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Acknowledgements

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OBASM Executive Committee

Jennifer Bennett
Erin Murphy
D.J. Ferguson
Lubna Abu-Niaaj
Christine Weingart
Chet Cooper
Laura Tuhela-Reuning
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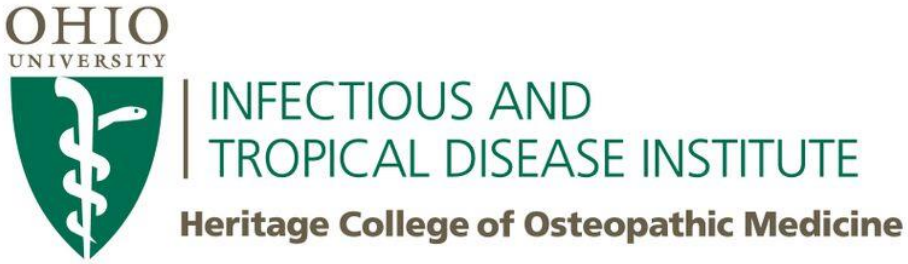
Kim Finer – Region 3 Branch Planning Coordinator
ASM Branch Lectureship Program
The American Society for Microbiology

Invited Speakers

Tara Smith
Henry Neal Williams
Karen Dannemiller
Xin Wang
Brian Lower
Mario Grijalva
Matthew Anderson
Arne Rietsch
Ibrahim Katampe
Ahmed Yousef
Jennifer Bennett



Program Schedule for OBASM 2018

Friday, March 23

6:00 – 7:00 pm **Registration –** Walter Hall Rotunda
Social – Walter Hall Rotunda

7:00 – 7:10 pm **Welcome Statement –** Walter Hall
Joseph Shields
Vice President for Research and Creative Activities
Dean of the Graduate College
Ohio University

7:15 – 8:15 **OBASM Keynote Lecture** – Walter Hall

"Vaccine denial and the internet: What can microbiologists do?"
Tara Smith
College of Public Health
Kent State University

8:15 – 9:00 pm **Social** – Walter Hall

Saturday, March 24

7:30 – 8:30 am **Registration** – Morton Hall
Poster set-up – Morton Hall

8:30 – 10:00 am **ASM Branch Lecture** – Morton Hall, Room 235

"Hunting the role of the world's smallest hunters, the *Bdellovibrionales*"

Henry Neal Williams
School of the Environment
Florida A&M University

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: Medical Microbiology

Session 1: Environmental and Applied Microbiology – Morton Hall, Room 237

Moderator: D.J. Ferguson

10:30-11:00 **Karen Dannemiller** – Department of Civil, Environmental, and Geodetic Engineering, The Ohio State University
"The indoor microbiome: Implications for childhood asthma and building design"

11:00-11:30 **Xin Wang** – Department of Microbiology, Miami University
"Metabolic rewiring to support efficient limonene production in Cyanobacteria"

11:30-12:00 **Brian Lower** – School of Environment and Natural Resources, The Ohio State University
"Magnetotactic Bacteria – Bugs with backbones"

Session 2: Medical Microbiology – Morton Hall, Room 235

Moderator: Nathan Weyand

10:30-11:00 **Mario Grijalva** – Director, Tropical Disease Institute, Miami University
"Chagas disease: The road from the lab bench to community interventions"

11:00-11:30 **Matthew Anderson** – Department of Microbiology, The Ohio State University
"Rapid evolution and diversification of an expanded gene family in *Candida albicans*"

11:30-12:00 **Arne Rietsch** – Department of Molecular Biology and Microbiology, Case Western Reserve University
"Inhibition of neutrophil ROS production by *Pseudomonas aeruginosa*"

12:00 – 1:30 pm **Noontime Activities:**

Lunch: Box or "on your own"
Only for those who pre-ordered box lunches
Pickup near main entrance of Morton Hall

OBASM Business Meeting – Morton Hall, Room 227

12:15 – 1:30 pm

Session 3: Education – Morton Hall, Room 226

Moderators: Laura Saltman, Stephanie Miller, Lubna Abu-Niaaj

"Strategies for engaging and sustaining undergraduate research students"
Ibrahim Katampe
Natural Sciences Department
Central State University

"Food microbiology projects: Student ownership from topic selection and experimentation to presentation in 10 weeks"
Ahmed Yousef
Department of Food Science and Technology
The Ohio State University

"Innovative Active Learning Strategies for the Microbiology Classroom"
Jennifer Bennett
Department of Biology and Earth Science
Otterbein University

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

3:30 – 5:40 pm **Podium presentations and judging** – Morton Hall, Room 235
Coordinator – Chet Cooper

3:30 – 3:45 pm

Host cell perforation by listeriolysin O (LLO) activates a Ca^{2+}-dependent cPKC/Rac1/Arp2/3 signaling pathway that promotes *Listeria monocytogenes* internalization independently of membrane resealing

Jonathan G.T. Lam* [1, 2], Stephen Vadia[2], Sarika Pathak-Sharma[1], Eric McLaughlin[3], Xiaoli Zhang[3], Joel Swanson[4], and Stephanie Seveau[1, 2]
[1]Department of Microbial Infection and Immunity, Infectious Diseases Institute, The Ohio State University Wexner Medical Center
[2]Department of Microbiology, The Ohio State University
[3]Center for Biostatistics, The Ohio State University
[4]Department of Microbiology and Immunology, University of Michigan Medical School

3:45 – 4:00 pm

Genetics-Based Discovery of *Histoplasma* Genes Reguired for Intramacrophage Growth

Qian Shen, Matthew J. Beucler, and Chad A. Rappleye
Department of Microbiology, Ohio State University

4:00 – 4:15 pm

Alternative electron flow supports rewired metabolism in Antarctic photopsychrophile *Chlamydomonas* sp. UWO241

Isha Kalra*, Xin Wang, and Rachael Morgan-Kiss
Department of Microbiology, Miami University

4:15 – 4:30 pm

RyfA and RyfB in *Shigella*: Which sRNA is the Evil Twin?

Megan E. Fris*[1], William H. Broach[1], Sarah E. Klim[1], Peter W. Coschigano[2], Ronan K. Carroll[1], Clayton C. Caswell[3], and Erin R. Murphy[2]
Ohio University[1], Ohio University College of Osteopathic Medicine[2], Virgina Tech[3]

10 minute break

4:40 – 4:55 pm

The role of neuraminidase-producing bacteria in exposing cryptic carbohydrate receptors for *Streptococcus gordonii* adherence

Alexander Wong*[1,2], Margaret A. Grau[1], Anirudh K. Singh[1], Shireen A. Woodiga[1], and Samantha J. King[1,3]
[1]Nationwide Children's Hospital Center for Microbial Pathogenesis, [2]The Ohio State University College of Medicine, [3]The Ohio State University Department of Pediatrics

4:55 – 5:10 pm

Target Identification of the New Antifungal Compound 41F5

Kristie Goughenour*[1], Keisuke Ishita[2], Werner Tjarks[2], and Chad Rappleye[1]
[1]Department of Microbiology, Ohio State University
[2]Division of Medical Chemistry and Pharmacology, Ohio State University

5:10 – 5:25 pm

Identification of Novel Anti-Adenoviral Small Molecules

Hannah Shows[1], Priyanka Sharma[1], Mahmoud Alghamri[1], Michael Raymer[2], Katherine Excoffon[1]
[1]Department of Biological Sciences, Wright State University;
[2]Department of Computer Science, Wright State University

5:25 – 5:40 pm

Role of Cell Fusion in Protection of the Placenta Against Infection

Lauren J. Johnson[1,2,3]*, Siavash Azari[1,2, 3], Joanna Marshall[1,2,3], William Ackerman[5], Stephen Thung[5], Kara Rood[5], John M. Robinson[4], Stephanie Seveau[1,2,3]
[1]Ohio State University, Department of Microbial Infection and Immunity, [2]Ohio State University, Department of Microbiology, [3]Ohio State University, Infectious Diseases Institute, [4]Ohio State University, Department of Physiology and Cell Biology, [5]Ohio State University,

Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine

5:40 – 6:30 pm **Mentoring in Microbiology** – Morton 237

Henry Neal Williams
School of the Environment
Florida A&M University

6:30 – 8:30 pm **Banquet and Student Awards Presentations** – Ohio University Inn
Cash Bar – 6:30 pm
Dinner Served – 7 pm

Abstracts of OBASM Poster Presentations
Saturday, March 24
1:30-3:20 pm

BOARD 1

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

Mahnur Khan, **Emily Verbrugge***, Chloe Murrell, Joseph Basalla, and Hans Wildschutte
Bowling Green State 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 four semesters that SWI has been offered at BGSU, this strategy has allowed students to isolate over 400 bacterial strains, of which three 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 2

Elucidation of the role of FCRL1 in B lymphocyte activation

Jenna M. DeLuca* and Timothy J. Wilson
Miami University

Fc receptors play a role in cellular and humoral immune responses. Fc receptors often pair with adaptor transmembrane proteins that possess immunoreceptor tyrosine based activation motifs (ITAMs) or immunoreceptor based inhibitory motifs (ITIMs). The presence of these activation or inhibitory motifs confers immunoregulatory roles to the molecules with which they are found. Fc receptor-like (FCRL) genes have been identified and are found to be differentially expressed by B-lineage cells. Among this family of molecules is FCRL1, which is unique in that it has two ITAM-like motifs in its intracellular domain. The presence of these motifs leads to the possibility that FCRL1 is involved in B lymphocyte activation. Additionally, it has the potential to be an activating co-receptor with the B cell receptor complex, which also has ITAM domains. The goal of this study is to determine where FCRL1 is expressed in mice. Determining expression patterns may give an indication of function. Additionally, determining the expression and possible function of murine FCRL1 may provide information on the function of human FCRL1 due to their homology. This study also aims to determine how immune response to vaccination are altered in FCRL1 -/- mice. This will help determine development of antibody responses in vivo along with elucidating the effects of FCRL1 on B cell development. While its role has yet to be fully determined, current publications and preliminary data suggests that it may be a co-receptor on B cells that could play a role in activation and signaling. Through flow cytometry of murine splenocytes, we determined that there is varying expression of FCRL1 on different B cell subtypes, which may aid in the explanation that FCRL1 is an activating co-receptor. Additionally, after challenge to the immune system with various antigens, differences in the individual cell subtypes was observed between the two genotypes.

BOARD 3

The Prevalence and Identification of Antibiotic-Resistant Bacteria in Isolated Ecological Zones

Orion Brock* and Jennifer Larson
Capital University

Studying antibiotic resistance in the environment is important for understanding the transfer of resistance between environmental and pathogenic bacteria. Previous studies have indicated that antibiotic resistance exists in environments unaffected by anthropogenic antibiotic use. A recent "crowdsourcing" approach to assay the prevalence of antibiotic resistance in the environment (PARE) has been taking place in undergraduate labs across the United States. Seven ecological zones (grassland, woodland, prairie, riparian, wetland, spring, and river) were evaluated from a private research property to determine if antibiotic-resistant bacteria were present. Two soil samples from each zone were collected, serially diluted, and plated on selective and non-selective media with or without tetracycline. To build off of the PARE project, we not only assessed antibiotic prevalence, but also characterized the resistant organisms by surveying colony diversity, testing for multi-drug resistance, and identifying bacteria through DNA sequencing of the 16S gene. We hypothesized that we would find antibiotic-resistant bacteria in all ecological zones studied, and also that these bacteria would be unique to their specific niche. Antibiotic resistance was discovered in all sites at varying percentages. Some morphotypes were present in all sites, with the woodland zone having the greatest diversity. Multi-drug resistance was found in 93% of the isolates using a Kirby Bauer disk assay. These results suggest that multi-drug resistance is prevalent even in the presence of naturally occurring pressures. Of the ten isolates sequenced for the 16S rRNA gene, six different types of bacteria were identified. Together, our findings indicate that antibiotic resistance is prevalent in environments isolated from human development and that this naturally occurring resistance may contribute to the rise in antibiotic resistance seen in clinical settings.

BOARD 4

HIV Infectivity Counteracted by the CCR5-delta32 Mutation Effect on the CXCR4 Gene

Riley Figueroa* [1,2], Leéna Boone[1,2], Jennifer Ortega[1,2], Kyle Patton[1,2], and H. W. Kestler[1]
1 Lorain County Community College Department of Biology
2 Lorain County Early College High School, Elyria, OH, USA

The Human Immunodeficiency Virus (HIV), which can progress to Acquired Immune Deficiency Syndrome, or AIDS, is a virus that targets and weakens the body's immune system. An infection initially occurs when HIV interacts with two receptors on a human T-cell; the primary receptor, CD4, and one co-receptor, either CXCR4 or CCR5. It is hypothesized that the CCR5-Delta32 mutation affects CCR5 expression and possibly CXCR4, as suggested by Agrawal *et al*. This research will determine the effects of the CCR5-Delta 32 mutation on CXCR4 and the possible role it has in HIV-resistance.

The *cxcr4* gene was isolated from a T-cell line using primers containing XhoI and EcoRI restriction sites. Using pCR4-TOPO, *cxcr4* was then subcloned into the retroviral shuttle vector pLXSN. This was done through the ligation of *cxcr4* and TOPO. *cxcr4* was digested with EcoRI and XhoI, then subclonder into pLXSN. Once pLXSN-CXCR4 is achieved, a bacterial transformation using competent *E. coli* cells will be performed to produce successful clones.

Cell cultures of the packaging cell line PT67 are being grown currently and fed twice a week with DMEM 10% FBS and 1% penstrep media. A kill-curve using G418 will be performed to determine the optimum concentration for selecting plasmids containing the *cxcr4* gene.

Retroviral particles from the pLXSN-CXCR4-transfected PT67 will be used to infect both primary and tumor cells. This process will be carried out with *cxcr4*, and repeated with *ccr5* alleles. The *ccr5* alleles will include the delta32 mutation, the wild type variation, and a novel point mutation called TG5. The novel point mutation was isolated from a possibly HIV-resistant subject. This will allow HIV-resistance to be tested by infecting cells expressing various *ccr5* alleles and *cxcr4.*

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

BOARD 5

The Effect of Coriander Extract on the Replication of T2 Bacteriophage

Gerald Goldstein, Sarah M. Bergman, **Marielle K. Buss*,** Hanna K. Cordes, Mary A. Cranley, Nick R. Reed, Mallory M. Wolfe,
Ohio Wesleyan University

Coriander, also known as cilantro or Chinese parsley, is an annual herb in the family Apiaceae. All parts of the plant are edible, but the fresh leaves and the dried seeds are the parts most traditionally used in cooking. Coriander has been shown to have antibacterial and antiviral activity for viruses of humans in vitro. Increasing concentrations of coriander seed extract added to Luria-Bertani broth have no effect on the growth of E. coli. The addition of E. coli coriander seed extract to Luria-Bertani broth at a concentration of 0.00012 g/ml enhances the yield of T2 bacteriophage up to 125% of the control culture. When the time of addition of coriander extract before infection of bacteria was varied, the longer the extract was in contact with the bacteria, the greater was the stimulatory effect on T2 bacteriophage yield. Increasing amounts of glucose added to E. coli cultures infected with T2 bacteriophage also caused an increase in bacteriophage yield. However, increasing concentrations of glucose added to E. coli cultures treated with coriander seed extract and then infected with T2 bacteriophage caused a decrease in the bacteriophage yield. Even very small concentrations of glucose inactivate adenylate cyclase enzyme activity in E. coli. These results suggest that stimulation of T2 bacteriophage caused by coriander seed extract is due to stimulation of cAMP production and adenylate cyclase enzyme activity in E. coli cells. Like other herbs and spices that enhance T2 bacteriophage yield, coriander seed extract exhibited no stimulatory effect on T4 bacteriophage yield.

