Annual Meeting
Ohio Branch of the American Society for Microbiology

Lorain County Community College
Spitzer Conference Center

Lorain County Community College

Elyria, Ohio
April 12 – April 13, 2019

Official Meeting Program
and
Conference Abstracts

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Acknowledgements

The assistance and dedication of the following have contributed to the success of OBASM 2019:

OBASM Executive Committee

Jennifer Bennett
Erin Murphy
D.J. Ferguson
Lubna Abu-Niaaj
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ASM Distinguished Lecturer Program
The American Society for Microbiology

Invited Speakers

Vaughn Cooper
Beronda Montgomery
Matthew Saxton
Brian Lower
Annette Rowe
Yvonne Sun
Vanessa Hale
Justin Pruneski
Liem Nguyen

Program Schedule for OBASM 2019

Friday, April 12

6:00 – 7:00 pm **Registration and Social –** Spitzer Conference Center Entrance
Poster set-up – Rooms 210 and 211, Spitzer Conference Center

7:00 – 7:10 pm **Introduction** – Reaser Grand Room, Spizer Conference Center

Hannah Newsome
President of the ASM Student Chapter at Lorain County Community College

Welcome Statement
Marcia Ballinger
President of Lorain County Community College

7:15 – 8:15 **OBASM Keynote Lecture** – Reaser Grand Room, Spitzer Conference Center

"Predictable host adaptations by Gram-negative opportunitsts caused by strong selection and short-term commitment"

Vaughn Cooper
Department of Microbiology and Molecular Genetics
University of Pittsburgh

8:15 – 9:00 pm **Social** – Culinary Arts Institute, Spitzer Conference Center

Saturday, April

7:30 – 8:30 am **Registration** – Spitzer Conference Center Entrance
Poster set-up – Rooms 210 and 211, Spitzer Conference Center

8:30 – 9:30 am **ASM Branch Lecture** – Reaser Grand Room, Spitzer Conference Center

"First insight into second messengers: Roles of cyclic Dinucleotides in environmental responses in Cyanobacteria"

Beronda Montgomery
MSU Foundation Professor, Department of Biochemistry & Molecular Biology and Microbiology & Molecular Genetics; MSU-DOE Plant Research Laboratory

9:30 am – 9:40 am **Five Minute Microbe Talks** – Reaser Grand Room, Spitzer Conference Center

Moderator: D.J. Ferguson

9:30 – 9:35 **Ruchita Solanki**
University of Cincinnati
***"*Characterization of a *Pseudomonas stutzeri* strain from marine sediments capable of iron oxidation and electrode oxidation"**

9:35 – 9:40 **Ariana Shannon**
Capital University
"An investigation into the isolation and prevalence of extended-spectrum beta lactamase bacteria in supermarket raw chicken and pork meat"

9:40 – 9:50 am **Break** - **Culinary Arts Institute, Spitzer Conference Center**
Snacks and beverages

9:50 am – 11:40 pm **Symposia – Concurrent Sessions and Five Minute Microbe Talks**

Session 1: Environmental and Applied Microbiology

Session 2: Medical Microbiology

Session 1: Environmental and Applied Microbiology – Spitzer Conference Center, Rooms 207 – 208

Moderator: D.J. Ferguson

9:50-9:55 **Ryan M. Mrofchak**
Youngstown State University
***"Enterococcus* species Isolated from Lake Newport in Mill Creek ParK"**

9:55-10:00 **Simran Chandawarkar**
New Albany High School
"Money Laundering: An effective way to disinfect paper currency"

10:00-10:30 **Matthew Saxton** – Biological Sciences Department, Miami University, Middletown
"Stinky, salty and covered with ice: methane and sulfur cycling in lakes of the McMurdo Dry Valleys, Antarctica"

10:30-11:00 **Brian Lower** – School of Environment and Natural Resources, The Ohio State University
"Magnetotactic Bacteria – Bugs with backbones"

11:00-11:05 **Jyoti Kashyap**
Miami University
"Characterizing a human fecal isolate capable of degrading choline in the human gut"

11:05-11:10 **Samuel Blechman**
University of Akron
"Single-cell analysis of inner membrane diffusion of *Escherichia coli* by FRAP"

11:10-11:40 **Annette Rowe** – Department of Biological Sciences, University of Cincinnati
"Methanogenic electron uptake from electrodes: better energy conservation through DIET"

Session 2: Medical Microbiology – Spitzer Conference Center, Rooms 214 – 215

Moderator: Harry Kestler

9:50-9:55 **Stephanie C. Ray**
Ohio State University
"Mammalian temperature and available copper dually regulate the *Histoplasma capsulatum* Ctr3 copper transporter"

9:55-10:00 **Amy Jindra**
Lorain County Early College High School/College Credit Plus

"The effects of CCR5 delta32 on surface expression of CCR5 and CXCR4"

10:00-10:30 **Yvonne Sun** – Department of Biology, University of Dayton
"Running out of oxygen: How anaerobic exposure affects *Listeria monocytogenes* pathogensis"

10:30-11:00 **Vanessa Hale** – Department of Biology, The Ohio State University
"Finding your niche: *C. difficile and the microbiome* "

11:00-11:05 **Eleana Cintron**
Lorain County Community College
"Examining hliA Primer Sets for *Planktothrix agardhii*"

11:05-11:10 **Paul Zdinak**
Ohio University
"Membrane associated complement inhibitors: here today, gonorrhea tomorrow"

11:10-11:40 **Liem Nguyen** – Department of Molecular Biology and Microbiology, Case Western Reserve University
"The antifolate resistome in mycobacteria"

11:40 – 1:00 pm **Noontime Activities:**

Lunch: Box or "on your own"
Box lunches available only for those who pre-ordered box lunches
Pickup outside of Rooms 210/211, Spitzer Conference Center

OBASM Business Meeting – Spitzer Conference Center, room 201

1:00 – 3:00 pm **Poster presentations and judging –** Rooms 210 – 211, Spitzer Conference Center
Coordinator – Chet Cooper

3:00 – 4:00 pm **Podium presentations and judging** – Rooms 207 – 208
Coordinator – Chet Cooper

3:00 – 3:15 pm

Methanogenesis from glycine betaine proceeds through a corrinoid-dependent methyltransferase pathway

Adam Creighbaum*[1], Tomislav Ticak[2], Shrameeta Shinde[1], Xin Wang[1], D.J. Ferguson Jr.[1,3],
[1]Department of Microbiology, Miami University; [2]Department of Biological Sciences, University of Idaho; [3]Miami University Hamilton

3:15 – 3:30 pm

Back from the dead: How changes in LPS structure alter its transport by $LptB_2FG$

Emily Lundstedt*, Brent Simpson, Rebecca Davis, Natividad Ruiz
The Ohio State University

3:30 – 3:45 pm

***Histoplasma* relies on gluconeogenic carbon substrates to proliferate in macrophages**

Qian Shen*, Stephanie Ray, and Chad Rappleye
Department of Microbiology, Ohio State University

3:45 – 4:00 pm

Tracking Diel Metabolism of the 2014 Lake Erie Toxic Microcystis Bloom Through Metatranscriptomics

Michelle Neudeck*[1], Paul Matson[1], R. Michael McKay[1,2], George S. Bullerjahn[1]
[1]Department of Biological Sciences, Bowling Green State University
[2]GLIER, University of Windsor

4:00 – 5:00 pm **Session 3: Education** – Spitzer Conference Center, Rooms 207 – 208

Moderators: Stephanie Miller and Lubna Abu-Niaaj

"Lighting the Way: Building Bridges to Access and Success"
Beronda Montgomery
MSU Foundation Professor, Department of Biochemistry & Molecular Biology and Microbiology & Molecular Genetics; MSU-DOE Plant Research Laboratory

“Engaging students with case studies: Not just for medical microbiology anymore”
Justin Pruneski
Biology & Environmental Sciences Department, School of Natural Sciences & Allied Health Sciences, Heidelberg University

5:15 – 7:45 pm **Banquet and Student Awards Presentations** – Reaser Grand Room, Spitzer Conference Center

Abstracts of OBASM Poster Presentations
Saturday, March 24
1:00-3:00 pm

BOARD 1

The Effectiveness of Norwex Microfiber Cloths in the Elimination of Bacteria from Common Kitchen Surfaces

Cassidy Hogue*[2, 3], Dulce Cintron[1], Kathryn A. Durham[2]
[1]Lorain County Early College High School; [2]Lorain County Community College; [3]Bowling Green State University

Norwex microfiber cloths claim to eliminate 99% of bacteria from common kitchen surfaces due in part to the silver embedded in the cloth. If true, it would eliminate the need for household cleaning products and be more environmentally friendly. The goal of this research project is to determine how efficiently Norwex cloths eliminate bacteria when compared to the more traditionally used household cleaners such as Clorox and Dawn dishwashing soap. Three different common kitchen surfaces: granite, wooden and plastic cutting boards were partitioned into four sections, cleaned with bleach and cultured. The surfaces were infected with *Salmonella enteritidis* and cultured. The bacteria were cleaned from each section using a different product: water, Dawn dishwashing soap, Clorox wipes and Norwex microfiber cloth and then cultured. All of the cultures were grown at 37°C on TSA media plates for twenty four hours and bacterial growth was assessed. There was minimal growth on all four granite cutting board sections suggesting that each cleaning product was effective, however, there was heavy bacterial growth on all cleaned sections of the plastic cutting board except the one cleaned with Clorox wipes. This research will continue with experiments on the wooden cutting board and using the spore-forming *Bacillus subtilis* on all three surfaces.

BOARD 2

Purification of Phage-Displayed HSA-Specific Peptide for Biosensor Production

Alexander D. Huber*[1], Jonathan Caguiat[1], Pedro Cortes[2], and Diana L. Fagan[1],
[1]Youngstown State University, Department of Biological Sciences, One University Plaza, Youngstown, OH 44555
[2]Youngstown State University, Department of Chemical Engineering, One University Plaza, Youngstown, OH 44555

The goal in our lab is to produce a biosensor that can detect blood, which can be used by the police or military. This biosensor would be able to detect a specific component of blood, human serum albumin (HSA), and send a distress signal for help. The specific goal of this study is to purify a heptapeptide, BR-1, from a M13 filamentous phage that was obtained through phage display. HSA was used as the bait protein for this process, therefore this peptide has a high affinity to HSA. The affinity of this peptide for HSA was confirmed by performing an Enzyme-linked Immunosorbent Assay (ELISA). The peptide was previously sequenced in this lab, and this sequence was utilized for this experiment. Primers were designed for a polymerase chain reaction process that incorporated the peptide sequence into the expression vector pMal-c5x, and this DNA will be subsequently purified, ligated, and sequenced again to confirm the presence of the peptide sequence in the vector. Next, this vector will be transformed into E. coli, and will be induced to express this peptide that is fused to a maltose-binding protein (MBP). The peptide will subsequently be purified from the MBP using an amylose resin column. After this process is complete, the affinity of this purified peptide can be tested

by performing another ELISA. Future efforts will be to use the peptide that our lab purified and incorporate it into a biosensor that can detect HSA.

