catalyze the isomerization between the cis and trans form of proline peptide bonds, thus accelerating the rate of refolding in proteins containing prolines. The absence of PPIase activity can lead to delayed/incorrect folding and a loss of protein activity. In bacteria, PPIases have been shown to assist in the folding of secreted virulence factors and in doing so contribute to virulence, but their role in S. aureus remains unknown. Previous work on Staphylococcal nuclease (Nuc), a secreted virulence factor, has demonstrated that the isomerization state of a single proline bond (K116-P117) controls the rate of Nuc refolding. In this work, we identify a Staphylococcal PPIase (PrsB) that is required for Nuc activity. We demonstrate that equal amounts of Nuc are secreted in a prsB mutant and wild-type strain, however, the secreted Nuc is less active when secreted from the prsB mutant. We also show that PrsB is active in a PPIase assay and is capable of Nuc folding in vitro. Future work includes identifying other targets for PrsB through co-immunoprecipitation, determining the amino acids essential for PrsB PPIase activity, and abcess models of infection to determine the role of PrsB PPIase activity in virulence.

BOARD 33

Detection of Heat-Resistant Mold Contamination by a Pan-fungal PCR Technique

Kayla Thieman*, Robert Nichols, and Andrew Rasmussen
Mount St. Joseph University

Despite robust thermal processing, spoilage of fruit products by heat resistant molds (HRMs) continues to be an issue in maintaining food product integrity. HRMs produce heat resistant ascospores or similar structures that approach bacterial endospores in survivability. In addition, detection of HRM contamination in fruit products is a time-consuming process. Typically, a 30-day culturing, incubation, and examination period is required to confirm the lack of HRM contamination, thereby delaying release of processed fruit products and reducing overall shelf life. In order to shorten the time required to screen fruit products for contamination, we have adapted a molecular approach utilized to detect medically important fungi to detecting HRMs. Specifically, we used a polymerase chain reaction (PCR) technique directed at conserved motifs in the rRNA genes of fungi to detect the presence of accumulated HRM DNA in processed fruit samples. We were able to detect the presence of eight of eight representative HRMs using this technique. We believe this warrants further study as a HRM detection technique that may reduce or eliminate long incubation and laborious screening procedures.

Abstracts of OBASM Podium Presentations

Saturday, April 9
3:00 – 5:00 pm

3:00 – 3:15 pm

Establishing a connection between anaerobic virulence regulation and metabolism in *Listeria monocytogenes*

Nathan Wallace*, Ashley Zani, Eric Newton, and Yvonne Sun

University of Dayton

Listeria monocytogenes (*Listeria*) is a Gram positive, facultative anaerobe responsible for gastrointestinal infections. *Listeria* pathogenesis has been investigated extensively, but mostly under aerobic conditions. Considering the ability of *Listeria* to proliferate and survive in places with low or no oxygen, including the lower gastrointestinal tract, little is known about how *Listeria* pathogenesis is affected by anoxic conditions. Anaerobicity serves as a significant strain to *Listeria* metabolism, potentially impacting pathogenesis. However, the role of *Listeria* anaerobic metabolism in pathogenesis is not clearly defined. To establish a connection between anaerobic metabolism and virulence regulation in *Listeria*, we first tested the effect of anaerobicity on tricarboxylic acid (TCA) cycle activity. By measuring the activity of aconitase, an enzyme in the TCA cycle, we observed a significantly decreased aconitase activity in anaerobically grown *Listeria* compared to aerobically grown *Listeria*. A result implying reduced TCA cycle activity under anaerobic conditions. Moreover, anaerobically grown *Listeria* also exhibited decreased levels of the toxin listeriolysin O (LLO) in the culture supernatant and increased invasion of cultured human colonic cells. To further investigate the role of anaerobic TCA cycle activity on LLO production and infections, we supplemented anaerobic cultures with the TCA cycle intermediate citrate to induce TCA cycle activity. Upon supplementation of citrate anaerobically grown *Listeria* exhibited an increase in LLO production and a decrease in cellular invasion. Our results highlighted for the first time a connection between *Listeria* anaerobic metabolism and virulence regulation.