BOARD 6

Identification of environmental Pseudomonas exhibiting inhibitory activity against multi-drug resistance pathogens

Ryan Harris*, Joe Basalla, Payel Chatterjee, Brittany Eggly, and Hans Wildschutte
Bowling Green State University

The evolution of multi-drug resistant (MDR) pathogens is becoming a serious threat to humanity due to the overuse and misuse of antibiotics, both in medicine and agriculture. These MDR pathogens are giving rise to completely untreatable infections leading to increasing mortality rates worldwide. Additionally, pharmaceutical companies are no longer investing in the research and development of novel alternatives leading to an increase in use of last resort antibiotics. Previously, we identified that bacterial strains isolated from Lake Erie were effective at inhibiting the opportunistic pathogen Pseudomonas aeruginosa. The aim of this study is to identify if lake-derived pseudomonads produce compounds that inhibit a panel of MDR pathogens isolated from the lungs of cystic fibrosis patients. A collection of 280 environmental pseudomonads were isolated from the Central Basin of Lake Erie and their gyrB gene was amplified, sequenced, and used to create a phylogenetic tree. Using a nutrient agar antagonistic plate assay, all Pseudomonas strains were competed against 28 different MDR pathogens consisting of P. aeruginosa, B. multivorans, B. vietnamiensis, B. dolosa, B. cepacian & B. cenocepacia. After 72 hours of co-culture incubation, the assays were screened to observe zones of inhibition. Results showed that amongst the 7,840 interaction between the environmental and pathogenic strains, 179 exhibited inhibition of the MDR pathogen and a total of 85 environmental strains showed inhibitory activity. This data show promise into the continued use of environmental bacteria as a source of novel compounds that inhibit MDR pathogens.

BOARD 7

Identification of Proteins Putatively Secreted by the Yeast Phase of *Talaromyces marneffei*

Victoria H. Silvis,* Sarah L. Eisnaugle, Cole P. McKenry, Joshua A. Engle, Rachael M. D'Auria, Julio E. Budde, Xiangjia Min, Chester R. Cooper, Jr.
Youngstown State University

The virulence of *Talaromyces marneffei*, a pathogen endemic to Southeast Asia, is associated with the yeast phase of this thermally dimorphic fungus. The yeast phase of *T. marneffei* thrives in the intracellular milieu of host macrophages. This ability to survive within a normally harsh environment prompted us to hypothesize that the yeast phase of *T. marneffei* may secrete proteinaceous factors which hinder the normal defensive response of the host macrophage.
To assess this hypothesis, 538 putatively secreted *T. marneffei* proteins were identified using the Fungal Secretome KnowledgeBase. DNA primers were designed to a selected number of these proteins and their functionality confirmed in a polymerase chain reaction (PCR) using *T. marneffei* genomic DNA. Functional primer pairs were then used to evaluate gene expression by both the mycelial and yeast phases of *T. marneffei*. RNA collected from these phases after incubation for 24-96 hours was employed in a reverse transcription PCR (RT-PCR) in conjunction with a specific pair of primers. Those RT-PCR results indicating exclusive or increased gene expression by the yeast phase were further subjected to quantitative RT-PCR (qRT-PCR) analysis.
Of 73 primer pairs initially screened, 24 produced results that warranted further examination of gene expression. RT-PCR analysis employing different primer pairs demonstrated that three distinct genes were preferentially expressed during yeast-phase growth of *T. marneffei*. These genes encode a multicopper oxidase, a catalase, and an unknown cell-wall protein. The first two genes are known to have roles in fungal virulence, whereas cell-wall changes are known to be associated with dimorphism as well as virulence. Subsequent qRT-PCR analysis revealed that each of these genes exhibited statistically significant increased expression in the yeast phase of *T. marneffei* as compared to the mycelial phase. Further efforts shall be directed towards demonstrating the relationship of these yeast-phase specific proteins to virulence.

BOARD 8

Glyphosate Inhibits Keratinolytic Activity of *Bacillus* spp. Isolated from Wild Songbirds

Serena E. George[1], **Elizabeth G. Urbanski*[1]**, Madeline M. Vroom[2], Laura Tuhela[1]
[1]Ohio Wesleyan University, [2]University of Florida

Glyphosate is a frequently-used herbicide both domestically and in the agricultural industry worldwide. While the effect of glyphosate ingestion on bird health has been studied, less is known about the potential impact of glyphosate on bacteria found on avian plumage. This study investigated the effect of glyphosate on the degradation of bird feathers by keratinolytic plumage bacteria. To determine the effect of glyphosate on bacterial feather degradation, two strains of *Bacillus* spp. isolated from the plumage of wild songbirds captured in mist nets were tested. A disk-diffusion assay was used to determine that 8.44 g/L of glyphosate inhibited the growth of these two isolates. Feather degradation assays were subsequently performed on each of the *Bacillus* isolates to determine whether the keratinolytic activity of the bacteria was affected. For each isolate, four tubes were prepared that contained a basal salts medium and a white goose feather as the sole carbon and nitrogen source. To two of those tubes, 8.44 g/L of glyphosate was added, then one tube with glyphosate and one tube without glyphosate were inoculated with a *Bacillus* isolate. The other tubes with and without glyphosate were left uninoculated as controls. All experiments were done in replicates of 10, and all tubes were incubated at 37ºC at 125 rpm for seven days. Aliquots from each tube were removed once every 24 hours. Feather degradation was determined by measuring the absorbance of the aliquots at 230 nm. At this wavelength, an increase in the absorbance indicated an increase in oligopeptides resulting from the degradation of keratin in the feathers. The results from uninoculated tubes indicated that glyphosate alone did not damage feathers. However, glyphosate

inhibited bacterial feather degradation from 86% to 98% depending upon the isolate. These data suggest that exposure to glyphosate disrupts feather degradation by *Bacillus* spp.

BOARD 9

The Role of CphA and CphB With Respect to Nitrogen Availability in *Planktothrix Agardhii*

Nicole M. Kern[1, 2], Jasmine Baxter[1,2], Jillian Buga[1,2], **Dulce Cintron*[3]**, Eleana Cintron[3], Arianna Diaz[3], Mia Diaz[3], Sijoon Jeon[2], Karlee Hetsler[2], Deanna A. Leatherwood[3], Zoe Lias[2], Tamanh Luong[1,2], Nicole Marks[1,2], Alexa Plantado[1, 2], John Spatafore[2],Taylor Webber[1, 2], Jacob R. Wyatt[1, 2], Sunny S. Dickerson, BS[1, 2], Michelle J. Neudeck, BS[1, 2], Kathryn A. Durham, Ph.D.[2], George S. Bullerjahn, Ph.D.[1], R. Michael McKay, Ph.D.[1]
[1]Bowling Green State University; [2]Lorain County Community College; [3]Lorain County Early College High School.

Unlike the phosphorus dependent algal blooms of Western Lake Erie, Sandusky Bay's *Planktothrix agardhii* blooms are often dependent on nitrogen inputs. Nitrogen levels of the bay drop significantly by midsummer, but the blooms persist, despite the fact that Pa is a nondiazatrophic organism. (Davis et al. 2015). Certain strains of cyanobacteria house two genes that are responsible for nitrogen storage and utilization. *cphA* encodes the enzyme cyanophycin synthetase that synthesizes a nitrogen storage polymer of arginine and aspartic acid called cyanophycin. *cphB* encodes the enzyme cyanophycinase that breaks down cyanophycin. The presence of these two genes in Sandusky Bay *Pa* strain was demonstrated through PCR. It is expected that *cphA* should be expressed when nitrogen is replete and that *cphB* should be expressed during nitrogen depletion. In this experiment, two cultures of *Planktothrix agardhii* were grown in BG-11 media. The culture was divided, centrifuged, rinsed, and resuspended: one in BG-11 and one in nitrogen free BG-11. Every three days a portion of each culture was filtered for chlorophyll *a,* phycocyanin, and RNA. Furthermore, the color of the cultures was observed daily for signs of nutrient stress. The experiment continued until the N free culture showed significant signs of chlorosis. The levels of chlorophyll a and phycocyanin were tested using a fluorometer to measure any difference between the two cultures. RNA was extracted and RT-PCR was performed using primers for *cphA* and *cphB* to monitor the expression of those genes.

BOARD 10

Substrate-Dependent Variability of Cell Shape in *Mycoplasma iowae*

Nathan R. Schwab*, Rebecca L. Wyma, Neena K. Patel, Steven L. Distelhorst, Mitchell F. Balish
Miami University

Cell shape is related to the function and lifestyle of the cell, *e.g.* aiding in nutrient uptake by increasing surface area or allowing streamlined motion with an elongated shape. In addition, some cells can alter their morphology to adapt to the environment in response to physical and chemical cues. *Mycoplasma iowae*, a bacterial poultry pathogen that causes diminished hatchability and leg abnormalities, has a distinct cell shape, with a polar attachment organelle that enables the bacterium to adhere to and glide across the host cell surface. *M. iowae* has a small genome conferring the absence of a cell wall and markedly reduced biosynthetic capacity, but its virulence mechanisms and life cycle are poorly characterized. We have observed that when a subset of amino acids and related compounds are added to the growth medium at high concentration, the typical elongated morphology shifts to a rounder shape. To understand the cause of this change, we measured cell shape by staining the nucleoid with 4', 6-diamidino-2-phenylindole (DAPI), which serves as a proxy for cell shape because it fills the body of the cell, excluding the attachment organelle, and calculated the aspect ratio of the fluorescence image using ImagePro 7.1 software. Several amino acids had no statistically significant effect, but others caused increased rounding as the concentration was increased, reaching a plateau at ~70 mM. Although this concentration is beyond the physiologically normal concentration of amino acids, the effects on rounding by individual amino acids were found to be additive, suggesting that the signal to modify cell shape could result from modest elevations of several amino acids. Future efforts will focus on more precisely identifying the shape

change signal, the molecular mechanisms through which this change occurs, and the physiological role of this change during infection.

BOARD 11

Cloning and Sequencing of Mercury Resistance genes from *Enterobacter sp. YSU* and *Stenotrophomonas maltophilia* OR02

Stephen Muhindi* and Jonathan Caguiat
Youngstown State University

Enterobacter sp. YSU and *Stenotrophomonas maltophilia* OR02 (*S. maltophilia* 02) were isolated from a heavy-metal contaminated site in Oak Ridge, TN. Both strain are resistant to salts of copper, cadmium, zinc, selenium, arsenic, gold and mercury. Known primers for the mercury resistance genes, *merR* and *merP*, were used to PCR amplify, clone and sequence DNA segments from each strain. Then, Basic Local Alignment Search Tool (BLAST) analysis of the sequences identified reference sequences used to sequence the rest of the *mer* genes in each strain. These reference sequences contained the genes, *merRTPCADE*, for *Enterobacter* sp. YSU and the genes, *merRTPADE*, for *S. maltophilia* 02. Alignments using other related *merR* and *merE* genes were used to design PCR primer to amplify both *mer* operons which were cloned into the plasmid, pSC-A-amp/kan, from the StrataClone PCR Cloning Kit. Liquid culture experiments in LB broth showed that the minimal inhibitory concentrations (MICs) were 150 µM and 190 µM $HgCl_2$, for the *Enterobacter* sp. YSU and *S. maltophilia* 02 cloned fragments, respectively, and 30 µM $HgCl_2$ for the vector control. The bioinformatics tool, Molecular Evolutionary Genetic Analysis (MEGA 7), generated a phylogenetic tree that showed that the MerA protein from *Enterobacter* sp. YSU is related to other *Enterobacter* MerA proteins and that the MerA protein from *S. maltophilia* 02 is related to other *Stenotrophomonas* MerA proteins.

BOARD 12

Molecular typing of *Streptococcus pyogenes* isolates collected at a Mongolian Hospital (Ullanbaatar, Mongolia)

Samantha Mackey*[1], Dipendra Thapaliya[1], Jhalka Kadariya[1], Bulgan Davaadash[2], and Tara C. Smith[1]
[1] Kent State University, College of Public Health, Department of Biostatistics, Environmental Health Sciences and Epidemiology, Kent, Ohio
[2] Bacteriological Reference Laboratory, National Center for Communicable Diseases, Ulaanbaatar, Mongolia

Streptococcus pyogenes is a significant cause of morbidity and mortality worldwide, causing an estimated 1.8 million cases and 517,000 deaths each year. *S. pyogenes* infection disproportionately affects low-income countries where routine surveillance is not available. The objective of this study was to investigate the molecular epidemiology and antibiotic resistance patters of clinically relevant *S. pyogenes* isolates to better understand the burden in this under-served population.

A convenience sample of 41 *S. pyogenes* isolates collected between 2007 and 2016 by the Bacteriological Reference Laboratory, National Center for Communicable Diseases, Ullanbaatar, Mongolia, and sent to our lab for molecular typing. The *emm* gene was sequenced and *emm* type was assigned. We also carried out multi-locus sequence typing (MLST); antibiotic susceptibility testing was carried out via the Vitek-2 system.

We observed 18 distinct *emm* types among the 41 S. pyogenes isolates. stG6792.0 was the most common *emm* type, accounting for almost one-third of the isolates followed by *emm*2.0 and *emm* 82.0. A total of seven sequence types (STs) were detected among 15 tested isolates. The most common ST type was ST55 accounting for one-third of the isolates. Additional MLST analysis is in progress. Most of the isolates were susceptible to all tested drugs.

The findings of this study provided some insights regarding the molecular characteristics of *S. pyogenes* in Mongolia that will be crucial for future surveillance studies. The information about antibiotic susceptibility

patterns and molecular types can help to devise better treatment strategies for *S. pyogenes* infections, and inform vaccine development.