BOARD 3

Characterization of two *Idiomarina* sp. isolated from cathodes and capable of solid substrate oxidation

Jorge Vinales*, Annette Rowe
Department of Biological Sciences, University of Cincinnati

The transfer of electrons to and from solid-substrates and cells, a process termed extracellular electron transport (EET), has remodeled our understanding of microbial physiology as well as the potential for technologies at the microbe-electric interface. While many mineral-reducing organisms have been described, very few mineral-oxidizing organisms have been characterized and as such, this metabolism is poorly understood. To better understand oxidative-EET, we isolated a variety of microbes on cathodes incubated in marine sediment taken from Catalina Island. This work focuses on the characterization of two of the strains isolated that display unique electron uptake characteristics on cathodes. Phylogenetic analysis of the 16S rRNA gene of strains SN11and FeNw1 show them belonging to the genus *Idiomarina*. SN11 is most closely related to *Idiomarina Loihiensis* L2-TR^T (99.77% sequence similarity), and FeN1w-70 is most closely related to *Idiomarina Maritima* (98.21%). Draft genomes of these microbes have been assembled. Analysis of the G + C content showed a value of 47—48% among all species. Average nucleotide identity of the genomes showed a similarity of 97.46% between SN11-90 and *I. Loihiensis* L2-TR[2] and 73.49% similarity between FeNw1-70 and *I. Maritima.*To further test the metabolic capabilities or these microbes, as well as compare electrochemical activity across these closely related *Idiomarina* strains, we have developed a various mediums that allow all of these strains to grow aerobically. We have been culturing these species under varying conditions using these media. Culturing methods have included using carbohydrates, amino acids, and sulfur species as electron donors.

BOARD 4

Interactions between *Bordetella pertussis* biofilms and host immune components

Audra R. Fullen*[1], Purnima Dubey[1], Rajendar Deora[1]
[1]Department of Microbial Infection and Immunity, The Ohio State University Wexner Medical Center

Pertussis, or whooping cough, is a re-emerging infectious disease caused by *Bordetella pertussis*, despite high vaccination coverage. Biofilms are sessile microbial communities which are enclosed in an extra-polymeric matrix to provide protection from host immunity. We hypothesize that biofilm formation by *B. pertussis* enables escape from immune defenses, resulting in persistence, transmission, and continued circulation of the bacteria. Bps (*Bordetella* polysaccharide) is antigenically and biochemically similar to the poly-β-1,6-N-acetylglucosamine (PNAG) family of polysaccharides, and is essential for *B. pertussis* biofilm formation *in vitro* and *in vivo*, as well as colonization of murine respiratory organs. We sought to determine the role of Bps in resistance to key host innate immune components known to be found in the respiratory tract. Compared to its isogenic wild type strain, the Δ*bps* strain was more susceptible to killing by polymyxin B and LL-37, as well as hydrogen peroxide, hypochlorous acid, and complement. We have recently shown that, compared to the prototypical laboratory-adapted strain (Bp536), currently circulating strains of *B. pertussis* exhibit an enhanced biofilm forming capacity. We show herein that many of these circulating strains produce

higher amounts of Bps. Furthermore, we show that one representative circulating strain is able to resist killing by each of the innate immune components better than Bp536, in both planktonic culture and established biofilms. These results will lead to a further understanding of interactions of *B. pertussis* with host immunity to determine how fitness differences in strains can result in infection and disease. Unravelling mechanisms by which *B. pertussis* continues to persist and circulate will lead to better therapeutic strategies and, ultimately, more efficacious vaccines.

BOARD 5

Evaluating the Efficacy of Newly-created Antimicrobial Peptides Using Confocal Fluorescence Microscopy

Louis Ray, **Kailey Christman*, Garrett Parker*,** and Michael Konopka
The University of Akron, Department of Chemistry

The growing threat of antibiotic-resistant bacteria continues to drive the investigation and development of new antimicrobials. Antimicrobial peptides show the potential to kill pathogens by destabilizing the inner membrane. Our work will investigate the efficacy of several newly-synthesized antimicrobial peptides (AMPs). These AMPs are positively charged, and consist of a polyester backbone with varying functionalization. We will use RedoxSensor™ Green (RSG), a redox sensitive dye, to monitor antibiotic action with confocal fluorescence microscopy. Cells were pre-stained with RedoxSensor Green and allowed to equilibrate with the dye prior to antibiotic treatment. Images will be recorded of the cells at intervals of 15 seconds per frame prior to and during antibiotic exposure to achieve nearly real-time imaging. The efficacy of each antibiotic at a given concentration will be determined by measuring the time until a five-fold increase in RedoxSensor Green signal. The efficacy of the antibiotics will be compared to conventional antibiotics such as carbenicillin and polymyxin B. Additionally, the interaction of these AMPs and *E. coli* cell membranes will be investigated through a fluorescently labeled variant of the AMPs.

BOARD 6

Immune evasion by *Neisseria gonorrhoeae* using human complement regulatory proteins

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

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

Neisseria gonorrhoeae (Ngo) is the causative agent of gonorrhea, the second most common bacterial sexually transmitted infection worldwide. Antibiotic resistance to Ngo is growing rapidly, even affecting countries with advanced healthcare systems. The World Health Organization (WHO) published a report labeling gonorrhea as an imminent threat due to its high levels of antibiotic resistance. If gonorrhea is left untreated, it can cause sterility, ectopic pregnancy and pelvic inflammatory disease. Many times, Ngo is not easily noticed due to its ability to cause asymptomatic infections. Some individuals infected asymptomatically are pharyngeal carriers. Ngo also has a high rate of infection, with more than 800,000 cases in the US alone. Currently there is no vaccine. Research efforts are focused on vaccine development and other preventative therapeutics.

Ngo may cause infection by attaching to human epithelial cells in the urogenital tract. Ngo persistence mechanisms in humans frequently allow evasion of human immune defenses such as the complement cascade. Our research focuses on complement regulatory proteins, which are proteins that our cells express to prevent autoimmunity. Our data suggests that Ngo is stealing complement regulatory proteins, like CD46, to prevent immune activation, allowing the bacteria to persist and cause infection. To test this, we are targeting genes, both human and bacterial, on opposing sides of this interaction to see if the bacteria become more susceptible to human complement immune defenses. We hypothesize that blocking the interaction between Ngo and human-cell associated complement inhibitors will result in decreased bacterial survival. The aim of this research is to find alternative therapeutics as opposed to antibiotics which are declining in efficiency. The data that we have collected may contribute to finding alternative therapeutics that prevent Ngo from recruiting human proteins during infection.

BOARD 7

Disruption of peroxisome function reduces iron scavenging and virulence in the human fungal pathogen *Histoplasma capsulatum*

Peter J. Brechting*, Qian Shen, Matt J. Beucler, and Chad A. Rappleye
Department of Microbiology, Ohio State University

The fungal pathogen *Histoplasma capsulatum* causes pulmonary and systemic infection among both immunocompromised and immunocompetent individuals. During infection, *H. capsulatum* is found almost exclusively inside the phagosomal compartment of macrophages. A forward genetic screen for *H. capsulatum* mutants which were unable to replicate in macrophages identified four peroxisome-associated genes (*PEX5, PEX10, PEX11, PEX33*) suggesting an important role for peroxisomes in *H. capsulatum* virulence. Peroxisomes are organelles found in nearly all eukaryotic organisms, but their functions beyond fatty acid oxidation are largely unknown. The β-oxidation and glyoxylate pathways as well as the peroxisomal catalase CatP are dispensable for *H. capsulatum* infection, indicating a novel function for peroxisomes in virulence. Pex5, Pex10, and Pex33 comprise the import machinery for peroxisomal proteins, which are targeted to the organelle by a peroxisomal targeting sequence (PTS1). Accordingly, a PTS1-tagged GFP fails to localize to peroxisomes in *pex5*, *pex10*, and *pex33* mutants. Peroxisome-targeted GFP in one of the four peroxisomal-associated gene mutants, *pex11,* still localizes to intracellular compartments, suggesting that Pex11 contributes to virulence without total disappearance of the organelle. Among PTS1-containing proteins are enzymes involved in siderophore biosynthesis, which *H. capsulatum* uses to acquire iron in iron-limited conditions in the host. Growth of the *pex5, pex10* and *pex33* mutants was inhibited in low iron conditions at a level similar to that of a siderophore knockdown strain, linking peroxisomes to siderophore production. However, the *pex11* mutant showed near wild-type growth in the presence of an iron chelator. Additionally, supplementation with high iron rescues the virulence of siderophore-deficient yeasts, but not peroxisome-deficient yeasts. These results indicate that the virulence function of the peroxisome is not limited to siderophore synthesis. Future efforts will be directed towards isolating the peroxisomes of pathogenic phase yeasts and using proteomics to elucidate candidate peroxisomal matrix enzymes involved in *Histoplasma* virulence.

BOARD 8

Presence of Megaviruses from Diverse Icelandic Environments

Delanie Baker*, Laura Tuhela, and Surendra Ambegaokar
Ohio Wesleyan University

The proposed Megavirales order comprises members of the previously known nucleocytoplasmic large DNA viruses (NCLDVs). Virus families in the Megavirales order include Poxviridae, Ascoviridae, and the recently explored families of megaviruses infecting free living amoeba such as Mimiviridae, Marseilleviridae, and Pandoraviridae. Megaviruses have been isolated from water and soil samples from Chile, France, India, and the United States. We chose to study the occurrence of megaviruses in Iceland because of the diverse habitats all within one island. No research has been carried out on the presence of megaviruses in Iceland. Samples of water and soil were collected from lava fields, moss/lichen mountain fields and mixed forestry plantations. *Acanthamoeba castellanii* (ATCC #30010) at a $5x10^5$ cells/ml concentration was used in an amoebal co-culture. A 1:10 dilution of soil or water sample preparation was added to each well. Cell confluence estimates were taken immediately after inoculation and plates were incubated at 28ºC. After 72 hours, wells containing mixed forestry plantation soil saw 48% lysis, followed by 44% lysis in wells containing lava field soil, and 59% lysis in wells containing moss/lichen mountain field soil. The co-cultures were subcultured onto *A. castellanii* plus antibiotics at a 1:10 dilution. Percent lysis for wells containing mixed forestry plantation soil, lava field soil, and moss/lichen mountain field soil was 19%, 22%, and 31% respectively. These data suggest *A. castellanii* may be infected with viruses, potentially megaviruses, originating from the various samples.