3:15 – 3:30 pm

Modification of Adenoviral Transduction Efficacy through Tat-myc-PDZ Protein Domains of MAGI-1 Protein

Ibrahim Alkhomsi*, James M. Readler, Shon Jergens, Mahmoud Alghamri, Priyanka Sharma, and Katherine Excoffon

Wright State University

Adenoviruses are common human pathogens that often cause cold-like symptoms in human populations. Adenoviruses enter the body through airway epithelial cells using the Coxsackievirus and adenovirus receptor (CAR). CAR availability is one of the major factors that limits viral infection. We have discovered that one of the alternatively spliced isoforms of CAR (CAREx8) is localized at the apical surface of polarized primary human airway epithelia, where mediates apical adenovirus infection. We hypothesize that an increase or decrease of CAREx8 will directly affect the susceptibility of the airway epithelium to adenovirus infection. We have recently discovered that apical CAREx8 abundance is regulated with opposing effects by two different domains within the PDZ-domain containing protein MAGI-1 (PDZ1 and PDZ3). The cell permeable MAGI-1 PDZ1 and PDZ3 domains, and PDZ2 (control), were purified by subcloning each domain with a cell permeable peptide TAT sequence and a myc tag. The domains have been successfully expressed in *E. coli*, purified in GST columns and endotoxin subsequently removed. Current work is focused on testing the ability for these domains to enter non-polarized and polarized epithelial cells, and either up or downregulate CAREx8 expression and adenovirus

infection. The basic knowledge of CAR function investigated in this study will lead to better understanding of adenovirus infections and may identify regulatory steps that can be manipulated to protect populations from wild type adenovirus infection or improve adenovirus-mediated gene therapy.

3:30 – 3:45 pm

A novel mechanism of toxicity amplification: ACD toxin-produced actin oligomers poison formin controlled actin polymerization

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Bacterial toxins are the deadliest compounds on Earth; a single copy is capable of compromising a host cell. Amplification of toxicity is typically achieved by enzymatically targeting signaling cascades or inhibiting vital host proteins found in relatively few copies. Conversely, the major cytoskeletal protein actin is a common target for toxins, and it is not clear how actin-targeting toxins achieve high efficiency. The actin crosslinking domain (ACD) catalyzes formation of an amide bond between actin monomers, forming actin oligomers. It was believed that ACD toxicity stems from the slow failure of the cytoskeleton due to the gradual accumulation of non-functional actin oligomers. We found that ACD is not required to crosslink all actin in the cell; suggesting that low doese of ACD-crosslinked oligomers are toxic. Since actin-binding domains of actin-regulatory proteins are organized in tandems, these proteins can potentially bind actin oligomers with abnormally high affinities. Formins, a family of actin binding proteins, nucleate filaments and, through their tandem poly-proline stretches, bind profilin-actin complexes to accelerate elongation. We found that formins preferentially bind crosslinked oligomers and are inhibited by sub-nanomolar concentrations of oligomers as revealed on the single filament level by total internal fluorescence reflection microscopy. In the presence of profilin, the oligomers caused reversible blocks of elongation of formin-controlled filaments. Mathematical modeling revealed that the oligomers potently inhibit nucleation and elongation of formin-controlled actin assembly. Our findings indicate that ACD employs a novel toxicity mechanism by converting actin into highly toxic oligomers to target key regulators of actin dynamics.

3:45 – 4:00 pm

Prevalence and characterization of *Staphylococcus aureus* on public recreational beaches in Northeast Ohio

Dipendra Thapaliya*, Emily J. Hellwig, Jhalka Kadariya, Mark Dalman, Kristen Kennedy, Mackenzi DiPerna, Adrienne Orihill, Tara C. Smith