BOARD 13

Construction of a Complete Knockout of the CCR5 Gene by Genome Editing

Weizhuan He* AS [1, 2],Kayla S. Zamborsky AS [1, 2]**,** and Harry W. Kestler[1]
[1] Lorain County Community College, Elyria, Ohio
[2] Bowling Green State University, Bowling Green, Ohio

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, *ccr5 delta 32*, confers resistance to *Yersenia pestis* and HIV infection. In the mid-1990's, it was discovered that some people who were exposed to but not infected with HIV were homozygous for *ccr5 delta 32*. This observation suggested that a cure or prevention for HIV might be possible by eliminating or down modulation of the ccr5 gene. Timothy Ray Brown, the only known person to be cured of both HIV and leukemia, received a bone marrow transplant from a donor who was homozygous for delta 32. CCR5 is one of two secondary receptors for entry of the virus into human T cells. 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, the other secondary co-receptor. This study was designed to determine the effect of the complete removal of the *ccr5* gene in human cells *in vivo*.

Gene editing was performed using the CRISPR/Cas9 system to generate mutations in the N-terminus of the *ccr5* gene. The human T cell line, H9, was co-transfected with one plasmid containing the guide RNA sequences that have homology to the amino-terminus of the *ccr5* gene and the CRISPR/Cas9 and the second plasmid contains genes for puromycin resistance for selection. Puromycin toxicity was determined by serial diluting puromycin. Transformants were obtained by puromycin selection 21 days after transfection. The presence of the puromycin resistance gene in transfected cells was confirmed by PCR. Various alleles will be co-transfected into the cells to test the effects of the delta 32 allele on wild type CCR5 and CXCR4.

BOARD 14

Nucleotide utilization and redox centers of an archaeal corrinoid reductase

Katherine A Huening*[1], Eric L Carter[2], Stephen W Ragsdale[2], and Joseph A Krzycki[1]
[1]The Ohio State University; [2]University of Michigan

The archaeal methanogen *Methanosarcina barkeri* is capable of reducing methyl groups from select methylamines to form the potent greenhouse gas methane. To generate methane, it utilizes three-component methyltransferase systems that are dependent on a corrinoid-binding protein. The cobalt ion in the corrinoid cofactor must be able to cycle between the methylated Co(III) state and the unmethyalted Co(I) state for methyl transfer to occur. Oxidation of the low potential Co(I) state results in the formation of the inactive Co(II) state, which halts methyl transfer. RamA, an enzyme consisting of an ATP-binding site and a ferredoxin domain containing two [Fe_4S_4] clusters, is capable of catalyzing the single electron transfer from the [Fe_4S_4] clusters to the Co(II) corrinoid protein in an ATP-dependent manner, thus restoring methyl transfer. Previously, we reported that RamA appears to do this by hydrolyzing two ATPs per corrinoid protein reduced. We have now found a similar stoichiometry using a coupled methyl transfer reaction to trap the low potential Co(I) corrinoid protein in the methylated Co(III) state in order to avoid error from oxidation. Additionally, we used an electrochemical cell to perform redox titrations on both RamA and the trimethylamine corrinoid protein. The midpoint potential of RamA's [Fe_4S_4] clusters is -390 ± 4 mV, while the midpoint potential of the Co(II) to Co(I) couple of the trimethylamine corrinoid protein appears to be below -700 mV. The electrochemical difference indicates a large energy gap that must be overcome in the reduction of Co(II) to Co(I) with RamA activity, consistent with the two ATP hydrolyzed per electron transferred we observe.

BOARD 15

Identification of a putative two-component gold-sensor histidine kinase regulator in *Stenotrophomonas maltophilia* OR02

Andrew Zack* and Jonathan Caguiat
Youngstown State University

Stenotrophomonas maltophilia Oak Ridge strain 02 (*S. maltophilia* 02) is a bacterial strain that was isolated from a heavy-metal contaminated site in Oak Ridge, TN. Previous research showed that it is resistant to metal salts of mercury, copper, cadmium, zinc, selenite, arsenite, chromate and gold. This strain demonstrated a minimal inhibitory concentration (MIC) of 250 µM for Au(III) in liquid cultures. Transposon mutagenesis generated a mutant strain, S02-A12, which failed to grow on agar plates containing 50 µM gold chloride. In addition, liquid culture experiments showed that the mutant's MIC was 175 µM Au(III). Gene rescue and Sanger sequencing and Basic Local Alignment Search Tool (BLAST) analysis suggested that the transposon inserted itself in the stop codon of a hypothetical protein that was located downstream of a DNA-binding protein that is part of a two-component sensor histidine kinase regulator. This membrane protein may detect the presence of Au(III) in the growth medium and activate the DNA-binding protein which activates the expression of the gold-resistance genes. Differential gene expressions studies using the wild type strain and mutant strain grown in the presence of Au(III) may reveal gold-resistance genes in *S. maltophilia* 02.

BOARD 16

An Intracellular Cyclophilin Has Additional Functions Not Limited To PPIase Activity in *Staphylococcus aureus*

Rebecca A. Keogh*, Rachel L. Zapf, Richard E. Wiemels, and Ronan K. Carroll.
Department of Biological Sciences, Ohio University

The virulence of *Staphylococcus aureus* is largely dependent on the successful secretion and processing of extracellular proteins. Peptidyl- prolyl *cis/trans* isomerases (PPIases) are a class of enzymes that assist in the secretion of proteins containing a proline residue in the *cis* confirmation. Previously we demonstrated that the *S. aureus* PPIase PpiB has PPIase activity that may contribute to protein folding and overall virulence. In this study we set out to determine if the PPIase activity of PpiB specifically contributes to *S. aureus* virulence. We first examined the contribution of PpiB to the activity of known secreted virulence factors. A direct role for PpiB in the refolding of secreted Staphylococcal nuclease is shown using a fluorescence resonance energy transfer (FRET) refolding assay. In addition erythrocyte lysis assays show decreased hemolytic activity in a *ppiB* mutant, suggesting the involvement of PpiB in the secretion of multiple toxins. Exoproteome analysis of a *ppiB* mutant compared to wild-type *S. aureus* suggests the secretion of over 30 proteins is affected by PpiB. A murine abscess model of infection with the *ppiB* mutant shows fewer recovered bacteria after 7 days of infection than in a wild-type strain, suggesting PpiB contributes to *S. aureus* virulence. These data suggest PpiB has multiple roles in *S. aureus* virulence and could be a novel target for drugs to combat bacterial infection. This work was supported by grant AI128376 from the National Institute for Allergies and Infectious Diseases (NIAID).

BOARD 17

Analysis Of A Novel Mutation Located In The CCR5 Gene With Potential Effects On HIV Infectivity

Octavia J. Whitfield*[1], Elianis G. Osorio[1], Ashley E. Innes[1], Audra L. Fincham[1], Savannah L. Herbert[1], and Harry W. Kestler
[1]Lorain County Community College, Elyria, Ohio

A novel mutation in the *ccr5* gene, which codes for the CCR5 protein, was found in a child of an African-American family. The mother contracted HIV before the birth of her first child in the early 1980s. After she was infected she unknowingly exposed all five of her children to HIV through vaginal delivery. Out of the five children, the second born did not acquire the infection even though she was exposed to the virus at birth. The mother and the infected children all carried genetically related virus and the similarity was inversely correlated with birth order. A missense point mutation in the cytoplasmic domain of the uninfected child's *ccr5* gene was discovered. The mutation (TG5), changes a lysine codon at position 314 into an arginine codon. The allele containing the TG5 mutation was cloned into pCR4-TOPO® Vector. The TOPO vector containing the TG5 mutated gene was sub-cloned into pLXSN, the retroviral plasmid vector. Clones made from pLXSN-TG5 will be used to transfect the packaging cell line PT67. The PT67 cell line will assemble viral particles containing the TG5 mutation. The retroviral particles will be recovered and introduced into an H9 cell line as well as other cells. The expression of the TG5 gene in H9 and its effect on HIV infectivity will be tested. It has been reported that CCR5 delta 32 can down-modulate wild-type CCR5 and CXCR4. The ability of TG5 to down-modulate wild-type CCR5 and CXCR4 will be evaluated.

BOARD 18

Quantitative trait loci (QTL) mapping in a parasexual species

Delanie Baker*[1], Robert Fillinger[2], and Matthew Anderson[2]
[1]Ohio Wesleyan University; [2]The Ohio State University

Candida species are the most clinically relevant fungal pathogens, with *C. albicans* being the most prevalent. The standard frontline treatment for *Candida* is the azole class antifungal drug fluconazole. Resistance to fluconazole has increased since its introduction into the clinic. Although characteristic mutations are known to contribute to fluconazole resistance in *C. albicans*, ~33% of resistant isolates do not encode these genetic signatures of resistance. A lack of unbiased genotype-phenotype analysis methods exist for *C. albicans* due to a lack of meiosis, making identifying the mechanism of resistance in these isolates challenging. Quantitative trait loci (QTL) mapping is a method that uses meiosis to identify genetic loci contributing to a phenotype. Here, we describe an approach utilizing the alternative parasexual cycle of *C. albicans* to perform QTL mapping of a strain encoding an unknown mechanism of fluconazole resistance. This methodology will provide an unbiased method for identification of loci involved in virulence traits in any parasexually replicating species.

BOARD 19

Demethylation of the Quaternary Amine γ-Butyrobetaine by the Human Gut Acetogen *Eubacterium limosum*

Jared B Ellenbogen*[1], Liwen Zhang[1], Duncan J Kountz[1], Jonathan W Picking[1], Rushieng Jiang[1], and Joseph A Krzycki[1].
[1]The Ohio State University

Recent work has found a novel relationship between the occurrence of cardiovascular disease (CVD) and gut microbial quaternary amine (QA) catabolism. Dietary QAs including *L*-carnitine, choline, glycine betaine, and γ-butyrobetaine are cleaved by gut microbial enzymes, releasing trimethylamine (TMA). TMA is subsequently oxidized by a human hepatic enzyme to trimethylamine-*N*-oxide (TMAO). High serum TMAO levels have been shown to correlate with the onset of atherosclerosis. An alternative route of gut microbial QA metabolism demethylates QAs without generating TMA. We have shown that a gut isolate of the acetogen *Eubacterium limosum* demethylates proatherogenic QAs. Acetogens are known to use three-component methyltransferase systems to demethylate substrates and methylate tetrahydrofolate for acetogenesis, however such QA-dependent systems were previously unknown. We previously identified a glycine betaine-dependent three-component system in the non-acetogenic *Desulfitobacterium hafniense*, and only recently identified an *E. limosum* carnitine-dependent system. In both cases, the QA-dependent methyltransferase components were members of the MttB superfamily of methyltransferases. Here, we undertook proteomic analysis of *E. limosum* grown with γ-butyrobetaine. Homologs of each member of a three-component 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 with other QAs. The MttB homolog is unique to γ-butyrobetaine-grown cells, suggesting this may be a γ-butyrobetaine-specific corrinoid-dependent methyltransferase. Early difficulties with the production of soluble, recombinant putative methyltransferase have recently been surmounted via the use of an *E. coli* expression strain possessing an additional plasmid with inducible chaperones. We are currently testing the hypothesis that this protein is part of a three-component γ-butyrobetaine:tetrahydrofolate methyltransferase system.

BOARD 20

Evaluation of commercial cleaners on two polyurethane degrading fungal species isolated from aircraft

Neal, A.R.[a, c] *, Crouch, A.L.[b, c], Drake, C.A. [b, c], Bojanowski, C.L. [c], Goodson-Crookes, W.J. [c]
[a] Azimuth, Inc.; [b] UES, Inc.; [c.] Soft Matter Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory

Many studies characterizing microbiomes of built environments focus on the impact of microbiology on occupant health, but there is additional concern that microbial communities can contribute to biodeterioration of materials. Materials at risk of biodeterioration on aircraft can include aerospace alloys, electronics, and coatings. To remediate microbial contamination of aircraft materials, cleaners, like Calla 1452®, are applied to aircraft surfaces. In this study, the detergents Calla 1452® and Navclean® were evaluated for their ability to decontaminate surfaces exposed to two fungal strains, *Papiliotrema laurentii* and *Aureobasidium pullulans*, both isolated from a cargo aircraft and identified as polyurethane degraders. Polyurethane coated aluminum coupons were either pretreated or treated with detergent at specified intervals after inoculation with one of the fungal strains at $5x10^5$ CFU cm^{-2}, an inoculum reflective of cell densities found on contaminated aircraft. The coupons were incubated at 27 °C and sampled on days 1, 3, 7, and 14. Cells were removed and assessed via CFU mL^{-1} counts and microscopy. It was found that both Calla 1452® and NavClean® were ineffective treatments against the inoculum tested; pre-treated coupons and treated coupons showed little change in viability of cells compared to the control coupons over the course of the experiment. Furthermore, an additional

month long study was performed to quantify *P. laurentii* and *A. pulluans* degradation of polyurethane coatings. Profilometry measurements of the coatings were taken after a month long incubation at 27°C in 99% relative humidity. These measurements demonstrated that *A. pulluans* caused deeper pits in the PU coating than *P. laurentii*. In conclusion, microbiology can cause degradation of PU coatings, and the current detergents used to mitigate these organisms are not effective in decontaminating typical environmental isolates. These findings indicate that a more effective decontamination method is required for remediation of aircraft surfaces.

BOARD 21

Identification of a novel riboswitch in the Gram-negative bacterium *Bartonella henselae*

Marcus A. Wittekind*[1], Richard E. Wiemels[1], Burt Anderson[2], and Ronan K. Carroll[1]
[1] Department of Biological Sciences, Ohio University, Athens, OH
[2] Morsani College of Medicine, University of South Florida, Tampa, FL

Bartonella henselae is a Gram-negative bacterium that is the causative agent of cat scratch fever. A unique regulatory RNA, named Brt1, was recently discovered, which could potentially assist *B. henselae* in causing disease. Computer analysis of the predicted structure of the Brt1 RNA suggests the presence of a hairpin in the 3' region. We hypothesize that this hairpin may function as a riboswitch controlling the transcription of the downstream *trp1* gene. Trp1 is a DNA binding transcription regulatory protein from the XRE family. To determine if computer predictions of Brt1 structure/function are correct, the Brt1 sequence was cloned into a plasmid downstream of a constitutive promoter and upstream of the *gfp* gene. Quantitative real-time PCR analysis of the resulting construct showed no expression of *gfp*, supporting the model of Brt1 acting as a transcription terminator. Site directed mutagenesis was then performed to destabilize the hairpin structure of the predicted Brt1 riboswitch. Quantitative real-time PCR analysis of the destabilized construct demonstrates a significant increase in *gfp* expression. However, western blots show a significant decrease in quantity of Gfp present in cultures after the hairpin was disrupted. To investigate the discrepancy between the qPCR and western blot data, further analysis of the Brt1 RNA will be preformed using RNAse T1 structural probing to precisely define the secondary structure of the 3' end of Brt1.