BOARD 9

A Novel Mutation Located In the C-terminal Cytoplasmic Domain of The CCR5 Gene With Potential Effects On HIV Infectivity

Ashley E. Innes*[1,2], Audra L. Fincham[1,2] Jabari Fuentes[1,4], Matthew Fox[1], Savannah L. Herbert[1,3], Harry W. Kestler
[1]Lorain County Community College, Elyria, Ohio;[2]Bowling Green State University; [3]Baldwin Wallace University;[4]Lorain County Community Early College High School

A novel mutation in the *ccr5* gene, which codes for the CCR5 protein, was found in one of 5 children in an African-American family. The mother contracted HIV before the birth of her first child in the early 1980s before HIV testing was widely used. The CDC did not recommend testing pregnant women until 1995. She unknowingly exposed all five of her children to HIV through vaginal birth. Out of the five children, the second born did not acquire the infection even though she was exposed to the virus at birth. The other 4 children were HIV infected. The mother and the infected children all carried genetically related HIV and the similarity was inversely correlated with birth order. A missense point mutation was discovered in the carboxyl terminal cytoplasmic domain of the uninfected child's *ccr5* gene. The mutation (TG5), changes a lysine codon at position 314 into an arginine codon. The allele containing the TG5 mutation was amplified by polymerase chain reaction. The DNA fragment containing the mutated TG5 gene was sub-cloned into pLXSN, a 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 the T-cell line H9 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 determined.

BOARD 10

Isolating phage on *Pseudomonas* plant and human pathogens

Alexandra Lake*, **Kaylee Wilburn***, and Hans Wildschutte
Department of Biological Sciences, Bowling green State University

It is predicted that 10 million deaths will occur worldwide by 2050 due to multi-drug resistant bacterial infections. Antibiotics were once the therapy of choice to treat infections but the overuse and misuse of these drugs has selected for the evolution of multi-drug resistant (MDR) pathogens. As antibiotic resistance increases, the discovery of new antibiotics and their approved for use decreases. Furthermore, recent data from the Wildschutte Lab suggests that there are few natural antibiotics to be discovered from bacteria that are effective against MDR pathogens necessitating the use of different treatments. Bacteriophage therapy has been successfully used to treat infections for over 100 years in countries other than the US and represents an alternative treatment against MDR pathogens. In this research, we optimized a protocol to isolate and purify phage that antagonize the plant pathogen *Pseudomonas syringae* acquired from atmospheric and leaf samples. Sixteen different *P. syringae* phage have been purified and their diversity verified through restriction digest, and subsequently stored as a stable lysate prior to genome sequencing. Currently, we are optimizing the protocol to isolate phage on MDR *Pseudomonas aeruginosa* obtained from the sputum of cystic fibrosis (CF) patient lungs. Of CF patients, 90% will harbor *P. aeruginosa* MDR infections in their lungs by the age of eighteen, and many will die from complications by their infection. Ultimately, we plan to use the phage to rescue human and plant infections of *Pseudomonas* pathogens. As the crisis of antibiotic resistance worsens, this data will be increasingly beneficial to health and survival against infections no longer susceptible to antibiotics.

BOARD 11

Investigation into the Target and Mechanism of Action of the Novel Antifungal 41F5

Kristie Goughenour*[1], Chad Rappleye[1], and Craig McElory[2]
[1]Department of Microbiology, Ohio State University, Columbus, OH
[2]College of Pharmacy Division of Medicinal Chemistry and Pharmacognosy, Ohio State University, Columbus, OH

Pathogenic fungi present a particular challenge for therapeutics development due to the shared eukaryotic nature of host and pathogen. Phenotypic screening of a small molecule library identified an aminothiazole compound, 41F5, with antifungal activity against two important fungal pathogens *H. capsulatum,* and *C. neoformans*. Importantly, 41F5 exhibits very low cytotoxicity to mammalian cells including macrophages, the host cells for *H. capsulatum.* 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 line has at least a 50-fold increase in 41F5 MIC with no significantly increased resistance to azole-class antifungals, suggesting their resistance does not derive from general drug resistant responses. Whole genome sequencing of the 41F5-resistant strains identified SNPs associated with drug resistance and validation of targets

gene(s) by ectopic over-expression of resistance-associated alleles and testing for increased resistance to 41F5 is currently ongoing. In addition, a biochemical approach to target identification using a non-tagged HPLC-MS/MS method for identifying 41F5 and co-fractionating proteins provides a more direct identification of drug-protein interactions.

BOARD 12

Extension of Tiny Earth: Bioinformatic analysis of two similar strains isolated a year apart by different TE students

Kayla Heimlich*, Marissa Kljun*, Haley Shore*, and Hans Wildschutte
Department of Biological Sciences, Bowling Green State University

Tiny Earth (TE) is a crowdsourcing citizen science network that engages students in real microbiology research with the goal of discovering new antibiotics. Unfortunately, pharmaceutical companies have stopped drug discovery due to low profitability, thus slowing the development and availability new antibiotics. Because TE has been implemented in 100s of colleges and universities worldwide, this crowdsourcing strategy has the potential to facilitate novel drug discovery. In the first semester of TE, students collect environmental soil samples, isolate bacteria, and perform an antagonistic assay to test for inhibition against the ESKAPE pathogen's "safe" relatives. Traditional TE courses use chemical techniques to extract compounds, however this method has proven to be difficult since no drugs have been isolated or characterized. At BGSU, we developed a transposon module to facilitate antibiotic production and extended TE to a second semester for bioinformatic research. Using mutagenesis, genes that encode antibiotics can be identified by aligning the arbitrary PCR product from the transposon mutant to the whole genome sequence of the wildtype strain. Using this data, bioinformatic analyses can be employed to characterize the antagonistic strain and genes involved in drug production. Here, we analyzed the genomes of two strains isolated on the campus of BGSU by TE students one year apart. Both strains are closely related based on the 16S rRNA phylogenetic gene tree and more than 99% identical based on average nucleotide identity of the whole genome. Differences in strains were attributed to integrated phage. Together, through a second semester of bioinformatics, we were able to analyze and compare genomes isolated through TE.

BOARD 13

Examining hliA Primer Sets for *Planktothrix agardhii*

Eleana Cintron,*[1] Dulce Cintron[1], Mia Diaz[1], Andy Nageotte[1], Paul Warkentien[1]
Michelle Neudeck[1,2], Kathy Durham[1]
[1]Lorain County Community College; [2]Bowling Green State University

Within photosynthesis in cyanobacteria the photosynthetic apparatus can be harmed during high light stress from oxidative damage. Cyanobacteria contain genes that can encode for proteins that protect during this high light stress one of which is called *hliA*. The purpose of this experiment is to look at the *hliA* gene within the cyanobacterial species *Planktothrix agardhii* and find optimal temperatures for six forward and reverse primers sequenced for this gene by method of gradient temperature PCRs. Gel electrophoresis was used to confirm the optimal temperature for each primer, and these primers will be used within future experiments with different manipulated environmental variables to measure *hliA* gene expression. Furthermore, primer sets will be used during DNA and RNA extractions to isolate the *hliA* gene within P. *agardhii* and receive the transcriptions that will show the stress on the cells during high light.

BOARD 14

Monitoring formation of intracytoplasmic membranes in *Methylotuvimicrobium alcaliphilum* comb.nov. 20Z, utilizing confocal fluorescence microscopy

James Stouffer*, Ravindra Gudneppanavar, and Michael C. Konopka
The University of Akron

Methanotrophic bacteria can utilize methane as the sole source of carbon and energy. These bacteria achieve this through an enzyme known as methane monooxygenase (MMO). There are two forms of this enzyme, soluble (sMMO) and particulate (pMMO) methane monooxygenase which can be found in the cytoplasm and membranes respectively. Our research focuses on *Methylotuvimicrobium alcaliphilum* comb. nov. 20Z, a Type I methanotroph which has only pMMO. Its catalytic site consists of copper, and it is known that intracytoplasmic membranes (ICMs) that form in these bacteria is linked to copper levels.

This research focuses on rapidly visualizing the formation of ICM over copper introduction using confocal fluorescence microscopy over a period of time. Fluorescence microscopy methods with a styryl membrane staining dye has the advantage of live cell imaging and monitoring ICM formation in real time. We describe the increase in ICMs over time at a single-cell level upon the addition of copper.

BOARD 15

Screening 'Knockout Sudoku' generated *Shewanella oneidensis MR-1* Mutants on a Cathode: What Genes Are Involved in Electron Uptake?

Leah R. Trutschel*[1], Buz M. Barstow[2], Annette R. Rowe[1]
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The mineral-reducing microorganism, *Shewanella oneidensis MR-1* is an important model organism for the study of extracellular electron transfer (EET)—the ability for an organism to either deposit and/or take up electrons from outside the cell, often from solid phase minerals or electrodes. The Mtr respiration pathway, which is composed of several c-type cytochromes spanning the inner and outer membranes in *S. oneidensis*, is known to be the main enzyme pathway involved in EET to minerals and/or electrodes and is essential for these processes. The reversibility of the Mtr pathway has led to the hypothesis that this group of proteins is also involved in the electron uptake phenotype observed in *S. oneidensis* on cathodes. However, testing of gene deletion mutants from the Mtr pathway, as well as other putative outer membrane proteins and cytochromes, highlighted the potential for a more complex network of proteins to be involved in cathode oxidation (Rowe, 2018). In order to assess what other genes may code for components of the *S. oneidensis* electron uptake pathway, we capitalized on a previously constructed whole genome knock out library (Baym et al, 2016). From this pool of mutants, 24 strains were deemed of interest and many have already been or are currently being screened on a cathode to assess their electron uptake capacity using a solid phase substrate. To this aim, these *S.oneidensis* mutants are grown in biological reactors containing a 3-electrode system. Comparing these mutants with the wild type *S. oneidensis,* 5 so far have demonstrated a marked reduction in electron uptake (40-50% reduction). Finding the genes that contribute to electron

uptake from electrodes is of particular interest to those investigating the systems biology of *S.oneidensis,* and to those interested in a variety of potential applications in microbial fuel cells and biofuel production.

Annette R. Rowe, Pournami Rajeev, Abhiney Jain, Sahand Pirbadian, Akihiro Okamoto, Jeffrey A. Gralnick, Mohamed Y. El-Naggar, Kenneth H. Nealson
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Michael Baym, Lev Shaket, Isao A. Anzai, Oluwakemi Adeshina, Buz Barstow, *Nature Communications* **volume 7**, Nov 2016, **DOI**: 10.1038/ncomms13270

BOARD 16

Money Laundering: An effective way to disinfect paper currency

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Community-acquired microbial infections affect susceptible populations and can lead to death. Currency bills change hands constantly and have been shown to act as fomites for microbes. They transmit pathogens and pose a global risk, especially to the sick, immunocompromised patients and the elderly. Robust methods to eliminate the contagion threat are non-existent. Treasuries focus on countering fraudsters and bill-durability and not on microbial threats. Here we test the efficacy of a novel UV-based device we have designed to sterilize currency-bills and neutralize the threat. First, currency bills from local sources, restaurants, groceries, gas stations and retail stores (Study-Group) were compared with fresh notes from a bank (Control-Group) to test whether microbes exist on bills and what types are commonly detected. Samples were obtained under sterile conditions and microbial culture in a CO2 incubator was performed. One hundred percent bills of the study-group were positive for microbes and only 33% of controls. Fungus, yeasts, molds, *Escherichia coli* and *Staphylococcus aureus* were detected. Next, a prototype for a new device, named “The Money-Launderer” (TML, patent application pending), was designed to sterilize currency bills. Structurally, the TML comprised a wooden case and fit 3 currency bills. An ultraviolet(c) bulb, fixed diagonally to the top, ensured uniform radiation. Contaminated bills were exposed for 1,3 and 10 minutes and re-examined for microbes after each time-point. Results were quantified and statistically analyzed using the Fisher two-tail Test. Seventy-eight % of bills, exposed to UV-rays for 10 minutes, were completely sterile (90% confidence; $p < 0.05$). In conclusion, the TML could sterilize most contaminated currency bills under optimal conditions, and mitigate their infective threat.