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), and due to its ever-changing epidemiology. Although MRSA has been isolated from marine water and intertidal beach sand, only few studies have conducted to assess the prevalence of *S. aureus* in freshwater recreational beaches. This study aimed to determine the prevalence and molecular characteristics of *S. aureus* at freshwater recreational beaches in Northeast Ohio. A total of 280 beach sand and water samples were collected from 10 public fresh water beaches in Northeast Ohio, USA between June 24, 2014 and April 30, 2015. Samples were analyzed using established microbiology methods, and resulting S. aureus isolates were typed by spa typing and multi-locus sequence typing (MLST). PCR was used to detect the presence of the Panton-Valentine Leukocidin (PVL) and

mecA genes. Antibiotic susceptibility was tested via the Vitek-2 System. The overall prevalence of S. aureus in sand and water samples was 22.9% (64/280). The prevalence of MRSA was 7.9% (22/280). The highest prevalence was observed in summer (45.8%; 55/120) compared to fall (4.2%; 5/120), and spring (10%; 4/40). The prevalence of PVL genes among S. aureus isolates was 23.4% (15/64). A total of 26 spa types were detected from 64 *S. aureus* isolates. Overall, t008 was the most common spa type. Two isolates were t002, a common human-associated strain. One isolate was t571 (ST398), a livestock-associated strain. Twenty-eight (43.8%) isolates were multi-drug resistant. The results of this study indicate that beach sand and fresh water of Northeast Ohio was contaminated with *S. aureus* and MRSA. The high prevalence of *S. aureus* in summer months and presence of human –associated strains may indicate the possible role of human beachgoers in *S. aureus* contamination of beach water and sand.

4:00 – 4:15 pm

Novel regulation of *Escherichia coli*'s ptrB mRNA involving an AUG triplet at the 5'-terminal and downstream coding sequence elements

Heather Beck*, Ian Fleming, and Gary Janssen
Miami University

Analysis of the *E.coli* transcriptome (Regulon DB) identified a unique subset of mRNAs that contain a conventional untranslated leader and Shine-Dalgarno (SD) sequence upstream to the gene's start codon while also containing an AUG triplet at the mRNA's 5' terminus. Focus was placed on the ptrB gene because although the reading frame specified by the 5'-terminal putative start codon was lowly expressed, the AUG triplet was necessary for coding sequence expression. A mutation of ptrB's 5-terminal AUG (5'-uAUG) reduced downstream ptrB expression by more than 90% even in the presence of the predicted ptrB SD sequence. Strengthening of the ptrB SD sequence did relieve the necessity of the 5'-uAUG, suggesting that the regulation imposed by the 5'-uAUG is distinct from SD regulation. Replacement of other 5'UTRs with the ptrB 5'UTR showed a similar dependence on the 5'-uAUG for downstream expression, suggesting that the regulatory features of the ptrB 5'UTR were sufficient to control expression of other CDS. However, internal RNA segments fused to the ptrB 5'UTR were not efficiently expressed implying the necessity of certain downstream sequence elements. These elements were identified in ptrB mRNA and found to be necessary for translation. When both the 5'-uAUG and the downstream coding sequence elements were mutated in concert, ptrB expression was abolished. These data imply that the additional upstream and downstream regulatory features are present due to the weak SD. This provides insight into a novel form of translation regulation that may be widespread in mRNAs with weak or no SD sequence.

4:15 – 4:30 pm

One of These Things is Not Like the Other: RyfAs in *Shigella dysenteriae*

Megan E. Fris*[1], William Broach[2], Sarah Klim[1], Tyler Sieron[3], Francis Essien[3], Ronan Carroll[1], Peter Coschigano[3], and Erin R. Murphy[3]
[1]Ohio University Department of Biological Sciences [2]University of South Florida Department of Cell Biology [3]Ohio University Heritage College of Osteopathic Medicine

As genome-wide searches have become standard and easy to access, scientists have uncovered interesting patterns in small RNA and small protein related research. One emerging concept is that these small RNAs/small proteins can exist as multiple siblings on an entire chromosome. However, teasing apart these siblings to determine if each have specific functions has proven difficult. Recently, we have discovered two sibling small RNAs/potential small proteins in *Shigella dysenteriae*, which are 95% identical to each other, but when overproduced, result in two vastly different phenotypes. RyfA1 overproduction limits cell-to-cell spread by *Shigella* in tissue culture assay, while RyfA2 overproduction inhibits eukaryotic cell invasion by the pathogen. Additionally, we have noticed two small transcripts just upstream of each *ryfA* gene, designated *ryfB1* and *ryfB2. In silico,* each *ryfB*

gene has a region of approximately 18 nucleotides with complementarity to the corresponding *ryfA* gene, a finding that suggests a regulatory function for the RyfBs. By understanding the RyfAs/RyfBs in *S. dysenteriae*, important biological questions can be answered. How do small RNAs/or small proteins contribute virulence in bacteria? What advantages do multiple sibling sRNAs/small proteins give to bacteria? And finally, can we demonstrate exact instances of evolutionary adaptation suited for sRNA regulation vs protein regulation?