BOARD 22

Role of Hofbauer Cells in Vertical Transmission of *Listeria monocytogenes*

Siavash Azari[1,2, 3*], Lauren J. Johnson[1,2,3], John M. Robinson[4], Catalin Buhimschi[5], Stephen Thung[5], Kara Rood[5], Stephanie Seveau[1,2,3]
[1] Ohio State University, Department of Microbial infection and Immunity
[2] Ohio State University, Department of Microbiology
[3] Ohio State University, Infectious Diseases Institute
[4]Ohio State University, Department of Physiology and Cell Biology
[5]Ohio State University, Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine

Listeria monocytogenes is a Gram-positive facultative intracellular bacterium and the causative agent of listeriosis, a severe foodborne disease associated with 20% mortality despite treatment. Pregnant women are at a significantly higher risk of infection, which can result in severe fetal-placental complications and subsequent fetal demise. In the placenta, *L. monocytogenes* can cross the trophoblast layer, proliferate in the chorionic villi, and eventually spread to the fetus. However, to date, no mechanistic study has addressed *L. monocytogenes* proliferation within the chorionic villi and its transmission to the fetus. The stroma of the chorionic villi contains fetal macrophages, called Hofbauer cells, fibroblasts, and endothelial cells. Hofbauer cells are motile and abundant within the intravillous space. Since tissue resident macrophages are known to have a significant role in infection and spread of *Listeria monocytogenes* in other tissues, we hypothesize that Hofbauer cells exert a major role in transmission of *L. monocytogenes* from trophoblasts to the fetus. To test this hypothesis, we

isolated Hofbauer cells from healthy human term placentas by sequential trypsin/DNase and collagenase/DNase tissue digestions followed by cell centrifugation on density gradients. For further purification, we performed a step of negative selection to deplete cytotrophoblasts and fibroblasts. Hofbauer cells were infected with wild type *L. monocytogenes* (serotype 1/2a strain 10403S isolated from a skin lesion) and the isogenic *hly* deletion mutant. Preliminary data show an increase in infection over time for the WT strain, while the *hly* mutant was cleared after 5 hours. These results support our hypothesis and are the primary steps in further studying the role of Hofbauer cells in transplacental propagation of *L. monocytogenes* from the trophoblasts to the fetus. The ultimate aim of our study is to establish a more detailed picture of the role of Hofbauer cell responses to infection in placental inflammation, immunity, and pathology.

BOARD 23

The Use of Membrane-Associated Complement Inhibitors by *Neisseria gonorrhoeae* in Immune Evasion

Donnie Pickel*[1], **Avital Savin*[1]**, Won Kim[2,3], Morgan Brown[2,3], Sanjay Ram[4], Magdalene So[2,3], Nathan J. Weyand[1]

[1]Ohio University, Department of Biological Science; Athens, OH, 45701
[2]University of Arizona, Department of Immunobiology; Tucson, AZ, 85721
[3]University of Arizona, BIO5 Institute, College of Medicine; Tucson, AZ, 85721
[4]University of Massachusetts Medical School, Division of Infectious Diseases and Immunology; Worcester, MA, 01655

Neisseria gonorrhoeae (Ngo), the causative agent of gonorrhea infections, is responsible for the second most prevalent bacterial sexually transmitted infection worldwide. The World Health Organization (WHO) recently published a report characterizing antibiotic resistant gonorrhea as an imminent threat, demonstrating an incidence of resistance to the final recommended antibiotics as high as 66%. Furthermore, the report states that the development of new therapeutics is not in line with the growing need for novel antibiotics, with only three in any stage of clinical testing. Left untreated, gonorrhea can progress to pelvic inflammatory disease, ectopic pregnancy, and sterility. With current therapeutic strategies dwindling, research if beginning to focus on preventative therapeutics such as vaccine development.

One difficulty in developing these therapies is the characteristic ability of Ngo to avoid host innate immune defenses, including the complement cascade. Inhibitors of the cascade regulate activation of the immune system to prevent autoimmunity on host cells. It has been demonstrated that Ngo interacts with fluid-phase complement regulators, such as factor H, to evade complement-mediated killing. However, much less is understood about the potential contribution of membrane-associated complement inhibitors (mCIs) in immune evasion. Previously, one mCI, CD46, has been shown to cluster underneath adherent Ngo during infection of human epithelial cells. We hypothesize that Ngo sequesters mCIs from infected host cells to evade host immune activation.

To test our hypothesis we developed a co-culture serum bactericidal assay (ccSBA) to monitor Ngo survival during infection of live epithelial cells. Using this assay we found: 1) The presence of living epithelial cells is necessary for Ngo survival; 2) Strains incapable of expressing the molecular motor PilT are more susceptible to killing during human serum challenge; 3) shRNA-mediate disruption of mCI expression levels alters Ngo survival. This study supports the hypothesis that Ngo sequesters mCIs to evade immune defenses.

BOARD 24

Investigating the contribution of the small RNA Teg41 to *Staphylococcus aureus* infection-related phenotypes

Andrew R. Calliet[1*], **Emily R. Trzeciak*[1]**, Rachel L. Zapf[1], Richard E. Wiemels[1], Jeffrey L. Bose[2], and Ronan K. Carroll[1]
[1]Department of Biological Sciences, Ohio University, Athens, OH
[2]Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS

Recent work in our lab has demonstrated a role for the *Staphylococcus aureus* small RNA (sRNA) Teg41 in regulating production of the alpha phenol soluble modulins (α-PSMs). The α-PSMs are well-characterized cytolytic peptides with potent activity against a range of host cells, including neutrophils and erythrocytes. While the biological role of the α-PSMs is well understood, the molecular mechanism underlying the regulation of the α-PSMs by Teg41 is still unknown. In this study we set out to investigate the contribution of Teg41 to infection-related phenotypes known to be influenced by α-PSM activity. Specifically, we investigated the contribution of Teg41 to (i) *S. aureus* biofilm formation and (ii) growth and survival of *S. aureus* in whole human blood. The α-PSMs have previously been shown to impact both of these phenotypes therefore we hypothesized that Teg41 would also influence these activities. Previous studies have shown that when the αPSM genes are knocked out, that the biofilms formed by the resulting strain will be thicker, and have a greater overall biovolume, as αPSMs play an important role in the detachment of biofilms. To confirm the link between Teg41 and the αPSMs, biofilm assays were performed using a Teg41 mutant strain and compared to those formed by the wild type. Our results indicate that biofilm formation is similar in a Teg41 mutant to an αPSM deletion mutant. Additionally, recent work in our lab has shown strong hemolytic activity of a Teg41 overproducing strain when grown on sheep blood agar plates. To further explore the contribution of Teg41 to *S. aureus* hemolytic activity whole human blood was infected with the wild type strain and a Teg41 overproducing strain. Similar rates of growth were observed for both strains however increased hemolytic activity was observed in whole human blood infected with the Teg41 overproducing strain.

BOARD 25

Neutrophils Influence Adenovirus-Host Interactions

James M Readler*[1], Meghan R. Jenkins[1], Timothy L. Williamson[1], Katherine J.D.A Excoffon[1]
[1]Wright State University

Neutrophils, or polymorphonuclear leukocytes (PMN), are the first responders of the innate immune system. PMN respond to and neutralize a variety of threats including environmental contaminants and a huge array of invading microorganisms. PMN utilize many well-characterized tactics for combating microbes, including: phagocytosis, reactive oxygen species production, release of digestive enzymes, and the formation of neutrophil extracellular traps (NETs). NETs are large strings of PMN DNA that contain digestive enzymes and proinflammatory mediators, such as danger associated molecular patterns (DAMPs). NETs effectively trap bacteria and fungi and aid in their clearance, however overproduction of NETs can damage host cells and induce chronic inflammation. By contrast, virus-NET interactions are less well understood. We have previously shown that neutrophils increase human adenovirus type 5(AdV5) entry and infection of polarized epithelia. We hypothesized that AdV5 activates NET production to increase viral infection. Using primary human neutrophils, we found that AdV5 can activate NET production in a donor-dependent manner. Furthermore, we demonstrate that, similar to intact PMN, the presence of NETs at the apical surface of polarized epithelia increases AdV5 infection of the epithelium. While increased epithelial susceptibility to AdV5 is not due to disruption of epithelial tight junctions or global changes in fluid phase endocytosis, pharmacologic inhibition of epithelial actin dynamics and certain steps of epithelial autophagy alter the effect. Current work is focused on identifying the exact mechanism by which neutrophils influence the infectivity of AdV infection. Understanding the mechanisms by

which microbes, such as AdV, coopt our innate immune system to improve infection may lead to novel antimicrobial therapeutic strategies as well as methods to improve AdV-mediated gene therapy.

BOARD 26

Differential Expression of the Streptomyces Conservon in a Cyclic di-GMP Mutant

Caroline Fulmer*
Otterbein University

Streptomyces coelicolor is a gram-positive Actinobacterium. It is a filamentous soil organism that performs complex multicellular development of physiological and morphological differentiation and produces active secondary metabolites. These metabolite products include antibiotics which can have important applications in pharmaceutical industries. Studies of S. coelicolor show that genes encoding cyclic di-GMP proteins regulate physiological and morphological development. S. coelicolor , has 13 copies of an operon named “conservon” because it is highly conserved. The 13 operons are named cvn1-13 and genes in each operon are denoted cvnA-D from 5’ to 3’. RNA-Seq data of S. coelicolor showed differential gene expression between the wild type and a cyclic di-GMP phosphodiesterase double mutant strain for many of the conservons. Cvn2, cvn3, cvn7, cvn9 and cvn11 all showed significant under expression while cvn1, cvn4, cvn8, cvn10 and cvn13 showed significant overexpression. Previous studies of S. coelicolor have shown varied effects of deleting these conservons. Kenji Ueda et al., 2011 made null mutants lacking all 4 or 5 coding sequences of the operon for all 13 copies of cvn in S.coelicolor which resulted in wild-type phenotypes for all cvn operons except cvn 1. Null mutants for each coding sequence of cvn 1 were made. cvnA1 showed a mutant phenotype but the other coding sequences presented wild-type phenotypes. Komatsu et al., 2006 showed similar results in that mutations for cvnA9, cvnD9, and cvnA10 exhibited mutant phenotypes while complete deletions of cvn9 and cvn10 presented wild-type phenotypes. These studies provide evidence that while deleting the cvn7 operon may not exhibit a phenotypic change, amplifying one gene of the operon could. Gene SCO6796 is of interest because according to strepdb it contains conserved domains from the bacterial regulatory protein arsR family. Members of this family contain a DNA-binding helix-turn-helix motif, which implies that the protein may be involved in gene expression. Gene SCO6798 is interesting because it is unnamed and at the end of the cvn7 operon. It contains a conserved GAF domain is in cyclic nucleotide specific phosphodiesterases. A distinct mutant phenotype of these two genes could shed light on the function of the specific gene and the complete cvn7 operon in the S.coelicolor genome.

BOARD 27

Bioinformatical characterization of a DeoR family transcriptional regulator

Tim D. Clinton Jr.* and Jennifer Bennett
Otterbein University Biochemistry and Molecular Biology Program

Streptomyces coelicolor is a gram-positive, nonmotile bacterium belonging to the soil-dwelling phylum Actinobacteria. The organism has long been utilized as a model specimen, providing valuable insight into studies on signaling pathways, morphology, and genomics. In addition to the organism’s vast usage in biological research, the bacterium is also exceptionally useful to the pharmacological industry. Streptomycetes produce over two-thirds of the world’s antibiotics as well as a host of other useful antiparasitic and antifungal drugs. In a previous study working with *S. coelicolor,* two genes were found to be heavily involved in the regulation of cyclic di-GMP and consequentially the regulation of aerial mycelium. In this study, both genes in question were inactivated to reveal what genes within the larger *S. coelicolor* transcriptome were consequentially up or down regulated. The current study was centered on a specific gene that was shown to have a log2FoldChange of -0.9316620276438. This gene, identified as SCO4920, was analyzed using a variety of different bioinformatical approaches. Examination of SCO4920 within the context of the surrounding genome was done using the

Streptomyces Annotation Server. This database was used to identify surrounding genes as well as those that act as an ortholog to SCO4920 in the greater *Streptomyces* family. These orthologs were then aligned with SCO4920 using the alignment tool Clustal Omega. The programs SMART and Pfam were used to identify potential domains within the gene's amino acid sequence. Two domains were determined using these programs: HTH_DEOR and DeoRC. The program RaptorX was used to provide a theoretical tertiary structure for SCO4920. This tertiary structured was then visualized in Cn3D and PyMOL before fabricating the protein using a 3D printer. These bioinformatics techniques culminated in a broad picture of the overall gene, providing insight into its role within the organism.

BOARD 28

Characterizing the Effects of the delta32 Mutation on CCR5 Expression and HIV Infectability

Hannah Newsome*[2], Raquel Dauch B.A.*[1], Megan Reynold[1], Gary Dodson B.A.[1] Brielle McCarthy[2], and Harry Kestler[1]
[1] Lorain County Community College, Elyria, Ohio
[2] Lorain County Early College, Elyria, Ohio

Human Immunodeficiency Virus (HIV) is a virus that disables the immune system and provides opportunity for further infections. For HIV to infect a cell, it first binds the primary receptor on the human T-cell, CD4, to one of two secondary receptors, CCR5 or CXCR4. After HIV glycoprotein 120 successfully binds, it uses glycoprotein 41 to pierce the cell membrane, allowing for insertion of its genetic material; creating an infected cell.

There is a mutation known to affect HIV infectivity. Delta32 is a deletion mutation of 32 base pairs of the *ccr5* human gene sequence. It is hypothesized that this mutation truncates the *CCR*5 receptor which then affects its ability to protrude and/or its conformation on the surface of the human T-Cell, making it unavailable to HIV. This mutation confers resistance to HIV when the individual is homozygous.

PCR primers that circumscribe a small portion of the *ccr5* gene where the mutation is located were designed. These PCR products will be 141bp or 109bp, dependent upon the alleles present in the individual's DNA. An individual has been identified, subject GD1, who is heterozygous for the *ccr5* delta32 allele. After confirmation, PCR amplification from the subject's DNA was completed using the circumscribing primers. The primers have restriction endonuclease recognition sites BamHI and XhoI allowing for the purification of both wild type and delta32 alleles. This product will be ligated into the mammalian/prokaryotic shuttle vector, pLXSN. The plasmid DNA will be analyzed by PCR amplification to confirm the presence of the *ccr*5 alleles.