BOARD 17

Expanding Tiny Earth to bioinformatics: characterization of a biosynthetic gene cluster involved in antagonistic activity

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Tiny Earth (TE) is a student crowdsourcing mechanism that is utilized by over a 100 universities across the country to engage students in authentic microbiology research with a common goal of antibiotic discovery. During the first semester, students collect soil samples, isolate bacteria, test for antagonistic activity, and identify the relatedness of strains by constructing a phylogenetic tree based on the 16S rRNA gene. At Bowling Green State University, a transposon mutagenesis module was

developed for TE to identify the genes that could be involved in antibiotic production. Utilizing transposon mutants generated in class, TE was expanded to a second semester that uses bioinformatic techniques to identify specific genes that encode for antibiotic production in order to facilitate drug discovery. Here, we use strain SWI6 for bioinformatic analyses. SWI6 was originally isolated by a TE student, optimized for transposon mutagenesis, and mutants were obtained. Through bioinformatics, we identified the gene cluster involved in antagonistic activity, predicted functions of all genes in the cluster, and performed average nucleotide identity to determine the species. This second semester of TE has provided us, the undergraduate students, with an understanding of how genes involved in antibiotic production are discovered.

BOARD 18

A mouse model to study neisserial colonization in the upper respiratory tract

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Human infections caused by *Neisseria gonorrhoeae*, the causative agent of gonorrhea are often asymptomatic. How *N. gonorrhoeae* establish asymptomatic colonization is still poorly understood. Studying *N. gonorrhoeae* in laboratory animals is challenging due to strict host tropism. Recently, *Neisseria musculi*, a commensal from the oral cavity of wild mice was used to study *Neisseria* host interactions in laboratory mice.

Our goal is to use *N. musculi* to model asymptomatic colonization of *N. gonorrhoeae* in the upper respiratory tract. The genome of *N. musculi* encodes many orthologs of host interaction factors found in human pathogenic *Neisseria* species. We are investigating the function of *N. musculi* orthologs of human pathogenic *Neisseria* colonization factors including factors involved in biofilm formation. Mutants lacking *nagZ* and *lctP,* that encode a biofilm and colonization factor, respectively were created in two *N. musculi* morphotype backgrounds, smooth and rough. The role of these genes in the upper respiratory tract colonization were analyzed both *in vitro and in vivo*.

Our preliminary results show that deletion of *nagZ* does not affect *in vitro* static biofilm formation under the conditions we tested. Deletion of *lctP* alters static biofilms in the rough morphotype background. *In vivo,* we observed no significant difference in the upper respiratory tract colonization between a wild type and Δ*lctP* strains in the smooth morphotype background. However, we observed a defect in colonization with the rough Δ*lctP* strains compared to the wild types. Our model allows the study of orthologs of *Neisseria* host interaction factors shared with *N. gonorrhoeae* in a natural host. We believe that our model will allow identification and understanding of shared mechanisms used by *N. musculi* and *N. gonorrhoeae* to asymptomatically colonize the upper respiratory tract.

BOARD 19

Identifying and Characterizing Transcription Factors for Enhanced Limonene Production in Cyanobacteria

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Limonene, a C10 monoterpene produced via the methylerythritol (MEP) pathway, is a potential biofuel candidate. Direct CO_2-to-limonene conversion in cyanobacteria is an attracting route for biofuel production due to its carbon neutral property. However, limonene production in cyanobacteria has been limited in its efficiency due to the decreased carbon flux towards the MEP pathway. Traditional metabolic engineering by targeting MEP pathway enzymes has led to marginal increase in terpene yield. Targeting global transcription factors (TFs) that can regulate carbon flux between the MEP pathway and other carbon metabolic pathways could lead to an increase in the terpene flux in cyanobacteria. By engineering TFs associated with carbon flux regulation, we can rewire the carbon metabolism to increase the production of limonene via metabolic engineering. To identify potential TFs, the engineered limonene producing strain was grown under various conditions, such as differing light intensity and salt conditions. Cultures grown in low light and no salt produced significantly higher limonene compared to the cultures grown in high light and salt stress. Proteomics was performed to determine potential TFs contributing to the carbon flux towards or away from the MEP pathway. Several potential TFs have been identified from the proteomics data and being analyzed for their contribution to the MEP pathway.

BOARD 20

Determining Specificity of a Biological Moiety to HSA in the Development of a Blood Biosensor

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Previous work on developing a wearable blood biosensor for use in military and law enforcement has identified a short-chain peptide through phage display technology which binds to human serum albumin. To determine the specificity of binding to HSA, various enzyme-linked immunosorbent assays have been performed to elucidate the most efficient testing conditions and stringencies and to evaluate the peptide's viability for such a biosensor. The phage from which the peptide is derived, and the peptide itself, are evaluated both for affinity of binding to HSA and for specificity of binding to HSA over other substances that may be present during typical everyday use. Once the peptide's viability has been confirmed, it will be tested for attachment to carbon nanotubes. The resulting peptide/nanotube structure will then be tested for signal transduction, affinity, and specificity for HSA.

BOARD 21

The effects of CCR5 delta32 on surface expression of CCR5 and CXCR4

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Human Immunodeficiency Virus (HIV) is a virus that cripples the patient's immune system. HIV glycoprotein 120 first binds to CD4 and one of two secondary receptors, CCR5 or CXCR4 on the surface of a T-cell, allowing it to insert its genetic material into the host cell. HIV can be divided into three strains: virus that prefers CCR5 as a co-receptor (R5 virus), virus that prefers CXCR4 (X4 virus) and virus that can infect both strains X4/R5 (virus). All viral replication needs to be suppressed to achieve a cure.

A mutation known to affect HIV infectivity, CCR5 delta32, is a deletion mutation of 32 base pairs of the *CCR5* gene sequence. This mutation prevents surface expression of the CCR5 receptor when an individual is homozygous, conferring resistance to HIV. Timothy Ray Brown, the first HIV positive patient to be functionally cured, was treated by a bone marrow transplant from a homozygous *CCR5* delta32 donor. He was cured despite harboring X4 and R5 virus at the time of bone marrow transplantation.

Test subject GD1 was identified as heterozygous for *CCR5* delta32. PCR amplification from the subject was performed using primers that circumscribe the *CCR5* ORF. Both wild type and delta32 alleles were obtained from this individual. This product was ligated into the mammalian/prokaryotic shuttle vector, pLXSN. This DNA was then transformed into *E. coli* for amplification. Following this, the DNA was removed by means of plasmid prep and subjected to digestion with several restriction endonucleases and further PCR to confirm the presence of the CCR5 allele.

The next phase in research will consist of transfecting this CCR5/pLXSN vector into PT67, a retroviral packaging cell line. The resulting retroviral products will be used to create stable and transient transductants into H9 Lymphoid cells, which will be tested for HIV infectability and CCR5 expression.

BOARD 22

An extension of Tiny Earth: using bioinformatics to characterize gene clusters involved in antibiotic production

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Tiny Earth (TE) is an innovative STEM teaching strategy and crowdsourcing method for discovering novel antibiotics. Students enrolled in TE learn real microbiology research which includes isolating environmental strains of bacteria from soil habitats and performing a series of experiments to test for antibiotic production and pathogenic growth inhibition. BGSU has modified the traditional TE protocol by employing transposon mutagenesis to help identify potential antibiotic-producing biosynthetic gene clusters, rather than using biochemical techniques to purify antibiotic compounds. Mutants are generated by random transposon insertion events and screened for transconjugants that have lost the antagonistic phenotype suggesting the transposon disrupted a gene involved in antibiotic production. With the use of whole genome sequencing and arbitrary PCR, the location of the transposon was

verified, and bioinformatic analyses was performed to identify the potential gene cluster involved in antibiotic production. We identified a gene region in SWI7 that may be involved in antibiotic production and utilized other bioinformatic applications to identify the species, build a whole genome tree, and identify potential prophages. This second semester of bioinformatics was a unique and productive teaching method to engage students and teach current applications of bioinformatics.

BOARD 23

Characterizing a human fecal isolate capable of degrading choline in the human gut

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Gut microbiota metabolism can have profound effects on human health. Choline, a quaternary amine (QA) highly abundant in our diet, is cleaved by a glycyl radical enzyme, choline trimethylamine lyase (CutC), and its SAM dependent radical activator, CutD. CutC cleaves choline to form trimethylamine (TMA) and acetaldehyde. TMA in the gut is oxidized to TMAO by FMO3 in the liver, which plays a role in causing atherosclerosis. Our *hypothesis* is that gut microbes are capable of demethylating choline via anaerobic respiration. Our hypothesis has been formulated on the basis of evidence that *Desulfitobacterium hafniense* Y51, a strict anaerobe encodes a pyrrolysine-lacking trimethylamine:corrinoid methyltransferase (MtgB) that carries out demethylation of a QA, glycine betaine. MtgB is a member of the widespread COG5598 superfamily predicted to be involved in QA demethylation, of which there are many members within the human gut microbiota. Therefore, it is expected that certain gut microbiota can carry out respiration by demethylating QAs such as choline. Little is known about these demethylators and their metabolism in the human gut.

We have isolated, from a human fecal sample, a strain of *Citrobacter amalonaticus* capable of anaerobically utilizing choline as its sole carbon and energy source. The *C. amalonaticus* genomes available on NCBI encode no known enzymes for degrading choline, such as CutC/D. We are sequencing the genome of our strain to better establish its identity and to be able to better examine its metabolic potential. Protein BLAST results with a CutC from *Proteus mirabilis and Klebsiella pneumoniae* indicated the presence of a gene encoding a related GRE, formate C acetyltransferase. However, this is not likely acting as a CutC due to a substitution at the amino acid proline with leucine in the critical CutC motif GCVEP. Another possible route of choline degradation is demethylation to *N*,*N*-dimethylethanolamine, but no relevant methyltransferases are encoded in the published genomes. We are investigating how choline is being utilized by *C. amalonaticus* if not via CutCD clusters or a non-pyrrolysine COG5598 methyltransferase. In addition to sequencing the genome for this strain we are performing metabolomic and proteomic analyses. Characterizing the pathway of choline degradation by *C. amalonaticus*, a human fecal isolate, may demonstrate that this strain has obtained the genes, by horizontal gene transfer, necessary for choline dependent growth or possibly reveal a novel pathway of choline degradation in the human gut.