4:30 – 4:45 pm

Adeno-associated virus-VEGF-165 mediated modification of adipose derived stem cells for cell therapy

Upasana Niyogi*[1], Priyanka Sharma[1], Gregory C. Gould[2], Sunishka M. Wimalawansa[2], R. Michael Johnson[2], and Katherine J.D.A. Excoffon[1,2]

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Chronic wounds have become a major clinical and economic burden in our society. New treatment approaches are desperately needed. Angiogenesis and vascularization play a critical role in healing. An essential angiogenic factor that promotes the formation of vascular beds is vascular endothelial growth factor (VEGF). Moreover, adipose-derived stem cells (ASC), through their regeneration and differentiation properties, may promote healing when transplanted into a wound bed. Our preliminary work has shown that nonpathogenic adeno-associated virus 5 (AAV5) is able to transiently transduce ASC with higher efficiency than other AAV serotypes. We hypothesize that administration of AAV-VEGF genetically-modified ASC directly into the wound site will accelerate rejuvenation of ischemic tissue. To test our hypothesis, the VEGF-165 gene was synthesized (IDT) and cloned (Clontech) into two AAV5 plasmids, pFBAAVCMVmcswtIRESeGFP and pFBAAVCAGmcswtIRESeGFP. The AAV-VEGF-165 and control plasmids were then transfected into HEK-293, COS-7, and primary ASC cells. The transfection efficiency was determined by number of GFP positive cells observed under fluorescence microscope. The amount of VEGF-165 produced was quantified by VEGF ELISA. Greater transfection and VEGF expression was observed in the cells transfected with pFBAAVCAGmcswtIRESeGFP than pFBAAVCMVmcswtIRESeGFP plasmid. Therefore, AAV-CAG-VEGF-wtIRES-eGFP virus produced from pFBAAVCAGmcswtIRESeGFP by the University of Iowa using the Baculovirus system was used to transduce the ASC. Wound healing, as measured by scratch assay, was performed on monolayers of ASC and healing was observed. Future work will be to test the wound healing efficacy of transduced ASC expressing VEGF relative to control transduced ASC in a novel multidimensional wound model culture system.

4:45 – 5:00 pm

Heme-Iron Starvation Enhances Stationary Phase Persistence of Nontypeable *Haemophilus influenzae* (NTHI)

Rachael L. Hardison*, Meghan O'Bryan, Sheryl S. Justice, and Kevin M. Mason

Nationwide Children's Hospital, Center for Microbial Pathogenesis

NTHI is a Gram negative opportunistic pathogen that often persists in chronic and recurrent otitis media despite repeated antibacterial therapies. As a heme-iron auxotroph, NTHI must overcome host nutritional immunity to survive and persist in the middle ear. We have developed an in vitro method of transient heme-iron restriction to model nutritional changes that occur as NTHI transitions from nasopharyngeal colonization to middle ear infection. Using this method, we observed that heme-iron restriction results in NTHI survival in spent media for weeks as compared to NTHI grown in the presence of heme. The persistent population displays repeated and uniform cycling between an increase and decrease in recoverable NTHI. We previously demonstrated that heme-iron restriction results in a significant increase in height of biofilm towers with architecture composed of

filamentous morphotypes, and increased formation of intracellular bacterial communities. The characteristic biofilm traits of tower height and filamentous architecture associated with heme-iron restricted NTHI were maintained with persistent NTHI. In addition, persistent NTHI are more resistant to oxidative stress, and form biofilms with a distinct metabolic and proteomic profile characterized by significant changes in arginine biosynthesis compared with the parent. Thus, we have demonstrated that transient heme-iron restriction induces a persistent NTHI state that appears genetically stable and enhances multiple phenotypes beneficial for survival during infection. Our data contribute to the knowledge of how NTHI may persist within the middle ear despite clinical therapies. Elucidating this mechanism will aid in the development of more effective treatment for chronic otitis media.

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.

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