The next phase in this research consists of transfecting the *ccr*5/pLXSN vector into PT67, a retroviral packaging cell line. The resulting retroviral products will then be used to create stable and transient transductants into H9 Lymphoid cells and tested for HIV infectability and *ccr*5 expression.

BOARD 29

Production of colony variants by *Pseudomonas aeruginosa* in response to tobramycin

Devin Sindeldecker*[1], Nicholas R. Farrar[1], Anthony Falzarano[1], Scott M. Diamond[1], Cory S. Knecht[1], Matthew Anderson[1], Devendra H. Dusane[1], and Paul Stoodley[1]
[1]The Ohio State University

Pseudomonas aeruginosa is an important opportunistic, bacterial pathogen that causes approximately 51,000 hospital acquired infections per year including periprosthetic joint infections. In an attempt to treat periprosthetic joint infections, orthopedic surgeons place tobramycin and vancomycin loaded bone cement beads into the surgical site to allow for local antibiotic therapy. Variant like antibiotic tolerant or resistant colony generation has previously been shown as a response to tobramycin loaded bone cement beads, and this could lead to recurrent or persistent infections. In this study, three distinct phenotypes were identified from the variant colonies which survive the antibiotic bone cement bead therapy – classically resistant colonies, and two novel variants, viable but non culturable colonies (VBNCs), and a tolerant phenotype which we call “phoenix” colonies. Phoenix colonies are variants which grow up within the zone of inhibition of the tobramycin bead while there are still very high levels of antibiotic present, but upon subculturing, return to wild-type susceptibility. They show no growth defects indicating that they are not persister cells, are morphologically similar to wild-type colonies aside from a pigmentation change from green to white, and are not efflux pump driven. While these variant colonies were produced in response to the tobramycin beads, there was also a consistent zone of killing around the bead from which nothing could be cultured. These findings suggest that resistant colonies, VBNCs, and phoenix colonies may be generated in response to antibiotic therapies, but by achieving high enough local antibiotic concentrations, we may be able to kill classically resistant mutants, phoenix colonies, VBNCs, and persister cells thus reducing the incidence of recurrent or persistent infection.

BOARD 30

Investigating the contribution of PrsA to virulence in *Staphylococcus aureus*

Kellie A. King*, Rebecca A. Keogh, Richard E. Wiemels, Nikki M. Meyer, and Ronan K. Carroll
Ohio University

Staphylococcus aureus is a bacteria responsible for a variety of severe infections in humans. Its virulence is largely due to its ability to secrete toxins and other virulence related proteins. During the secretion process, proteins are in a denatured state, and they must fold once outside of the cell to become functional. PrsA, a prolyl isomerase, has been shown to assist the folding of secreted proteins in numerous Gram-positive bacteria, however its role in *S. aureus* is not fully understood. In this study, we test the hypothesis that PrsA in *S. aureus* functions as a prolyl isomerase to assist the folding of secreted virulence factors and that this folding activity contributes to pathogenesis. Site directed mutagenesis will be performed to identify the amino acids required for PrsA prolyl isomerase activity. A strain of *S. aureus* will then be constructed that expresses an inactive form of PrsA. This substituted strain will be compared to wild-type *S. aureus* and a *prsA* mutant strain in a mouse model of infection to identify the role of PrsA and prolyl isomerase activity in *S. aureus* virulence.

BOARD 31

Bioinformatic analysis of a putative *Streptomyces* sporulation gene

Derek T. Wei* and Jennifer A. Bennett
*Department of Biology and Earth Science, Otterbein University

Streptomyces coelicolor is a nonmotile soil-bacterium belonging to the family through which two thirds of the world's antibiotics are produced. Studies concerning the organism have the potential to increase understanding of the life cycle, morphologies, and signaling pathways within this pharmacologically important bacterium. A previous study identified two genes critical in the regulation of the second messenger signaling molecule, cyclic di-GMP and in the formation of an aerial mycelium. Transcriptome analysis of the double mutant using RNAseq revealed differential regulation corresponding to the atypical phenotype when compared with the wild type S. *coelicolor*. The current study was focused on the investigation of one of the genes revealed to have a log2FoldChange of approximately -6.7 in the double mutant, suggesting down-regulation from the wild-type *S. coelicolor*. The gene of interest, SCO2560, was subjected to a series of bioinformatics techniques. Visualization of SCO2560 within its genomic neighborhood was performed using the *Streptomyces* Annotation Server. In addition, the server was used alongside Clustal Omega to align SCO2560 with any similar protein sequences within *S. coelicolor* and to show how similar reported orthologues were to the gene of interest. Further, the domain prediction programs SMART and Pfam revealed three domains; HATPase C, GAF, and PP2C SIG. The RaptorX program was utilized to determine a theoretical tertiary structure for the SCO2560 gene. Provided secondary structural elements of the SCO2560 gene were explored through helical wheel diagrams and Kyte & Doolittle and Eisenburg hydropathy plots. The bioinformatics techniques performed on this gene revealed a wealth of information that will likely shed some more light on its possible function.

BOARD 32

Transplanting phytoplankton within a chemically stratified Antarctic lake shows the effects of incubating isolates back in their natural environment and can assess the resilience of natural communities to predicted environmental change

Shasten Sherwell[1]**, Zev Cariani***[1], and Rachael Morgan-Kiss[1]
Miami University Microbiology Department, Oxford Ohio

Lake Bonney is a perennially ice-covered and chemically stratified lake in the McMurdo Dry Valleys of Antarctica. Over the past decade, three experiments have been conducted transplanting natural communities and representative algal isolates to various depths in Lake Bonney for observation of the transplanted culture's physiological and photochemical responses. These experiments involved inoculating laboratory-grown isolates or natural communities into filtered lake water and suspending those cultures at appropriate depths in dialysis tubing, which allows the microalgae to respond to in situ light, temperature, and dissolved ions. The objective of the first transplant experiment was to determine the response of a representative chlorophyte, *Chlamydomonas* sp. UWO241, to the summer-winter transition in Lake Bonney. As light levels declined, so did the inorganic carbon fixation and gene expression of essential photosynthetic proteins in the transplanted cultures. The objective of the second transplant experiment was to determine the effects of incubation at various depths and over time on a Lake Bonney haptophyte, *Isochrysis* sp. MDV, and chlorophyte, *Chlamydomonas* sp. ICE-MDV. The results showed that both organisms were able to survive at specific depths in the photic zone, and both were able to acclimate to their natural environment within a short period of time. The objective of the last transplant experiment was to simulate lake level rise by moving natural communities 3m deeper than their original depths. Shallow communities were found to be the most sensitive to this change, showing the highest reductions in biomass and photochemical downregulation. These transplant experiments with natural communities and isolate phytoplankton cultures have provided an invaluable way of determining the effects of incubation within the Antarctic lake's natural environment and assessing resilience to changing environmental conditions.

BOARD 33

Metatranscriptomic Evaluation of Planktothrix agardhii: Reaction to Stress

Michelle J Neudeck*[1,2], George S Bullerjahn[1]
[1]Bowling Green State University, [2]Lorain County Community College

Sandusky Bay is being plagued by cyanobacterial harmful algal blooms (cHABs). A persistent bloom occurred in 2015 from June into October even though the levels of nitrogen dropped to near undetectable levels from mid-July on. *Planktothrix agardhii* produces microcystin, a hepatotoxin. This cyanobacterium is the main organism of the cHABs despite being non-diazotrophic. The Sandusky Bay waters were sampled every two weeks. RNA was extracted from the samples and sequenced. Metatranscriptomes from 10 water samples obtained throughout the summer were analyzed for genes pertaining to nitrogen storage and retrieval. *Planktothrix* stores N as cyanophycin, a polymer of arginine and aspartic acid. This is produced by cyanophycin synthetase which is encoded by *cphA*. *Planktothrix* has two paralogs of this gene. *cphA* is associated with *cphB*, the gene that encodes cyanophycinase that hydrolyzes cyanophycin into arginine-aspartic acid dimers. By contrast, the alternate cyanophycin synthetase gene, *cphA`*, is monocistronic. N was replete from June until mid-July, while the discharge of the Sandusky River was high, *cphA`* was transcribed at this time. When the N levels dropped to 0, *cphB* and *cphA* were cotranscribed.

Degradation of the phycobilisome is another source of N that can be scavenged by *Planktothrix*. A small protein that triggers the degradation of the phycobilisome is encoded by *nblA*. The transcripts from *Planktothrix* demonstrated that *nblA* was not transcribed until after *cphB* was transcribed. This indicated that *Planktothrix* utilized the N stores of cyanophycin before utilizing the N sequestered in the phycobilisome.

Sandusky Bay was turbid throughout the sampling season which is ideal for low light tolerant *Planktothrix*. The transcripts of *hliA*, which encodes for a protein that protects the photosynthetic apparatus from oxidative damage, were almost continuously present in the samples.

BOARD 34

Characterizing the substrate specificities of *Histoplasma capsulatum* amino acid permeases

Stephanie C. Ray*[1] and Chad A. Rappleye[1]
[1]Ohio State University, Columbus, OH

The dimorphic fungal pathogen *Histoplasma capsulatum* is unusual among pathogenic fungi in its ability to infect both immunocompromised and immunocompetent hosts, causing disease which can range in severity from a mild flulike illness to a deadly disseminated infection. As an intracellular pathogen, *Histoplasma* yeasts readily infect host macrophages, where they grow and divide within the phagolysosomal compartment. However, the carbon sources for *Histoplasma* within the phagosome are unknown. Mutant *Histoplasma* yeasts which are deficient in glutamate catabolism are attenuated in their ability to grow both in cultured macrophages and in a murine host, suggesting that glutamate or its related amino acids are a major carbon source for *Histoplasma* inside the macrophage. Consistent with this, *Histoplasma* upregulates expression of certain amino acid permeases upon transitioning to the pathogenic yeast phase. To better understand the substrate specificities of *Histoplasma's* amino acid permeases, *Saccharomyces cerevisiae* lacking amino acid permeases were transformed with individual *Histoplasma* amino acid transporters and screened for the transporter's ability to confer growth on individual amino acids. Multiple *Histoplasma* transporters were found to have overlapping specificities, particularly for glutamate, glutamine, and alanine, each of which can be utilized by Histoplasma yeasts for carbon. As many of these transporters are redundant in function, future work will probe each transporter's affinities for specific amino acids. By comparing these findings with gene expression data from intracellular yeasts, we will identify the permeases and amino acids which are most crucial for *Histoplasma* growth within the phagolysosome, and thereby define the major carbon source for intracellular yeasts.

BOARD 35

Survey of camelids for *Neisseria* species and bacteria associated with disease

Jocelyn Fields[*1], Clara Miller, Eliza Thapa[1], and Nathan J. Weyand[1]
[1]Ohio University, Athens, Ohio

The genus *Neisseria* is composed of many species. Most characterized species have been isolated from mucosal surfaces of man and other mammals. In an effort to learn more about mammalian host bacteria interactions we are working to identify *Neisseria* species in members of the biological family Camelidae. A strain collection of over two hundred bacterial isolates from domestic camelids (llamas and alpacas) is being screened for *Neisseria* species and bacteria associated with ungulate disease. Isolates were cultured from different body sites including the conjunctiva, dental plaque, nose, tongue, soft palate and oropharynx. *Neisseria* genus specific primers and 16S rDNA sequencing were also utilized to identify candidate *Neisseria* species. Thirty seven isolates amplified with genus-specific PCR primers. These candidate *Neisseria* isolates were further analyzed with oxidase testing and Gram staining to look for Gram negative diplococci. To date, of four Gram negative diplococci that have have been genotyped three were identified to belong to the genus *Moraxella* and one to the genus *Neisseria*. We have started to sequence the genomes of several isolates to search for genes that encode host-interaction factors. Our presentation will detail our culture and identification methods and present some preliminary analysis of host-interaction factors identified in sequenced isolates.

BOARD 36

Identifying Pathways for Quaternary Amine Degradation in Environmental Samples

Sarah Soppe*[1], Jyoti Kashyap[1], D.J. Ferguson[1]
[1]Miami University, Oxford, OH

In recent years, production of trimethylamine (TMA) by human microbiota from quaternary amine substrates has been linked to increased risk of cardiovascular disease, a leading cause of death worldwide. Quaternary amines, such as carnitine, choline, and glycine betaine (GB), are typically consumed in red meats and poultry. Recent studies have shown that some methanogens and anaerobically respiring bacteria have the ability to demethylate these quaternary amines, thereby preventing the formation of TMA. Our laboratory, and colleagues, have recently demonstrated that a homolog of the TMA methyltransferases of methanogens, from the nonpyrrolysine *Desulfitobacterium hafniense* Y51, was capable of demethylating GB as part of a corrinoid-dependent pathway of anaerobic respiration. One enzyme predicted to be required for this pathway is an activation enzyme (RamA) for the corrinoid protein. A *ramA* homolog (DSY3152) was identified in an apparent operon that also contains the genes encoding the GB methyltransferase (DSY3156) and its predicted cognate corrinoid protein (DSY3155). We aim to investigate the roles of the RamA enzyme and corrinoid protein in the demethylation of glycine betaine by heterologously producing RamA and the corrinoid protein in *Escherichia coli* sg13009 and a methanogenic strain, respectively. DSY3155 has already been cloned, and DSY3152 will be inserted into a vector and transformed into a housing strain. PCR and gene sequencing will then be used to confirm if the transformation was successful. In addition, brackish water Pulicat lake samples are being enriched for methanogens or bacteria that can degrade carnitine. Thus far, enrichments have been generated and monitored for methane production and growth on carnitine. The next step is to obtain pure cultures on these enrichments so that certain novel carnitine degrading microorganisms from the environment can be identified. By studying these pathways in a variety of communities, more insight will be gained into the catabolism quaternary amines.

BOARD 37

The *Staphylococcus aureus* small RNA Teg41 contributes to virulence via regulation of the alpha phenol-soluble modulins

Rachel L. Zapf[1*], Richard E. Wiemels[1], Rebecca A. Keogh[1], Kayla M. Howell[1], Andrew R. Caillet[1], Emily R. Trzeciak[1], Kellie A. King[1], Samantha A. Selhorst[1], Donald L. Holzschu[1], Jeffrey L. Bose[2], and Ronan K. Carroll[1]
[1]Department of Biological Sciences, Ohio University, Athens, OH
[2]Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS

Staphylococcus aureus is a Gram positive bacterial pathogen responsible for a variety of infections in humans and is responsible for approximately 18,000 deaths per year in the United States. Many species of bacteria, including *S. aureus*, have genetic regulatory elements known as small RNAs (sRNAs). sRNAs have a variety of cellular functions and often affect gene expression of neighboring transcripts. Previous studies in our lab have annotated all known sRNAs in *S. aureus*, and we now report the function of a previously uncharacterized sRNA, Teg41, which is encoded immediately downstream of the αPSM locus. The αPSM transcript encodes four alpha phenol-soluble modulins, which belong to a class of potent cytolytic toxins necessary for infection. We have found that overproduction of Teg41 in *S. aureus* leads to increased hemolysis of human red blood cells, as well as an approximately two-fold increase in αPSM production. Furthermore, we have identified, via *in silico* analysis, a 24-nucleotide region on the extreme 3' end of Teg41 that is predicted to interact with αPSM transcript. Deletion of this 24-nucleotide region reduces *S. aureus* hemolysis, decreases expression of αPSMs, and significantly attenuates virulence in a murine abscess model of infection.