BOARD 24

Microbial exposures in the bedrooms of asthma patients

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We spend almost 90% of our time indoors and roughly 30% of our lives in our bedrooms. In these spaces we are exposed to a combination of chemicals and microbes that may influence our health. These exposures occur simultaneously, and interactions may occur, such as traffic pollutants contributing to respiratory allergen sensitization. The objective for this study was to determined associations between indoor exposures to microbes and chemicals in the bedroom and asthma control, focusing on sleep disruption. A total of 51 dust samples were collected from doorframes in bedrooms of adult women with asthma. DNA sequencing was performed to identify indoor fungal and bacterial species. qPCR was used to measure total fungal and total bacterial concentrations. Viral detection used novel methods to attempt detection of viruses in dust on a small subset of samples. Outcome variables of interest include asthma control measured by a standard asthma control test and a Mini Asthma Quality of Life Questionnaire (MiniAQLQ). Additional measurements include bedroom temperature and relative humidity, tobacco use, modifications to the home environment, and environmental exposures in work and daily life. Bacterial concentrations measured in our samples ranged from 879 cells per swab to 9.46 million cells per swab. Fungal concentrations ranged from 577 spore equivalents per swab to 1.15 million spore equivalents per swab. Temperature and relative humidity varied greatly from home to home. The mean temperature for all 51 homes ranged from 66.3°F to 88.7°F and the mean relative humidity ranged from 26.3% to 60.7%. A lower than average score on the MiniAQLQ, which indicates more asthma impairment, was associated with a higher fungal concentration (p-value=0.043). This work will inform future studies that seeks to explore the “indoor exposome” and the interactions between microbial and chemical exposures.

BOARD 25

Characterization of a *Pseudomonas stutzeri* strain from marine sediments capable of iron oxidation and electrode oxidation

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Little is known about the ability of microorganisms to utilize solid phase minerals in environmental systems, in part due to the limited number of model systems available. To this aim, we previously cultivated a wide range of microbes from a marine sediment capable of extracellular electron uptake from poised potential electrodes (Rowe et al. 2015). Strains of *Pseudomonas stutzeri*, an easily grown, cosmopolitan gram- negative bacteria, were isolated in this work, and have been highlighted as a potential model systems for understanding extracellular electron transport (EET) for electron uptake. Our preliminary characterization of this organism has investigated growth and cell density of this organism in minimal media with glucose. Using this system, we are investigating the range of temperatures, pH and salinities that are compatible with growth of this strain. Preliminary results suggest growth of this organism occurs from +10℃ to +35℃ temperature, and can grow in 5 pH to a basic pH. Further characterization across cathode isolated and the type strains of *Pseudomonas* we will investigate the ability of the organisms to grow on electrodes, inorganic minerals, and using alternate terminal electron acceptors (i.e. nitrate). Further understanding their growth physiology will

allow us to better utilize *P. stutzeri* as a model system for investigations of extracellular electron uptake.

BOARD 26

Quantification of microplastics in the Ohio River basin and investigation of impacts on zooplankton reproduction

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Plastic pollution has been a growing global issue since the production and widespread consumption of plastic began more than 60 years ago. Microplastics are plastic pieces or fibers (less than 5mm) that often contaminate aquatic systems and may impact zooplankton and other aquatic life. Zooplankton play and important role in aquatic food webs as primary consumers that ingest phytoplankton or other zooplankton, which can be similar in size to microplastics. This study quantified microplastics in the Black Fork and Clear Fork Rivers, and investigated the impact of microplastic exposure on zooplankton using *Daphnia magna* as a model organism. Riverine water samples were obtained to quantify and classify microplastics. As the Black Fork and Clear Fork Rivers feed recreational lakes (Charles Mill Lake and Pleasant Hill Lake, respectively), it was hypothesized that water samples downstream of the lakes would contain greater quantities of microplastics than upstream samples due to human impact on the lakes. Quantification yielded a concentration of 11.96-49.96 microplastics/L where fibers were the most abundant microplastic type. Controlled laboratory experiments using an environmentally relevant microplastic concentration and type showed that although *D. magna* do not ingest microfibers, their reproductive success is significantly decreased in the presence of the fibers. In order to better understand the impacts that microplastics have on zooplankton and other levels in the freshwater food web, further analysis in the natural environment is necessary.

BOARD 27

Effects of Light Wavelength on Growth and Stress in *Planktothrix agardhii*

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Planktothrix agardhii has become a recurrent nuisance of Sandusky Bay as the culprit of harmful algal blooms. Like its cyanobacterial relatives, the *P. agardhii* of Sandusky Bay are dependent on photosynthetic pigments. Under high light intensities, these pigments destabilize, leading to an increased chlorosis rate. However, many cyanobacterial species, including *P. agardhii,* possess genes that maintain pigment structure under stress, ultimately delaying chlorosis . *hliA* is a gene that, when expressed, preserves pigment structure under high-light conditions. *P. agardhii* has been observed to undergo severe stress under intense white light conditions, but habitability under varying light conditions remains to be examined in respects to *hliA* expression. In order to investigate its response to variations in wavelength of light, five cultures of *P. agardhii* and a mixed culture from Sandusky Bay will each be grown on BG-11 media and under red, yellow, green, blue, and white light conditions for 14 days or until chlorosis is overwhelming. Cell counts and RNA extraction from each culture will be performed, the latter of which will undergo RT-PCR with an *hliA* primer for gene expression analysis.

BOARD 28

Characterization of a reductive activase from *Methanosarcina barkeri*

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The archaeal methanogen *Methanosarcina barkeri* is capable of reducing methyl groups from select methylamines to form the potent greenhouse gas methane utilizing three-component methyltransferase systems that are dependent on corrinoid-binding proteins. The cobalt ion in the corrinoid cofactor must cycle between the methylated Co(III) state and the unmethylated reduced 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 thus 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. We have characterized RamA to better understand its role in rescuing methyl transfer and have determined that RamA can catalyze the reductive activation of the corrinoid protein MttC at a rate of 8.8 ± 0.26 min^{-1} under optimized conditions. The Km of RamA for ATP is 0.25 ± 0.030 mM. Furthermore, gel filtration experiments indicate that RamA is a monomer in solution and will bind to a single corrinoid protein in the Co(II) redox state in the absence of ATP. Although RamA is dependent on ATP to catalyze reduction, its ATP hydrolysis activity is highly gated and occurs primarily in the presence of oxidized Co(II) corrinoid protein and a source of electrons. These aspects further set RamA apart from activating enzymes found in bacterial three component methyltransferase systems, which share homologous ATP binding domains but have highly dissimilar ferredoxin domains.

BOARD 29

Environmental *Pseudomonas* strains inhibit cystic fibrosis derived multi-drug resistant pathogens

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The emergence of pathogens resistant to all known antibiotics has become a serious threat to our healthcare system. In 2050, deaths from antibiotic resistant bacteria are predicted to become the leading cause of death worldwide, reaching over 300,000 per year in the U.S. alone. Unfortunately, pharmaceutical companies have stopped research and development of new drugs due to low profitability, so university research labs have begun leading efforts to discover new compounds. In concordance with this effort, the Wildschutte lab uses environmental *Pseudomonas* spp. as a source for novel antibiotics. We hypothesize that pathogenic bacteria have adapted to their human host, and as a result, exhibit a decreased fitness when competed against wild bacteria from ecological environments that pathogens likely do not encounter. To support this hypothesis, we isolated environmental *Pseudomonas* strains from different habitats and tested for their activity against a panel of drug resistant pathogens. Specifically, 192 of environmental *Pseudomonas* strains were isolated from soil in downtown Indianapolis, IN and water from an Indianapolis Canal. We generated a phylogenetic tree using the *gyrB* gene to determine the relatedness of the strains and if certain groups correlate to antagonistic activity. The environmental strains were competed against a panel of 25 multi-drug resistant pathogens isolated from the lungs of cystic fibrosis patients. In total, nearly 300 of these interactions were inhibitory, suggesting that the environmental strains represent an

underutilized source of antimicrobial factors. Some environmental strains were shown to inhibit a number of pathogens and will be optimized for using transposon mutagenesis to uncover the underlying genetic elements responsible for inhibition. Through this process, we will identify putative metabolites capable of inhibiting the growth of human pathogens

BOARD 30

Glycogen synthesis jump-starts photosynthesis through the G6P shunt in cyanobacteria

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An important route for photosynthesis fixed carbon is to store as glycogen. The photosynthesis output 3-phosphoglycerate (PGA) is an activator for the ADP-glucose pyrophosphorylase (AGPase), a key enzyme for glycogen synthesis. When photosynthesis starts, PGA accumulation would activate glycogen synthesis, diverting carbon toward a high-flux metabolic process. Glycogen synthesized in the light can be used for cellular respiration in the dark. Photosynthesis carbon fixation and cellular respiration are closely linked to each other through many common metabolites. In cyanobacteria, many electron carriers in the thylakoid membrane are also shared between the light reactions and oxidative phosphorylation. Two previous studies have shown that glycogen mutant had delayed activation of the Calvin-Benson cycle. Understanding on the underlying mechanism is lacking. In our study, we found that glycogen synthesis helps jump-start photosynthesis in *Synechococcus elongatus* PCC 7942. This ingenious strategy acts as a buffering system for photosynthesis and could ensure growth advantage for cyanobacteria in light limited conditions.

BOARD 31

Enterococcus species Isolated from Lake Newport in Mill Creek Park

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Several lakes located in Mill Creek Park, Youngstown, Ohio, were closed to recreational use in 2015 due to raw sewage overflows resulting in high levels of fecal bacteria. The present, more recent study monitored levels of *Enterococcus*, an accepted standard to assess water quality, at regular intervals from samples collected over several months from Lake Newport in Mill Creek Park. The number of bacteria consistently exceeded public health standards. In addition, molecular-based assays were employed to confirm which species of *Enterococcus* were isolated and if any possessed genes associated with vancomycin resistance. The results presented here show that *Enterococcus* species in addition to *E. faecalis* were recovered. Of the 44 isolates examined, only 17 were determined to be strains of *E. faecalis*, whereas 7 others were identified as either *E. hirae*, *E. gallinarum*, or *E. casseliflavus/flavescens*. Interestingly, no isolates of *E. faecium* were detected. The remaining 20 isolates could not be speciated with the molecular assay employed. However, biochemical characterization indicated that these isolates were enterococci. With regard to the detection of vancomycin resistance genes, six unknown enterococcal species possessed the VanC2 gene as did the lone isolate identified as *E. casseliflavus/flavescens*. One isolate of *E. gallinarum* possessed the VanC2 gene and another the VanA gene. Kirby-Bauer assays of 29 isolates detected 10 that were vancomycin resistant. Of these, only two occurred in strains identified as harboring a VanC2 (unknown *Enterococcus* species) or VanA (*E. gallinarum*) gene. The remaining vancomycin-resistant isolates included *E. faecalis* (7) and *E. hirae* (1). In summary, Lake Newport continues to appear

polluted with fecal material, but the species distribution of *Enterococcus* may partly originate from the wildlife that inhabits the area. Caution should be exercised in the recreational use of this lake, particularly given the presence of vancomycin-resistant genes among the *Enterococcus* isolates recovered.