BOARD 38

Observing differences in hand washing practices on campus among undergraduate students across academic disciplines

Elise Westenbarger*[1], Kathryn J. Hermanowski[1], Austin T. Keith[1], and Stephanie Miller, MS[1]
[1]Ohio University

Hand washing is known to reduce the occurrence of infectious disease in a variety of settings, including college campuses. College students who are majoring in a health science field receive instruction on proper clinical hand washing technique as part of their curriculum. Students who do not major in a health science field may or may not receive instruction on proper hand washing during college. The purpose of this study is to assess whether undergraduate health science students at Ohio University are practicing more effective daily hand washing protocol than other majors. Data was collected across campus at Ohio University over a period of 6 weeks. Students were asked to fill out a survey indicating their academic major and to plate finger pad samples before and after washing their hands. For each sample, the effectiveness was measured by whether or not the number of colonies decreased following hand washing. A Pearson's chi-squared analysis comparing hand washing effectiveness across different academic disciplines resulted in a P-value ≈ 0.15. Overall, only 52% (n=146) of students were effective at reducing bacteria on their hands using their preferred daily hand washing method. In conclusion, the observed differences in daily hand washing effectiveness of students in the health science field compared to students in other academic disciplines were not statistically significant. The results of this study will allow for discussion on hand washing curriculum in the health science field.

BOARD 39

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

Mahnur Khan*, Emily Verbrugge*, Chloe Murrell, Joseph Basalla, and Hans Wildschutte
Bowling Green State 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 to engage students in Science, Technology, Engineering, and Math programs but also to identify novel antibiotics. At Bowling Green State University, 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 (Tn) mutagenesis to identify biosynthetic gene clusters involved in toxigenic compound production. Over the four semesters that SWI has been offered at BGSU, this strategy has allowed students to isolate over 400 bacterial strains, of which three have been optimized for Tn mutagenesis used in order to identify mutants with loss of antagonistic activity. With the success of methodology and the generation of useful genomic data, a second semester of SWI has been developed to expand the workflow and include bioinformatics involving whole genome analyses. This approach includes online databases and software to further characterize the inhibitory gene clusters identified in the sequenced genomes. Overall, we have developed an effective SWI workflow that includes a molecular approach to identify genes involved in antagonistic activity and bioinformatic analyses involving whole genome comparisons to facilitate antibiotic discovery.

BOARD 40

An *in vivo* model to study colonisation and persistence of antimicrobial resistant *Neisseria* in the pharynx

Eliza Thapa[*1], Hanna Knauss[1], Benjamin Colvin[1], Benjamin Fischer[1], and Nathan J. Weyand[1]
[1]Ohio University, Athens, Ohio

Neisseria are gram negative aerobic diplococci that colonise the mucosal surfaces of humans and other animals. *Neisseria* species in humans are mostly commensals and only two species, *N. meningitidis* and *N. gonorrhoeae* are strict human pathogens with global heath significance.

Pharyngeal colonisation by pathogenic *Neisseria* causes some serious concerns. *Neisseria* are naturally competent thus allowing the pathogenic species to acquire antimicrobial resistance (AMR) determinants from commensal reservoirs cohabiting the pharynx. *In vivo* studies of *Neisseria* are difficult because of the strict host tropism of *Neisseria* species. In this study, we have used the upper respiratory tract (URT) of rhesus macaques (RM) as a model to study the pharyngeal colonisation and persistence of *Neisseria* in the pharynx. Four animals used in a previous study were treated with Enrofloxacin (a fluoroquinolone) intramuscularly daily for two weeks to remove their pre-existing neisserial flora. Interestingly, Enrofloxacin did not remove the pre-existing flora from two animals.

We show that RMs are commonly colonised by *Neisseria* and at least four strains can inhabit the animal simultaneously. Failure to remove pre-existing *Neisseria* after Enrofloxacin treatment was due to their reduced susceptibility to Enrofloxacin. Putative mutations conferring Enrofloxacin resistance were identified in their *gyrA* and *parC* genes. Loci bearing these mutations were transformed into sensitive strains resulting in reduced susceptibility to the antibiotic. Further, following Enrofloxacin treatment the persistence of resistant strains was likely associated with their low MICs in that pre-existing flora with higher MICs were eventually lost from the animals.

We believe that our model will contribute better understanding of asymptomatic carriage and persistence of antimicrobial resistant *Neisseria* species in the pharyngeal niche. Further, it will help to provide more insight about development of neisserial resistance mechanisms in a niche where resistance is suggested to evolve in humans.

BOARD 41

Analysis of a choline-degrading enrichment culture from the human gut

Jyoti Kashyap*[1], Jeff Ringeisn[1], D.J. Ferguson[1]
[1]Miami University, Oxford

Choline, a quaternary amine (QA) is found in a wide variety of foods such as dairy, egg-based dishes, rice, and meat. This compound, when in our gut, is degraded into trimethyamine (TMA), which is further oxidized in the liver to form trimethylamine N-oxide (TMAO). Increased concentrations of TMAO lead to heart risks such as atherosclerosis. However, an alternate pathway has been proposed in which gut bacteria use methyltranferases to degrade QAs by removing methyl groups. These methyltranferases are members of the TMA methyltransferase (MttB) superfamily (COG5598) which lack the pyrrolysine (pyl) residue. These non-pyl enzymes are proposed to demethylate the QA so that a demethylated product is formed instead of TMA, thus reducing the risk of atherosclerosis. Fecal enrichment cultures supplemented with choline were prepared and stable enrichments obtained from the choline enrichment cultures and identified by 16S rRNA amplicon sequencing. Sequence results showed putatively isolated choline-degrading organisms from the classes Deltaproteobacteria (*Bilophila wadsworthia*) and Gammaproteobacteria (*Citrobacter amalonaticus*). *B. wadsworthia* is an opportunistic anaerobe and makes up approximately 0.01% of the human gut microbiome. Genomic fragments of this organism contained hypothetical genes that encode non-pyl MttB homologs. These results confirm that QA degrading microbes can be obtained using our current strategy. iTAG sequencing results indicated the enrichment having percentage abundance of 90% of *B. wadsworthia*, 6.12% Citrobacter *amalonaticus*, and minute percentages of other organisms which encourages us to obtain a pure culture of this organism. Work towards isolation of *B. wadsworthia* is ongoing. We will analyze the metabolic end products during growth on choline and TMA using GC-MS and sequence the genome of the organism. Understanding the pathways that the gut microbe utilizes to breakdown QAs could help us identify other organisms that could be using similar pathways and better understand the role of gut microbiota in heart disease.

BOARD 42

Identification and Characterization of an RNA Thermometers in *Staphylococcus aureus*

Hebaallaha Hussein*[1,3], Megan E. Fris[2], Ronan K. Carroll[2] and Erin R. Murphy[3]
[1]National Research Centre, Cairo Egypt
[2]Ohio University, Athens OH
[3]Ohio University Heritage College of Osteopathic Medicine, Athens OH

Staphylococcus aureus causes many community acquired and nosocomial infections in humans. it is a commensal organism 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 diseases. 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 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), regulatory elements located in the 5′ untranslated region (UTR) of a regulated gene. The basic principle underlying the activity of an RNAT is 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 bind and facilitating the translation. This is the first study to examine the impact of RNA thermometers in *S. aureus*. One RNA thermometer *cidA* exhibits significant thermo-dependent regulation, in which sequences of the cidA 5'UTR mediate higher expression of the gene at 30°C than at 37°C. Interestingly, the here identified *cidA* RNA thermometer is one of the first shown to mediate increased expression of the regulated gene at lower temperatures. The implications of this research suggest that RNA thermometers exist in *S. aureus* and, importantly, that they may function in previously unrecognized ways to mediate target gene expression.

BOARD 43

Come to the Dark Side: We Have Light Sensors Blue Light Sensing and Regulation in *Acinetobacter baumannii*

Mariah Squire*, Luis Actis, and Natosha Finley
Department of Microbiology – Miami University – Oxford, Ohio

The human pathogen *Acinetobacter baumannii* is a major cause of nosocomial infections. Considering *A. baumannii*'s ability to persist on abiotic surfaces and the fact that various strains are becoming antibiotic resistant, the need to study this pathogen is critical. It can be argued that *A. baumannii* benefits from sensing and responding to various environmental stimuli in the hospital setting. Indeed, *A. baumannii* regulates motility, biofilm formation, and other functions in a blue light-dependent manner using the blue light sensing using flavin (BLUF) protein BlsA. Regarding BlsA's mechanism of action, it is understood that light is sensed by the BLUF domain, which comprises the majority of the protein (~100/156 residues). Although it is not clear how the protein functions as a regulator, the remaining C-terminal residues are implied. We have used homology modeling to predict that BlsA forms a decameric structure that requires the C-terminal residues to polymerize. We have generated BlsA C-terminal mutations and analyzed regulation of motility by resulting BlsA derivatives. Deletion of the protein's fourteen most C-terminal residues does not affect motility regulation. However, deletion of residues predicted to form the most C-terminal α-helix of the protein inhibits motility regulation. Additionally, mutating a lysine residue in this same helix to glutamic acid inhibits BlsA's ability to regulate motility. In our most recent work, we have shown that BlsA may work in tandem with another potential blue light-sensing protein to control motility in response to blue light. Future work includes determining the full extent of BlsA-protein interactions and how *A. baumannii* uses different pathways to respond to blue light. We anticipate that this investigation will lead to a deeper understanding of how

light is used to control cellular processes, and that the information gained will provide novel drug targets for the treatment of this pathogen.

BOARD 44

Influence of different sequencing platforms on the evaluation of response of soil microbial communities to the intrusion of coal mine-derived AMD

Shagun Sharma[1*], **Jenna Pumneo[1]**, Matthew Lee[2], Casey S. Pritchard[2], Teresa J Cutright[1,3] John M. Senko[1,2]
[1]Department of Biology, [1]Integrated Bioscience Program, The University of Akron, Akron OH USA
[2]Department of Geosciences, The University of Akron, Akron OH USA
[3]Department of Civil Engineering, The University of Akron, Akron OH USA

Coal mine-derived acid mine drainage (AMD) is formed upon intrusion of oxygenated water into abandoned mines. Fluid produced is low in pH and contains high dissolved Fe(II) concentrations due to the oxidation of pyrite (FeS_2). When AMD enters into circumneutral streams, dissolved Fe(II) oxidizes and precipitates as Fe(III) (hydro)oxides that affect the quality of surface water. Currently, AMD affects over 10,000 km of streams in the Appalachian regions of the United States. We have found cases where AMD emerges and mixes with formerly pristine soil while flowing as a fluid "sheet". Mixing of AMD and soil in "sheet flow" systems give rise to microbial communities that efficiently oxidatively precipitate Fe(II) as insoluble Fe(III) (hydr)oxides. In this study, we incubated previously pristine soil with AMD and evaluated the changes in the microbial communities that accompanied development of Fe(II) oxidizing activities. Both Illumina and 454 sequencing platforms were used for 16S rRNA gene sequencing to compare the effect of sequencing approach on detected changes in the microbial community during the incubations. We observed increased rates of Fe(II) biooxidation concurrently with changes in the microbial communities. Both sequencing efforts showed similar trends in the microbial communities shifts with biogeochemical changes, confirming there was indeed a development of microbial distinct microbial communities capable of efficient Fe(II) oxidation. We also observed increase in relative abundances of microorganism uncommon to AMD conditions as the Fe(II) oxidizing conditions progressed. However, changes in the 16S rRNA gene libraries generated by Illumina sequencing were more consisted with geochemical changes as the incubations progressed in comparison to 454 sequencing.

BOARD 45

Growth hormone alters the gut microbial abundance and diversity in male growth hormone deficient mice

Elizabeth A. Jensen*[1,2,3], Joshua Busken[3,4], Edward O. List[3,5], Ronan K. Carroll[6], John J. Kopchick[3,5,7], Erin R. Murphy[1,7] and Darlene E. Berryman[1,3,5,7]
[1]Translational Biomedical Sciences, Graduate College, Ohio University, Athens, OH; [2]Ohio University Heritage College of Osteopathic Medicine, Athens, OH; [3]The Diabetes Institute, Ohio University, Athens, OH; [4]College of Health Sciences and Professions, Ohio University, Athens, OH; [5]Edison Biotechnology Institute, Athens, OH; [6]Department of Biological Sciences, College of Arts and Sciences, Ohio University, Athens, OH; [7] Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Ohio University, Athens, Ohio.

Pediatric Crohn's disease, chronic undernutrition, intestinal fibrosis and colon cancer all share gut dysbiosis and abnormalities in growth hormone (GH) action. Furthermore, several studies have shown the gut microbiota can regulate the GH/insulin-like growth factor 1 (IGF-1) axis, specifically IGF-1 levels. Yet, the influence of GH on the gut microbiome remains unexplored. Thus, the purpose of this study was to examine how lack of GH alters the microbial abundance, diversity and predictive biological function in GH deficient (GH-/-) mice as compared to littermate controls. Microbial composition was quantified by targeting the 16S ribosomal RNA gene in fecal pellets of adult male GH-/- mice and littermate controls (n=10 per group) at the Microbiome and Host Response

core of Mouse Metabolic Phenotyping center at University of California, Davis. Bioinformatics analysis of the resultant sequences revealed a shift in the abundance of *Firmicutes* to *Bacteroidetes* and a significant reduction in the abundance of *Proteobacteria* phylum in GH-/- mice (2.81%) compared to controls (6.6%) ($p < 0.01$). Richness and diversity in the microbial populations were also lower in GH-/- mice. Additional multivariate analyses identified unique microbial signatures to the GH-/- mice in the *Parasutterella* and *Helicobacter* genera. Biological function of the microbial communities in GH-/- mice was predicted to have increased carbohydrate absorption and digestion, increased intrinsic resistance to drugs and decreased biosynthesis of antibiotics. Collectively, absence of GH is correlated with a unique microbial profile with decreased bacterial diversity, potential microbial immaturity and shifts in microbial abundance. Additional research will be needed to evaluate the influence of the microbiome due to GH on the intestinal and metabolic health of the host.

BOARD 46

Deletion and Characterization of Two Signaling Genes in *Streptomyces*

Bridget Bowman*, William King, and Jennifer Bennett
Otterbein University

Streptomyces coelicolor is a filamentous, high G-C (guanine-cytosine), gram-positive bacterium that produces an aerial mycelium. The genome consists of a 8,667,507 base pair linear chromosome for this organism and contains the largest number of genes so far discovered in a bacterium (Bentley et al 2002). It belongs to the phylum *Actinobacteria* (McCormick 2011). In addition, streptomycetes are used to produce the majority of antibiotics in the medical field (Alam et al 2010). This study focuses on the effect of the deletion of Sco5218, which encodes a putative integral membrane protein and Sco5219, which encodes a putative lipoprotein in *Streptomyces coelicolor*. Both of these genes are found in a potential operon. The goal of this research is to construct the double mutant and discern differences in phenotype compared to the single deletion mutants of each gene. We hypothesize that the double mutant will have an enhanced effect on the sporulation defect of *Streptomyces*. Sco5218 is believed to contain an HD-GYP domain through bioinformatics analysis and is highly conserved throughout the *Streptomyces* genus. The HD-GYP domain is typically associated with cyclic di-GMP specific phosphodiesterase activity. The relevance of this research allows us to see the importance of the HD-GYP domain in bacteria. If we witness a delay in the sporulation in *Streptomyces* we can deduce that it plays a role in bacterial development. These findings will allows us to further delve into the study of other bacteria and have a basis for other orthologs containing the HD-GYP domain.

BOARD 47

SAMHD1 Impairs HIV-1 Gene Expression and Reactivation of Viral Latency in CD4$^+$ T-cells

Jenna M. Antonucci[a,b,c], Sun Hee Kim[a,b], Corine St. Gelais[a,b], Serena Bonifati[a,b], Olga Buzovetsky[d], Kirsten M. Knecht[d], Alice A. Duchon[a,e,f,], Yong Xiong[d], Karin Musier-Forsyth[a,e,f], and Li Wu[a,b,c,e]
[a] Center for Retroviral Research, [b] Department of Veterinary Biosciences, [c] Department of Microbiology, [e] Center for RNA Biology, and [f] Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio, USA
[d] Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA

Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) restricts human immunodeficiency virus type 1 (HIV-1) replication in non-dividing cells by degrading intracellular deoxynucleoside triphosphates (dNTPs). SAMHD1 is highly expressed in resting CD4$^+$ T-cells that are important for the HIV-1 reservoir and viral latency; however, whether SAMHD1 affects HIV-1 latency is unknown. Recombinant SAMHD1 binds HIV-1 DNA or RNA fragments *in vitro*, but the function of this binding remains unclear. Here we investigate the effect of SAMHD1 on HIV-1 gene expression and reactivation of viral latency. We found that endogenous SAMHD1 impaired HIV-1 LTR activity in monocytic THP-1 cells and HIV-1 reactivation in latently infected primary CD4$^+$ T-cells. Overexpression of wild-type (WT) SAMHD1 suppressed HIV-1 long terminal repeat (LTR)-driven gene expression at the level of transcription. SAMHD1 overexpression also suppressed LTR activity from human T-cell leukemia virus type 1 (HTLV-1), but not from murine leukemia virus (MLV), suggesting specific suppression of retroviral LTR-driven gene expression. WT SAMHD1 bound to proviral DNA and impaired reactivation of HIV-1 gene expression in latently infected J-Lat cells. In contrast, a nonphosphorylated mutant (T592A) and a dNTP triphosphohydrolase (dNTPase) inactive mutant (H206D/R207N, or HD/RN) of SAMHD1 failed to efficiently suppress HIV-1 LTR-driven gene expression and reactivation of latent virus. Purified recombinant WT SAMHD1, but not T592A and HD/RN mutants, bound to fragments of the HIV-1 LTR *in vitro*. These findings suggest that SAMHD1-mediated suppression of HIV-1 LTR-driven gene expression contributes to regulation of viral latency in CD4$^+$ T-cells.

BOARD 48

Evaluation of novel cell-permeable PDZ domains to modulate adenovirus infection

Meghan R. Jenkins*[1], Timothy L. Williamson[1], Ibrahim A.M. Alkhomsi[1], James M. Readler[1], Priyanka Sharma[1], and Katherine J.D.A. Excoffon[1]
[1]Department of Biological Science, Wright State University, Dayton OH 45435

Creating a means of regulating the expression and localization of the Coxsackievirus and Adenovirus Receptor (CAR) within the cell would be useful, as downregulation could prevent opportunistic adenovirus infections, while upregulation could increase the chance of transduction for the purposes of adenovirus-based gene therapy. CAR^{Ex8}, a transmembrane isoform of CAR encoded by all 8 exons of the *CXADR* gene, is tightly regulated by the PDZ1 and PDZ3 domains found within the cellular scaffolding protein, Membrane Associated Guanylate Kinase, WW And PDZ Domain-Containing Protein 1 (MAGI-1). MAGI-1 PDZ3 can sequester CAR^{Ex8} within the cell, preventing adenovirus binding and subsequent infection. MAGI-1 PDZ1 prevents this sequestration, allowing CAR^{Ex8} to localize to the apical surface of polarized epithelial cells, where adenovirus can bind it and infect epithelial cells. Preliminary data has shown that decoy PDZ1 or 3 domains, connected to the HIV cell-permeable TAT sequence and a myc-tag for detection, allow the up- or downregulation of CAR^{Ex8}, respectively. We hypothesized that PDZ1 or 3 domain regulation of CAR^{Ex8} would, in turn, increase or decrease adenovirus infection. PDZ domains were cloned into a prokaryotic expression vector and purified from Rosetta *E. coli*. MDCK epithelial cells were treated with purified TAT-myc-PDZ1 or TAT-myc-PDZ3 domains. Recombinant adenovirus carrying a β-galactosidase reporter gene was used to infect treated cells, and adenoviral transduction was determined using a β-galactosidase assay. Results indicate that conjugation to the cell-permeating TAT peptide is an effective way to deliver PDZ1 and PDZ3 domains into cells for CAR^{Ex8}

regulation. Surprisingly, treatment with PDZ domains did not affect adenovirus infection. Future work will explore the effect of PDZ1 and 3 domains on cell surface CAR^{Ex8} expression as well as each domain contextualized by adjacent MAGI-1 domains on CAR^{Ex8} regulation and adenovirus infection.

BOARD 49

How LPS structure affects its transport by $LptB_2FG$

Emily Lundstedt* and Natividad Ruiz
The Ohio State University

The cell envelope is what defines a bacterium from its environment and protects it from the outside world. Gram-negative bacteria are diderms, and thus have a cell envelope composed of both an inner membrane and an outer membrane. The outermost leaflet of the outer membrane is made up of lipopolysaccharide (LPS). Tight packing of LPS molecules makes the outer membrane a potent permeability barrier, protecting the cell from entry of harmful compounds such as antibiotics. LPS is synthesized at the inner membrane and is transported to the outer leaflet of the outer membrane by the LPS transport (Lpt) machinery. The movement of LPS through this machinery is powered by the ATP-binding cassette (ABC) transporter $LptB_2FG$. Although it is clear that ATP hydrolysis is required for LPS transport, it is not known how the ATP-hydrolysis cycle is coupled to the extraction of LPS. To elucidate this mechanism, we performed structure-function analysis of mutants that alter residues along the interaction interface between the ATPase subunits $LptB_2$ and transmembrane subunits LptFG. We identified a new essential residue within LptB that is critical for connecting ATP hydrolysis and LPS transport. The lethality of a mutation that alters this residue in LptB could be suppressed by deletion of an LPS biosynthesis gene, *lpxM*. Since the loss of LpxM results in an altered LPS structure, these findings suggest that this residue in LptB is responsible for conferring conformational changes associated with ATP hydrolysis to drive transport of LPS by lptFG.

BOARD 50

Identification and analysis of *Staphylococcus aureus* small RNAs (sRNAs) that contribute to biofilm formation

Caleb A. Burke* and Ronan K. Carroll
Department of Biological Sciences, Ohio University, Athens, OH

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 each sRNA to biofilm growth we identified and changed its level of expression using both and overexpressing and a knockdown approach.

BOARD 51

Defining the structure and function of serine-rich repeat proteins in *Streptococcus oralis*

Allen L. Ronis[1,2], Anirudh K. Singh[1], Alex Wong[1], Kenneth Brockman, Mark van der Linden[3] & Samantha J. King[1,2]
[1]Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital, and [2]The Ohio State University, Columbus, OH, and [3]University Hospital RWTH Aachen, Aachen, Germany

Serine-rich repeat proteins (SRRPs) are a family of adhesins in bacteria, mediating binding to host and bacterial surfaces. We are studying the role of SRRPs in oral colonization and platelet binding, which is a critical step in sub-acute infective endocarditis. Microscopy images of *Streptococcus oralis* subsp. *dentisani* endocarditis isolates showed surface fibrils of multiple lengths, localized to one side of the cell. This fibril distribution resembles *Streptococcus cristatus,* which produces two distinct fibrils on one side of the cell, one of which is a SRRP. Genomic analysis of both species revealed multiple genes which may encode for distinct SRRPs. These findings led to the novel hypothesis that *S. oralis* subsp. *dentisani* expresses multiple SRRPs. Isolate F0392, which contains genes for three putative SRRPs, was chosen for further study as it is genome sequenced and genetically amenable. A mutation in the SRRP secretion system of F0392 resulted in bacteria devoid of surface fibrils, suggesting the different fibrils are SRRPs. This mutant showed reduced binding to saliva, suggesting the fibrils may effect oral colonization. We are currently mutating the genes predicted to encode SRRPs to define the contribution of these loci to fibril expression and adherence. Defining the structure and function of fibrils expressed by *S. oralis* subsp. *dentisani* will likely identify mechanisms by which this bacterium colonizes oral surfaces and causes sub-acute endocarditis.

BOARD 52

In vitro reconstitution of glycine betaine:coenzyme M methyl-transfer pathway from *Methanolobus vulcani* B1d

Adam J. Creighbaum*[1#], Tomislav Ticak[2], Xin Wang[1], D.J. Ferguson Jr.[1,3#]
[1]Department of Microbiology, Miami University, Oxford, OH 45056; [2]Department of Biological Sciences, University of Idaho, Moscow, ID 83844; [3]Miami University Hamilton, Hamilton, OH 45011

Methanogenesis from quaternary amines (QAs) is relatively uncharacterized in the spectrum of one-carbon metabolism. Previous studies have shown select strains in the genus *Methanococcoides* were capable of growth using tetramethylammonium (QMA), glycine betaine (GB), and choline but the physiological pathways were not elucidated. We recently described the first strain in the genus *Methanolobus* that catabolizes GB to 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 rare amino acid L-pyrrolysine (pyl)-containing enzyme (MttB), we have demonstrated the function of a MttB homolog that lacks pyl, MtgB. This demonstration is critical as the majority of the MttB superfamily lacks pyl; thus, leaving a gap in knowledge of functionality of 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 a corrinoid reductive activation enzyme (RamA: 2107). Through proteomic analysis we were able to determine that MV1727, MV1728, MV2107, and MV2112 were in high abundance when *M. vulcani* B1d was grown on GB relative to growth on TMA. We produced these highly abundant proteins recombinantly in either *Escherichia coli* or *Methanosarcina acetivorans*. Activity of MV1727 catalyzes GB dependent methylation of free cob(I)alamin and recombinant MV0221, MV0257, and MV2112 each catalyze methylcobalamin dependent methylation of CoM. MV1727 and either MV0221, MV0257, or MV2112 were then combined with MV1728, and MV2107 to successfully reconstitute a GB:CoM pathway from this organism. To our knowledge, this is the first demonstration of reconstitution of this pathway *in vitro* using purified enzymes. In addition to the discovery of a

novel methanogenic pathway, we have also gained further insight into the largely uncharacterized MttB superfamily.

BOARD 53

Elucidating how the LptB motor drives lipopolysaccharide transport

Brent W. Simpson*, Rebecca Davis, Alex Mestre, and Natividad Ruiz
Department of Microbiology, The Ohio State University

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 their cell surface with a glycolipid 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 must be transported across the periplasm and the outer membrane in order to be assembled at the cell surface. This process has been 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 powers LPS transport. The dimer of LptB utilizes the conserved features of ABC-motor domains to hydrolyze ATP and undergo conformational movements. These conformational changes are then transmitted to transmembrane partners LptFG to drive 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. Using structure-function and suppressor analyses, we demonstrate that this C-terminal domain functions with ATP-binding motifs in LptB to coordinate conformational movements of ATP hydrolysis with conformational movements of transmembrane proteins LptFG.

BOARD 54

Functional diversification accompanies gene family expansion of *MED2* homologs in *Candida albicans*

Matthew J. Dunn*[1], Griffin M. Kinney[1], Pamela M. Washington[1], Judith Berman[3], and Matthew Z. Anderson[1,2]
[1]Department of Microbiology, The Ohio State University, Columbus, OH, 43210, USA.
[2]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, 43210, USA.
[3]Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv, Israel.

Gene duplication facilitates functional diversification and provides greater phenotypic flexibility to an organism. Expanded gene families arise through repeated gene duplication but the extent of functional divergence that accompanies each paralogous gene is generally unexplored because of the difficulty in isolating the effects of single family members. The telomere-associated *(TLO*) gene family is a remarkable example of gene family expansion, with 14 family members in the more pathogenic *Candida albicans* relative to two *TLO* genes in the closely-related species *C. dubliniensis*. These genes encode interchangeable Med2 subunits of the major transcriptional regulatory complex Mediator although the function of individual family members is unclear. To identify biological functions associated with each *C. albicans TLO*, individual family members were misexpressed using a Tet-ON system and the strains were assessed across a range of phenotypes involved in growth and virulence traits. All *TLO*s affected multiple phenotypes and a single phenotype was often affected by multiple *TLO*s, including simple phenotypes such as cell aggregation and complex phenotypes including virulence in a *Galleria mellonella* model of infection. No phenotype was regulated by all *TLO*s, suggesting neofunctionalization or subfunctionalization of ancestral properties of different family members. Importantly, regulation of three phenotypes could be mapped to individual polymorphic sites among the *TLO* genes, including an indel correlated with two phenotypes, growth in sucrose and macrophage killing. Different selective pressures have operated on the *TLO* sequence, with the 5' conserved Med2 domain experiencing purifying selection and the gene/clade-specific 3' end undergoing extensive positive selection that may contribute to the impact of individual *TLO*s on phenotypic variability. Therefore, expansion of the *TLO* gene family has conferred

unique regulatory properties to each paralog such that it influences a range of phenotypes. We posit that the diversity associated with this expansion contributed to its success as a commensal and opportunistic pathogen.