BOARD 32

Succession of heterotrophic bacteria in enrichment cultures of ammonia-oxidizing bacteria

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The microbially mediated process of nitrification is responsible for the conversion of ammonia to nitrate. Chemolithoautotrophic ammonia-oxidizing bacteria (AOB) are responsible for the first step of nitrification in which ammonia is aerobically oxidized to nitrite. While generating energy from the oxidation of ammonia to nitrite, AOB fix carbon dioxide into organic carbon via the Calvin cycle. In natural systems, AOB co-exist and interact with heterotrophic bacteria. It is well known that AOB grow better in the presence of heterotrophic bacteria in enrichment cultures as well as natural systems. However, the impact of AOB on the composition and succession of the surrounding heterotrophic community is not well understood. Here, we present a project studying the succession of the heterotrophic community associated with different AOB in enrichment cultures from three different freshwater systems: Lake Acton (ACW), Lake Superior (SUP), and Lake Drontmeer (G5-7). The cultures were inoculated in mineral salts media in batch culture with 2.5mM NH_4^+. $NH_4^+$and NO_2^- were regularly measured. DNA was isolated from the cultures at different time points to determine the changes in the microbial community during the growth of the AOB. 16S rDNA paired-end amplicon sequencing was used to determine the identity and relative abundance of the AOB and associated heterotrophs. The cultures ACW and G5-7 contained AOB belonging to the genus *Nitrosomonas*, while SUP contained an AOB from the genus *Nitrosospira*. Most heterotrophic bacteria in the enrichment cultures belonged to *Alpha-* and *Gamma-Proteobacteria* and *Bacteroidetes*. The dominant heterotroph in all enrichment cultures was *Pseudomonas* spp. All three communities exhibited changes in the relative abundance of the heterotrophic community corresponding to the growth and decline in substrate resulting in shifts of the overall community composition. These changes indicate that the chemolithoautotrophic AOB can sustain a heterotrophic community and that these communities undergo successional changes during the growth of AOB.

BOARD 33

Automated Quantification of Biofilm-Related Phenotypes in Fungal Species

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Biofilm formation in fungi promotes persistence, pathogen dissemination, and recalcitrance to antifungal drugs and immune surveillance. The process of biofilm formation in *Candida* species proceeds sequentially with adherence of yeast cells to a surface, invasion into the substrate, initiation of aerial projections, deposition of extracellular matrix, and then release of disperser cells. Interference with the ability to adhere, invade, or filament can prevent formation of a robust biofilm leading to community fragmentation and clearance. Consequently, significant effort has been directed

to identifying the genetic determinants of biofilm development although the methods used for analysis are typically highly qualitative and lack power. Here, we describe an informatics pipeline for image quantification of filamentation, adhesion, and invasion of colonies on solid agar media plates. The constructed scripts can phenotype individual colonies from full plate images across *Candida* species and media types making it highly adaptable for assessing fungal colony growth and biofilm-associated phenotypes. The approach was applied to quantification of filamentation, adhesion, and invasion for 10 *Candida* clade species including 23 *Candida albicans* clinical isolates across three conditions ranging from nutrient-rich to minimal media. Our approach increased reproducibility of *C. albicans* filamentation by 3-fold compared to previous investigation of these strains. Furthermore, quantification of defects in adhesion, invasion, and filamentation did not additively predict biofilm formation under these conditions. While some conditions produced consistent phenotypes across all *C. albicans* strains, other conditions yielded strain-specific effects, highlighting how underlying genetic diversity across the species contributes to phenotypic plasticity. Taken together, development of a high-throughput, unbiased approach to score *Candida* biofilm-related phenotypes on solid media can facilitate the genetic dissection of these critical processes to pathogenesis.

BOARD 34

The role of *P. aeruginosa* exopolysaccharide Psl in antagonizing *S. aureus*

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Chronic infections in Cystic Fibrosis (CF) patients remain a problem. The ineffective clearing of the lung mucus provides a habitat for bacteria colonization. The airways of CF patients are typically colonized with bacteria *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA). It is known that co-infection causes more severe disease outcomes than mono-infection, so it is of great importance to study the interaction between these two pathogens. Biofilms are key factors contributing to PA and SA caused chronic infection. One of the key exopolysaccharides PA produces is Psl. It mainly functions in initiating biofilm formation and maintaining biofilm structures. There are two forms of Psl, cell-associated form which is bound to the cell membrane, and cell-free form which is released into the outer environment.
The **broad objective** of this project is to identify Psl's function in antagonizing SA *in vitro* and *in vivo*. Our preliminary data revealed that cell-free form of Psl played an important role in modulating PA antagonism towards SA in planktonic co-culture *in vitro*. Our **hypothesis** is that Psl mediated antagonism towards SA is due to one or some of the following mechanisms: 1) Psl itself is bactericidal and can cause SA autolysis; 2) Psl can signal gene expressions of PA virulence factors including PQS, LasA and others that are known to antagonize SA; 3) Psl can bind and sequester iron from SA. Future directions are to apply the findings in planktonic culture to biofilm and eventually to animal models. A better understanding in how these two pathogens coexist and cooperatively infect CF patients will help us better understand the progression of chronic lung disease in CF patients and provide new strategies for therapeutic exploitation of the CF lung microbiome.

BOARD 35

Isolation and Initial Characterization of Pseudomonas-Specific Bacteriophages from Soil

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The study of host-pathogen interactions afforded by investigating bacteria in the genus *Pseudomonas* and *Pseudomonas*-specific bacteriophages could facilitate the identification of novel enzymes with broad biological applicability. Although many bacteriophages specific to the human pathogen *Pseudomonas aeruginosa* have been identified and characterized, bacteriophages that infect other strains in the genus *Pseudomonas* (*Pseudomonas putida, Pseudomonas fluorescens*) have not been well- studied. Studies of such novel bacteriophages have the potential to broaden our understanding of host-pathogen relationships in *Pseudomonas*. From thirty soil samples collected from in and around the College of Wooster campus in Wooster, OH, bacteriophages specific to two host strains of *Pseudomonas* were isolated. Initial host-range experiments suggest the thirty independent isolates show differential infectivity across 19 different species of *Pseudomonas*. Three of these bacteriophage strains, PC1, PC3, and PC10, show broad infectivity against 7 of the 19 *Pseudomonas* strains tested. PC1, PC3 and PC10 show different plaque morphology on the *Pseudomonas* host strain used for isolation, and isolation of bacteriophage genetic material and subsequent digestion by restriction endonuclease EcoRI show different fragmentation patterns suggesting the phages may represent unique isolates. On-going work is focused on cloning portions of the phage genome, in preparation for sequencing and bioinformatics analysis.

BOARD 36

Identification of environmental pseudomonads that inhibit multi-drug resistant cystic fibrosis-derived pathogens

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Pathogens have evolved multi-drug resistance (MDR) due to the overuse and misuse of antibiotics, both in medicine and agriculture. Infections by pathogens are resulting in increased mortality rates worldwide whereby 10 million deaths are predicted by 2050. Unfortunately, pharmaceutical companies no longer invest in antibiotic discovery and development due to lack of profits. Moreover, previous work in our lab suggests that fewer natural drugs are available for discovery against MDR pathogens necessitating a streamlined workflow for the discovery of effective drugs. Here, we demonstrate a systematic strategy to identify strains that inhibit MDR pathogens and identify genes associated with antibiotic production. 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. An antagonistic plate assay was performed against a panel of 28 MDR pathogens consisting *of Pseudomonas aeruginosa and Burkholderia species including B. multivorans, B. vietnamiensis, B. dolosa, B. cepacian & B. cenocepacia.* Finally, transposon mutagenesis was optimized with a prolific antagonistic strain to identify genes associated with inhibition. We identified a biosynthetic gene cluster that is predicted to encode a NRPS involved in inhibitory compound production. We are currently issuing this strategy to identify other strains and gene cluster that inhibit MDR pathogens.

BOARD 37

Epidemiology of Staphylococci collected from Boston-area wild rodents

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As *Staphylococcus aureus* strains evolve and gain resistance to antibiotics, the risk of bidirectional transmission of resistant strains between humans and animals increases. The objective of this study was to identify and type *S. aureus* among wild rodents in Boston, Massachusetts, to examine their genetic relationship to common human and animal isolates. A total of 168 bacterial isolates collected from 45 Brown rats (*Rattus norvegicus*) in Boston proper were analyzed. Polymerase chain reaction was used to detect the *mecA* and PVL genes. All *S. aureus* isolates were *spa* typed. A subset of isolates was characterized via multi-locus sequence typing (MLST). All *S. aureus* isolates were tested for antibiotic susceptibility. Overall prevalence of *S. aureus* was 11.9% (20/168). Of all *S. aureus*, 5.0% (1/20) were MRSA (based on detection of the *mecA* gene which encodes methicillin-resistance) and 95.0% (19/20) were methicillin-susceptible *S. aureus* (MSSA). All isolates tested resistant to benzylpenicillin. Two isolates were resistant to erythromycin and one isolate was resistant to four antibiotics, including oxacillin. Of the 20 isolates, 75.0% (15/20) were *spa* type t933. MLST results to date show that these are sequence type (ST) 1094. Additional molecular testing is ongoing. Our results indicate that wild rats from Boston, MA are carriers of *S. aureus.* Additional study is needed to examine the distribution of t933/ST1094, an uncommon strain previously found in ewes in Tunisia. Further research is warranted to identify and characterize lineages of *S. aureus* strains in order to minimize the risk of Staphylococcal infection from city rodents.

BOARD 38

Mammalian temperature and available copper dually regulate the *Histoplasma capsulatum* Ctr3 copper transporter

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The dimorphic fungal pathogen *Histoplasma capsulatum* replicates within the phagosomes of host macrophages, where it must scavenge nutrients such as copper from the host cell to survive. After the onset of adaptive immunity, copper becomes restricted in the macrophage phagosome necessitating upregulation of the high-affinity copper transporter Ctr3 for full *Histoplasma* virulence. In *Histoplasma, CTR3* expression is induced both by transition from avirulent mycelia to the virulent yeast phase in response to mammalian temperature and by limitation of available copper, but what underlies this dual regulation is unknown. To define the minimal promoter that regulates *CTR3* expression, we fused *CTR3* promoter truncations to a GFP reporter and found 335bp was sufficient for full induction of GFP. Interrogation of this 335bp region identified putative binding sites for Ryp1, a transcription factor necessary for transition to the yeast phase at 37°C, and Mac1, a transcription factor with copper-dependent DNA binding. To assess the requirement of these putative binding sites for *CTR3* expression in low copper, we scrambled the binding sites individually or in combination. Simultaneous disruption of two putative Mac1-binding sites, but not individual disruptions, abolished copper-regulation of *CTR3* expression. In addition, a *MAC1*-RNAi strain showed loss of induction of CTR3 expression in response to low copper, confirming the role of Mac1 in the regulation of CTR3 expression. In a mouse model of respiratory infection, loss of Mac1 function caused more severe

attenuation than the loss of Ctr3 function suggesting that Mac1 controls additional genes important for *Histoplasma* virulence.