Abstracts of OBASM Podium Presentations

Saturday, March 24
3:30 – 5:40 pm

3:30 – 3:45 pm

Host cell perforation by listeriolysin O (LLO) activates a Ca^{2+}-dependent cPKC/Rac1/Arp2/3 signaling pathway that promotes *Listeria monocytogenes* internalization independently of membrane resealing

Jonathan G.T. Lam* [1, 2], Stephen Vadia[2], Sarika Pathak-Sharma[1], Eric McLaughlin[3], Xiaoli Zhang[3], Joel Swanson[4], and Stephanie Seveau[1, 2]

[1]Department of Microbial Infection and Immunity, Infectious Diseases Institute, The Ohio State University Wexner Medical Center, Columbus, OH 43210

[2]Department of Microbiology, The Ohio State University, Columbus, OH 43210

[3]Center for Biostatistics, The Ohio State University, Columbus, OH 43210

[4]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-5624

Host cell invasion is an indispensable step for a successful infection by intracellular pathogens. Recent studies identified pathogen-induced host cell plasma membrane perforation as a novel mechanism used by diverse pathogens (*Trypanosoma cruzi*, *Listeria monocytogenes*, and adenovirus) to promote their internalization into target cells. It was concluded that *T. cruzi* and adenovirus damage the host cell plasma membrane to hijack the endocytic-dependent membrane resealing machinery, thereby invading the host cell. We studied *L. monocytogenes* and its secreted pore-forming toxin listeriolysin O (LLO) to identify key signaling events activated upon plasma membrane perforation that lead to bacterial internalization. Using various approaches, including fluorescence resonance energy transfer imaging, we found that the influx of extracellular Ca^{2+} subsequent to LLO-mediated plasma membrane perforation is required for the activation of a conventional protein kinase C (cPKC). cPKC is positioned upstream of Rac1 and the Arp2/3 complex, which activation leads to F-actin–dependent bacterial internalization. Inhibition of this pathway did not prevent membrane resealing, revealing that perforation-dependent *L. monocytogenes* endocytosis is distinct from the resealing machinery. These studies identified the LLO-dependent endocytic pathway of *L. monocytogenes* and support a novel model for pathogen uptake promoted by plasma membrane injury that is independent of membrane resealing.

3:45 – 4:00 pm

Genetics-Based Discovery of *Histoplasma* Genes Required for Intramacrophage Growth

Qian Shen, Matthew J. Beucler, and Chad A. Rappleye
Department of Microbiology, Ohio State University

The fungal pathogen *Histoplasma capsulatum* causes disease in both immunocompromised and immunocompetent hosts, by parasitizing phagocytes. To discover new virulence factors of *Histoplasma*, specifically those that enable survival and growth within macrophages, we used a forward genetics approach. Random mutants of *Histoplasma* yeasts were created through insertional mutagenesis using *Agrobacterium tumefaciens*-mediated transformation and mutants were screened for: (1) the ability to survive and proliferate within macrophages and (2) the ability to lyse host macrophages. To enable high-throughput screening, we engineered *Histoplasma* to express red-fluorescence protein (RFP) facilitating fluorescence-based enumeration of intramacrophage yeasts. For efficient monitoring of macrophage numbers, a transgenic beta-galactosidase-expressing macrophage line is used. We screened 40,000 mutants and isolated 150 mutants with decreased proliferation within macrophages. Among the set of attenuated mutants, four mutations mapped to genes that contribute to peroxisome structure and function (the *PEX5*, *PEX10*, *PEX11*, and *PEX14* genes) suggesting peroxisomal functions are critical to *Histoplasma*'s intracellular proliferation. Complementation of the perxisomal

mutant strains confirms the attenuation phenotype is linked to the disrupted *PEX* genes. Expression of *gfp* tagged with a putative peroxisome-targeting signal (PTS) in *Histoplasma* yeasts showed that the PTS causes localization of the *gfp* to puncta within the yeast cell consistent with peroxisome organelles. In a murine model of respiratory histoplasmosis, loss of *PEX* gene function severely attenuated *Histoplasma*'s virulence. These data indicate that peroxisome functions are critical for *Histoplasma* pathogenesis and begin to reveal the mechanisms required for intramacrophage growth of this fungal pathogen.

4:00 – 4:15 pm

Alternative electron flow supports rewired metabolism in Antarctic photopsychrophile *Chlamydomonas* sp. UWO241

Isha Kalra*, Xin Wang, and Rachael Morgan-Kiss
Department of Microbiology, Miami University

Photosynthetic organisms adapted to survive in cold environments (photopsychrophiles) have evolved unique strategies to maintain photosynthesis and growth rate in such extreme conditions. The Antarctic green alga *Chlamydomonas* sp. UWO241 represents a model for cold-adaptation of photosynthesis. UWO241 is not only adapted to cold temperatures, it also survives high salinity and low light in its natural environment. As a result, UWO241 has remodeled its photosynthetic apparatus which includes downregulation of photosystem I (PSI), large photosystem II light harvesting antenna and unique thylakoid phosphorylation pattern. Interestingly, UWO241 also forms relatively large oil bodies and has poly-unsaturated fatty acid enriched membranes. To deal with the long-term stress in its environment, UWO241 has downregulated short-term stress acclimation mechanisms such as state-transitions and has instead upregulated the major alternative electron transport pathway in photosynthesis, i.e. cyclic electron flow (CEF) around PSI. In photosynthetic organisms, CEF is responsible for supplying the extra ATP necessary to fulfill the ATP:NADPH ratio for carbon fixation and maintaining energy balance between photosynthesis and downstream metabolism. This study was aimed to understand the mechanism and role of sustained strong CEF in UWO241. Proteomics and biochemical data revealed the presence of a stable PSI-supercomplex to support sustained high-rates of CEF in UWO241. Additionally, comparative proteomics analysis indicated upregulation of a secondary metabolite pathway for synthesis of aromatic amino acids and other compounds in UWO241 strain under conditions of low-temperature and high salinity. We propose that PSI-supercomplex associated strong CEF in UWO241 supports increased ATP production for energy sinks accompanying adaption to low temperature and/or high salinity.

4:15 – 4:30 pm

RyfA and RyfB in *Shigella*: Which sRNA is the Evil Twin?

Megan E. Fris*[1], William H. Broach[1], Sarah E. Klim[1], Peter W. Coschigano[2], Ronan K. Carroll[1], Clayton C. Caswell[3], and Erin R. Murphy[2]
Ohio University[1], Ohio University College of Osteopathic Medicine[2], Virgina Tech[3]

Shigella infections result in 161 million infections and 1.1 million deaths worldwide. *Shigella* cause cramping, fever, and bloody diarrhea which is known as dysentery. No vaccines exist to prevent the transmission of *Shigella* and antibiotics are considered contra-indicated for patients infected with *Shigella dysenteriae*. Lack of treatment for dysentery has spurned researchers to examine the molecular pathways by which *Shigella* causes an infection in hopes of creating novel therapeutics. One area of research in *Shigella* and other pathogens are small non-coding RNAs (sRNAs). RyfA is a sRNA that was discovered in *E. coli* and conserved in other species of *Shigella*. Interestingly, twin copies of RyfA exist in *S. dysenteriae*, termed RyfA1 and RyfA2. Each RyfA from *S. dysenteriae* is 95% identical to one another. The largest dissimilarity occurs in a five-nucleotide variable region which is in a predicted single-stranded region of the sRNA molecules. Given that sRNAs target mRNA molecules in single-stranded regions, RyfA1 and RyfA2 were predicted to have different molecular targets. To explore the role of each RyfA in *S. dysenteriae*, the pathogen overexpressed each sRNA and was measured for

the ability to infect in plaque assay. RyfA1 overproduction inhibited the ability of *S. dysenteriae* to spread, while RyfA2 overproduction did not impact pathogenesis. Interestingly, upstream of both RyfA1 and RyfA2, two additional sRNAs exist termed RyfB1 and RyfB2. The RyfB molecules are only 60% identical to one another. Furthermore, each RyfB shares a region of complementarity to each respective RyfA molecule which overlaps the five-nucleotide variable region; indicating a method for specific regulation. Further examination revealed RyfB1 inhibits RyfA1 levels. Interestingly, RyfB2 inhibits both RyfA1 and RyfA2 levels. Additionally, RyfB2 overproduction allows for *S. dysenteriae* to become hypervirulent in plaque assay. The RyfAs and RyfBs highlight the importance of sRNAs in *Shigella* pathogenesis.

10 minute break

4:40 – 4:55 pm

The role of neuraminidase-producing bacteria in exposing cryptic carbohydrate receptors for *Streptococcus gordonii* adherence

Alexander Wong*[1,2], Margaret A. Grau[1], Anirudh K. Singh[1], Shireen A. Woodiga[1], and Samantha J. King[1,3]
[1]Nationwide Children's Hospital Center for Microbial Pathogenesis, [2]The Ohio State University College of Medicine, [3]The Ohio State University Department of Pediatrics

Streptococcus gordonii is an early colonizer of the oral cavity. Although a variety of *S. gordonii* adherence mechanisms have been described, current dogma is that the major receptor for *S. gordonii* is sialic acid. However, as many bacterial species in the oral cavity produce neuraminidase that can cleave terminal sialic acid, it is unclear whether *S. gordonii* relies on sialic acid for adherence to oral surfaces or if this species has developed alternative binding strategies. Previous studies have examined adherence to immobilized glycoconjugates and identified binding to additional glycans, but no prior studies have defined the contribution of these different glycan structures in adherence to oral epithelial cells. We determined that the majority of *S. gordonii* strains tested did not rely on sialic acid for efficient adherence. In fact, adherence of some strains was significantly increased following neuraminidase treatment. Further investigation of representative strains that do not rely on sialic acid for adherence revealed binding not only to sialic acid via the serine-rich repeat protein GspB, but also to β-1,4-linked galactose. Adherence to this carbohydrate occurs via an unknown adhesin distinct from those utilized by *Streptococcus oralis* and *Streptococcus pneumoniae*. Demonstrating the potential biological relevance of binding to this cryptic receptor, we established that *S. oralis* increases *S. gordonii* adherence in a neuraminidase-dependent manner. These data suggest that *S. gordonii* has evolved to simultaneously utilize both terminal and cryptic receptors in response to the production of neuraminidase by other species in the oral environment.

4:55 – 5:10 pm

Target Identification of the New Antifungal Compound 41F5

Kristie Goughenour*[1], Keisuke Ishita[2], Werner Tjarks[2], and Chad Rappleye[1]
[1]Department of Microbiology, Ohio State University
[2]Division of Medical Chemistry and Pharmacology, Ohio State University

Pathogenic fungi present a particular challenge for therapeutics development due to the shared eukaryotic nature of host and pathogen. Although the recently developed echinocandins have reduced host toxicity, the pathogens *Histoplasma capsulatum* and *Cryptococcus neoformans* are naturally resistant to this new class of antifungals. Phenotypic screening of a small molecule library identified an aminothiazole compound, 41F5, with antifungal activity against *H. capsulatum*, and *C. neoformans*. Importantly, 41F5 exhibits very low cytotoxicity to mammalian cells including macrophages, the host cells for *H. capsulatum* and *C. neoformans*. Identification of the cellular target of 41F5 and its mechanism of action are necessary to advance this new antifungal compound. To identify the target, repeated passage of *C. neoformans* yeasts in subinhibitory concentrations of 41F5 led to isolation of six independent lines of 41F5-resistant yeast. Each exhibits at least a 50-fold increase in the MICs. These resistant strains have no significantly increased resistance to azole-class antifungals suggesting their resistance does not derive from general drug efflux. To identify the gene(s) mutated in these 41F5-resistant strains, whole genome sequencing is being performed to identify SNPs associated with drug resistance. A complementary biochemical approach to target identification will be used in conjunction with our genetic approach. A modified co-elution and Mass Spectrometry (MS) strategy using non-modified 41F5 will be used to determine which proteins may interact with 41F5 and therefore may be the drug's target. We are currently optimizing our compound detection ability using MALDI. Once completed, we will be able to identify 41F5 in cell lysate fractions along with co-eluting proteins by LC-MS/MS as potential targets. Targets will then be validated by ectopic expression of resistance-associated alleles in wild-type *C. neoformans* and testing for increased resistance to 41F5.

5:10 – 5:25 pm

Identification of Novel Anti-Adenoviral Small Molecules

Hannah Shows[1], Priyanka Sharma[1], Mahmoud Alghamri[1], Michael Raymer[2], Katherine Excoffon[1]
[1]Department of Biological Sciences, Wright State University; [2]Department of Computer Science, Wright State University

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 including 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.

5:25 – 5:40 pm

Role of Cell Fusion in Protection of the Placenta Against Infection

Lauren J. Johnson[1,2,3]*, Siavash Azari[1,2, 3], Joanna Marshall[1,2,3], William Ackerman[5], Stephen Thung[5], Kara Rood[5], John M. Robinson[4], Stephanie Seveau[1,2,3]

[1]Ohio State University, Department of Microbial Infection and Immunity, [2]Ohio State University, Department of Microbiology, [3]Ohio State University, Infectious Diseases Institute, [4]Ohio State University, Department of Physiology and Cell Biology, [5]Ohio State University, Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine

The placenta is a highly effective barrier for protecting the fetus against most infections. However, during pregnancy, women are more susceptible to infection by some pathogens, including *Listeria monocytogenes,* resulting in fetal demise or neonatal sequelae. 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. The syncytiotrophoblast forms a first barrier at the maternal-fetal interface and consists of a continuous cell layer formed by fusion of underlying cytotrophoblastic cells. We evaluated the antimicrobial mechanisms of the syncytiotrophoblast in comparison to cytotrophoblasts. The choriocarcinoma cell line BeWo which retains major properties of cytotrophoblasts, such as their ability to undergo fusion and produce βhCG, has been used to explore if cell fusion is accompanied with an increase in the antimicrobial response. As a second cell model, we isolated primary human cytotrophoblasts from term placentas. We infected cells with *L. monocytogenes* for up to 3 hours and enumerated the efficiencies of bacterial association and internalization into fused versus non-fused cells by fluorescence microscopy. We found that bacterial association is decreased by13-fold and their internalization by 3-fold upon cell fusion. Moreover, bacterial intracellular proliferation was 3-fold decreased in fused cells. Together, these results indicate that fusion markedly protects cells from *L. monocytogenes* infection by impeding several steps of the invasion process, e.g. bacterial association, internalization, and proliferation. This analysis could serve as a first approach to establish the syncytiotrophoblast mechanisms of resistance to infection.

Description of Awards for Presentations

N. Paul Hudson Award for Research Excellence

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

Donald C. Cox Award for Research Excellence

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

J. Robie Vestal Award for Research Excellence

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

Ohio Branch ASM Award for Research Excellence

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

Allan A. 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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