BOARD 39

An investigation into the isolation and prevalence of extended-spectrum beta lactamase bacteria in supermarket raw chicken and pork meat

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Antibiotic resistance is an increasing issue all over the world. Resistance is on the rise and has been linked to many hospital infections all over the world. Extended-spectrum beta lactamases (ESBLs) are a class of gram-negative bacteria that have previously been shown to be a source of antibiotic-resistant infections. ESBL bacteria include *E. coli*, *K. pneumoniae*, *S. enterica*, and *H. influenzae*. [JL1] This purpose of this investigation was to form a method to isolate bacterial DNA directly from raw meat, as well as identify antibiotic-resistant genes within supermarket raw chicken and pork samples. The first portion of this investigation looked into the most effective method of isolating bacteria from raw chicken and pork meat without culturing. It was found that using a rinsing procedure paired with a bacterial DNA isolation kit yielded a sufficient amount of bacterial DNA to perform PCR. The second portion of this study investigated the presence of extended-spectrum beta lactamase bacteria in raw chicken and pork meat that was obtained from a local supermarket. The implications of the information from this study could be further used in experimentation that aims to isolate bacterial DNA in a safer, non-cultivation method. A non-cultivation method of bacterial isolation lowers the need for higher biosafety precautions.

BOARD 40

Characterization of a γ-Butyrobetaine-Dependent Methyltransferase from the Human Gut Acetogen *Eubacterium limosum*

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Recent work has found a novel relationship between the occurrence of cardiovascular disease (CVD) and gut microbial quaternary amine (QA) catabolism. Dietary QAs 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 previously 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 have been unknown. We previously identified a glycine betaine-dependent three-component system in the nitrite-respiring *Desulfitobacterium hafniense*, and recently Kountz and Krzycki identified an *E. limosum L*-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 in order to identify candidate proteins underlying catabolic demethylation of the growth substrate. 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 (MtyB), a corrinoid-binding protein (MtqC), and a corrinoid:tetrahydrofolate methyltransferase (MtqA). We also identified a homolog of the reductive activation protein required to rescue oxidized corrinoid proteins (RamQ). The MttB family member MtyB is unique to ɣ-butyrobetaine-grown cells, and recombinant MtyB has activity as a ɣ-butyrobetaine:MtqC methyltransferase. We have also found MtyB to possess *L*-carnitine-dependent methyltransferase activity, though at a lower rate than with ɣ-butyrobetaine. Further work is underway to kinetically characterize the activity of the enzyme. We are currently testing the hypothesis that the candidate proteins identified by proteomics constitute a specific three-component ɣ-butyrobetaine:tetrahydrofolate methyltransferase system.

BOARD 41

SLAMF9-Mediated Immunomodulatory Signaling

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SLAMF9 may play an important role in the initiation of innate immunity in response to infection. Inflammation, a critical component of innate immunity, protects the human body from invading pathogens through mechanisms such as vasodilation, leukocyte recruitment, and fever induction. The activation of pathogen-recognition receptors, such as Toll-like receptors, on macrophages leads to the production of cytokines. Cytokines are small proteins that largely function to mediate inflammation and their production is either upregulated or downregulated based on the pathogen causing infection. As a cell surface protein, SLAMF9 has the potential for a signaling cascade that affects cytokine production and consequently affects pathogen clearance. In a mouse model lacking SLAMF9 expression, a dual phenotype is observed where mice are more susceptible to infection with bacteria but are more resistant to viral infection. The overall goal of this research is to elucidate the role of SLAMF9 in the human inflammatory response. Since the signaling pathway of SLAMF9 has yet to be described, we are aiming to identify potential candidate proteins that associate with SLAMF9 in addition to analyzing the response of differentiated THP-1 monocytes to interferon-inducing stimuli.

BOARD 42

Microscopic investigations of physiology and respiration in electrode oxidizing microorganisms

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The ability of organisms to utilize reduced electrons from an electrode for respiration and biosynthesis has recently been highlighted. However, the mechanistic and bioenergetic understanding of direct electron uptake from electrodes is limited. Using microscopic techniques coupled to electrochemical conditions probing for electron uptake, we sought to investigate the conditions that support respiration and NADH production in an electrode oxidizing model system(s). The *Alphaproteobacteria, Thioclava electrotropha* and *Gamaproteobacteria, Idomarina loihiensis* were both isolated from a marine sediment using electrodes poised at electron donating potentials. These organisms can also grow heterotrophically, making them a potential ideal model system for studying electron uptake. To investigate the metabolic consequences of electron uptake, we propose to utilize microscopic techniques that relate to cellular electrochemical gradient (Nernstian voltage indicator, Tht) and NADH concentration (NADH-binding protein Peredox). To establish the validity of fluorescent markers for biosynthetic capacity and respiration, *T. electrotropha* and *I. loihiensis* were studied under different

growth conditions where the concentration of acetate and/or the presences of oxygen was used to modulate energetic state. It was confirmed under the microscope that certain concentrations of acetate affect the NADH concentration of *I. loihiensis*. Also that the electrochemical gradient of *T. electrotropha* is proven to be affected by different acetate concentrations and oxygen states. Given the preliminary results that ground truth in the utility of these fluorescent markers, we can begin to investigate the effects of electron uptake in both *T. electrotropha* and *I. loihiensis* under various poised electrochemical conditions. This study will help in understanding the bioenergetic consequences of electron uptake in microorganisms and potentially help understand the link between respiration and biosynthesis in electrode oxidizing organisms. This process has both applied and environmental relevance as these organisms are common marine sediment microbes, and electrode uptake processes are of potential applied importance for electrosynthesis.

BOARD 43

Analysis of a Complete Knockout of the CCR5 Gene by CRISPR/Cas9

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Some survivors of the Black Plague, caused by *Yersenia pestis*, have a selective advantage by lacking a functional *ccr5* gene. This advantage comes from a 32 base pair deletion mutation, *ccr5 delta 32*, which confers resistance to *Yersenia pestis* and HIV infections. Timothy Ray Brown, the Berlin patient, is the first known person to be cured of HIV. While living with HIV, he was diagnosed with leukemia. To treat both HIV and leukemia, his doctor, Gero Hütter, performed a bone marrow transplant from a donor who was homozygous for the *ccr5 delta 32* mutation. Recently, another person dubbed the London patient went through a very similar treatment taking advantage of the *ccr5 delta 32* mutation and was announced as the second person cured of HIV. CCR5 and CXCR4 are secondary receptors for entry of HIV into human T-cells. It's been shown that the amino-terminus of the CCR5 delta 32 protein exerts a down-modulation effect on wild type CCR5 as well as CXCR4. 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 eliminate the expression of the CCR5 protein by removing a section of the sequence from both copies of the *ccr5* gene. The human T-cell line, H9, was co-transfected with two plasmids. One plasmid contained the guide RNA sequences that have homology to the amino-terminus of the *ccr5* gene and the CRISPR/Cas9, and another plasmid contained a puromycin resistance gene. Puromycin toxicity was determined by a kill curve. Transformants were obtained by puromycin selection 21 days after transfection. The presence of the gene in transfected cells was confirmed by PCR of the puromycin gene.

BOARD 44

Single-cell analysis of inner membrane diffusion of *Escherichia coli* by FRAP

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Several recent studies have suggested the lipid composition of the inner membrane of *E. coli* can affect respiration rate, by changing the behavior of the electron transport chain. Unsaturated fatty acids increase the fluidity of the membrane, and therefore the diffusivity of coenzyme Q, providing a mechanism by which respiration rate can be altered. FabB catalyzes the rate limiting, elongation step of unsaturated fatty acid biosynthesis in *E. coli*. We transformed *E. coli* cells with a plasmid containing the *fabB* promoter and a gene expressing the fluorescence protein mCherry, providing a proxy for *fabB* expression. Fluorescence Recovery After Photobleaching (FRAP) was performed on cells labelled with the pyridinium cyanine derivative of 2-(2-hydroxyphenyl)benzoxazole (HBO-PyCy). This novel dye preferentially stains the inner membrane and the FRAP measurement allows us to calculate the diffusion coefficient of the dye. This value was plotted against the intensity of mCherry for each cell. Our data show a positive correlation between the diffusion of HBO-PyCy and mCherry expression, indicating an increase in inner membrane fluidity in cells expressing higher levels of FabB.

BOARD 45

Elucidating the role of *Salmonella* Typhimurium-produced polyamine agmatine during macrophage infection

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Typhoid fever continues to be a major global public health threat resulting in over 11 million new infections and 129,000 deaths every year, and it is caused by consuming food or water contaminated with the human-specific *Salmonella enterica* serovar Typhi. One of the major characteristics of *Salmonella* during systemic infection is its ability to survival and thrive within host macrophages. This allows the bacteria to disseminate through the body to organs rich in these host cells. Polyamines are small polycationic molecules found ubiquitously in all organisms and function in a wide variety of biological processes. They are present at high concentrations in macrophages. In our study, mouse macrophages infected with *Salmonella* Typhimurium, a murine surrogate for the human-specific S. Typhi, were investigated to determine the role of the polyamine agmatine during the host-pathogen interaction. Our results revealed that *S*. Typhimurium-produced agmatine influenced host macrophage function, as macrophages infected with *S*. Typhimurium mutants unable to produce or secrete agmatine showed altered host cytokine and nitric oxide production, as well as arginase activity. Disrupting the bacteria's ability to produce or secrete this metabolite yielded a significantly more aggressive infection which contributes to the hypothesis that this molecule plays an important role in *Salmonella* pathogenesis.

BOARD 46

Screening for *Salmonella* Mutants that Fail to Recover from Fructose-Asparagine Intoxication

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The *fra* locus allows *Salmonella enterica* to utilize Fructose-Asparagine (F-Asn) as a metabolic nutrient. The *fra* locus is comprised of the *fraBDAE* operon and an upstream transcription factor encoded by *fraR*. In a *fraB* mutant, F-Asn is bacteriostatic to *Salmonella* due to a buildup of the toxic metabolic intermediate 6-phospho-fructose-aspartate (6-P-F-Asp). Removal of F-Asn allows the bacteria to recover and resume growth. Inhibitors of FraB could potentially be used to treat *Salmonella* infections. However, an important question is the mechanism by which 6-P-F-Asp inhibits the growth of *Salmonella*. My project aims to determine the mechanism(s) by identifying *Salmonella* mutants that 'fail to recover' (ftr) from F-Asn intoxication. 5,000 mutants were created using a transposon known as T-POP, which can both disrupt and activate genes adjacent to it on the chromosome. Mutants were exposed individually to F-Asn in minimal media for two hours to intoxicate them, and then moved to broth lacking F-Asn to recover. Growth of all mutants was monitored using a spectrophotometer (600 nm). Mutants that failed to recover from exposure to F-Asn were struck to isolation and saved. We identified six promising mutants. In several of the mutants the phenotype does not appear to be due to the transposon, suggesting that the phenotype may be due to secondary mutations. These mutants are being submitted for whole genome sequencing. In one mutant the phenotype is due to the transposon, and the phenotype is exacerbated by the tet-dependent promoter of the transposon, suggesting that the phenotype is due to activation of a downstream gene. We mapped this transposon to the end of the *polA* gene. The downstream gene that may be activated by the transposon is called *spf*. My results indicate that *spf* may be involved in 6-P-F-Asp toxicity. Future experiments will be needed to examine this relationship further.

BOARD 47

Distribution and phenotypes of SLAMF9+ myeloid cells in mouse tissues

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SLAM family receptors are critical mediators of immune function, whose expression varies across tissue and cell type. However, the expression of SLAM Family Member 9 (SLAMF9), the most recently discovered protein in the family, has yet to be investigated in mouse or human tissues. In this study, we sought to discover in what tissues and the degree to which SLAMF9 is expressed in the mouse model, as well as determine whether the cells expressing SLAMF9 belong to either a known or a novel subset. We discovered that, within the non-classical monocyte subset (CD45+CD11b+Ly6c-), two broad groups emerged based on SLAMF9 expression. The SLAMF9+ cells in this subset appear to constitute a homogenous group in both the kidney and liver. Renal SLAMF9+ cells also demonstrate higher expression of MHC-II, CX3CR1, and CD11c than their SLAMF9- counterparts, as well as low F4/80 expression and no CD103 expression. However, a cell type with largely similar marker expression and lower frequency appears in the hepatic non-classical monocyte gate, indicating that the cell type may be conserved across tissues. Therefore, SLAMF9 marks a

subset of CD103-, CD172a+ classical dendritic cells and may designate a specialized subset of these cells with similar functions in both the liver and kidneys.

BOARD 48

Salmonella biofilm extracellular polymeric substances alter the oxidative response from human phagocytes

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Each year, 21 million cases of Typhoid fever caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) lead to 200,000 deaths. Chronically-infected human carriers are the only known reservoir for *S.* Typhi and transmit it by fecal-oral contamination. Chronic gallbladder infections form biofilms on cholesterol gallstones encased in extracellular polymeric substances (EPSs). EPSs are thought to permit evasion of host immunity, but the involvement of specific components (EPSCs) in immune interference is poorly characterized. We investigated the contribution of EPSCs to innate phagocyte dysfunction using *S.* Typhi and *S.* Typhimurium with one or more EPSC mutations. Differentiated human neutrophils and macrophages were challenged with planktonic or biofilm cultures to determine how specific EPSCs influence the oxidative burst. Stimulation with planktonic *S.* Typhi produced less ROS than planktonic *S.* Typhimurium due to the presence of Vi-antigen in *S.* Typhi. However, biofilms from both serovars caused similar ROS responses regardless of Vi-antigen. Addition of the Vi-antigen to *S*. Typhimurium resulted in a significant reduction of the NO response to both planktonic and biofilm cultures. Wildtype and EPS mutant planktonic *S.* Typhimurium stimulated similar ROS responses, however; stimulation with colanic acid and/or cellulose mutant biofilms demonstrated a major reduction in ROS. These data suggest Vi-antigen has a crucial role in phagocyte dysfunction when expressed by planktonic *S.* Typhi but may have cellular-dependent effects as part of the EPS. Colanic acid is required for neutrophil recognition of *S.* Typhimurium biofilms; thus, *S.* Typhi has an advantage in biofilm recalcitrance because it lacks this slime polysaccharide. However, *S.* Typhi produces cellulose, which our data suggest has a similar effect as colanic acid. Therefore, we predict localization of cellulose alone to the biofilm surface would reduce stealth, but that the presence of the other *S.* Typhi EPSCs *in vivo* must prevent this interaction.

BOARD 49

Ecophysiological characterization of Complete ammonia oxidizers (Comammox)

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Complete ammonia oxidizers (comammox) are a group of ubiquitous chemolithoautotrophic bacteria that can derive their energy from the oxidation of ammonia to nitrate through nitrite and in turn use that energy to fix carbon into biomass. Comammox carry out the complete oxidation of ammonia to nitrate while Ammonia-oxidizing Archaea and Bacteria carry out only the first step of ammonia oxidation, i.e., the oxidation of ammonia to nitrite. These bacteria were discovered in 2015, however their ecophysiology has not been studied widely yet. In this study, we determined the impact of different environmental factors on the growth of comammox in an enrichment culture. Comammox was enriched from the sediment of Lake Superior. Based on the *amo*A gene, the ammonia oxidizer in the enrichment culture clustered within the *Nitrospira* Comammox cluster A and is closest related to sequences from a drinking water treatment plant. Growth experiments were performed under controlled laboratory conditions to characterize the effect of different environmental factors on the growth of Comammox. Enrichment cultures were grown in mineral salts (MS) medium under different conditions. Samples were taken in regular intervals to measure ammonium, nitrite and nitrate and to calculate the growth rates of Comammox. The comammox enrichment culture grew at ammonium concentrations up to 2 mM. Growth rates decreased and lag phases increased with increasing ammonium concentrations. The enrichment culture grew at pH values between 7 and 8.9. The highest growth rate was observed at pH 7.5. The enrichment culture was also able to utilize nitrite in the presence and absence of ammonium at concentrations up to 0.5 mM nitrite. Future experiments will include additional growth experiments under different conditions and analysis of the metagenome of the enrichment culture.

BOARD 50

Classroom education on hand washing needs intervention to result in effective student hand washing practices on a college campus

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Hand washing is known to be the best way to prevent communicable diseases. College students who are majoring in health sciences are required to learn and demonstrate proper hand washing protocol. Students with previous hand washing education may practice daily hand washing methods that are not more effective than students with no previous education. The purpose of this study is to assess if an on-site intervention that educates students about the proper hand washing technique will affect the hand washing of students in college restrooms. Data was collected over the academic school year on campus. Students volunteered for research and completed a survey about personal information, academic information and previous hand washing training. The student volunteers then plated finger pad samples on nutrient agar plates before and after being asked to wash their hands as they normally do in a campus restroom. Each plate was measured for hand washing effectiveness based on quantitative and qualitative data.. Results showed that only 47% of students at Ohio University effectively washed their hands, meaning they reduced the bacterial load on their hands. Chi-squared analysis comparing hand washing effectiveness across different college majors resulted in no significant differences. No significant differences in hand washing effectiveness between gender or among different sampling locations. When comparing hand washing effectiveness to students who had educational intervention to students that were not exposed to more information at the site of sampling, statistics reported a significant difference with Chi-square statistic of 9.8 with a p-value of

<0.05. In conclusion, hand washing effectiveness was affected when educational signs were placed in restrooms.

BOARD 51

Comparing the formal potential of *Geobacter metallireducens* (GS-15) oxidizing ethanol or acetate under anodic electrochemical conditions

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Direct interspecies electron transfer has been demonstrated to occur with *G. metallireducens* and other species grown in co-culture (Summers et al. 2010, Rotaru 2014, Rotaru 2015). This is due to its distinct ability to utilize ethanol as an electron donor, and in the absence of a usable electron acceptor such as iron, transfer electrons to a cell capable of using alternate and available electron acceptors (such as, fumarate or carbon dioxide). The bioenergetics of this processes are poorly understood. Our recent work investigating the ability of a methanogen shown to pair with *G. metallireducens*, *Methanosarcina barkeri,* is capable of taking up electrons from an electrode (likely as a surrogate for a cell) at very low redox potentials (<-450 mV vs. SHE) (Rowe et al. 2019). As the majority of electrochemical work in *Geobacter* species to date utilize acetate as an electron donor. We set out to compare the electrochemical activity of *G. metallireducens* using ethanol or acetate under traditional anodic redox potentials, as well as lower redox potentials than have previously been reported for *Geobacters*. In addition to looking at current production, we used cyclic voltammetry to determine the formal potential at which electron transfer to electrodes occurs under both conditions. This work will compare these data, and look at the potential for multiple, and energetically distinct pathways for extracellular electron transfer in *G. metallireducens*.

BOARD 52

Antibacterial Assessment of Pomegranate Peel, *Punica granatum L*., Extracts

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Fruit peels are usually discarded without valuable uses regardless of its high content of bioactive compounds. The Pomegranate, *Punica granatum L,* fruit is reputed for its sweetness and its juice is consumed because of its high contents of vitamins and mineral. The fruit peel is a part that is used in traditional medicine to treat some illness including infection and fever. This study aims to evaluate and compare the antibacterial activity of the peel methanolic (MeOH) and butanolic (BuOH) extracts on selected bacteria known to be associated with human diseases and food spoilage. Two Gram negative bacteria were used, *Escherichia coli* and *Sarcina aurantiaca* while the Gram positive tested were (*Staphylococcus epidermidis, Micrococcus luteus, Staphylococcus aureus, Rhodococcus rhodochrous, Bacillus sphaericus*). The peel was extracted using the organic solvents at room temperature and the extracts were used in a concentration of 40ug/ul.
Using LB agar and the well diffusion method, the desired bacteria was spread on the agar using cultures and the antibacterial assessment was by the measurement of the inhibition zones around the wells after 24 hr. Both pomegranate peel extracts showed inhibition of bacterial growth on all tested bacteria. The inhibition lasted for up to 72 hr and the range of the inhibition zones was between 6mm and 26mm. In conclusion, the pomegranate peel extracts ultimately contain bioactive compounds that are antimicrobials. This suggest the potential uses of pomegranate peel in the pharmaceutical and commercial applications.

BOARD 53

Membrane associated complement inhibitors: here today, gonorrhea tomorrow

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Neisseria gonorrhoeae is a Gram-negative bacterium and the causative agent of gonorrhea, a sexually transmitted infection. *N. gonorrhoeae* is classified as a high priority pathogen by the World Health Organization because of the emergence of strains that are resistant to antibiotics. Additionally, no commercial vaccine exists for *N. gonorrhoeae*. Furthermore, individuals can be infected multiple times with the same strain of *N. gonorrhoeae* because of the bacteria's ability to evade the immune response of the human host. To develop novel methods of targeting the bacterium, we are investigating how *N. gonorrhoeae* evades innate immune defenses. Specifically, we are investigating how complement regulatory proteins, normally associated with human cells, are manipulated by the bacterium. In our co-culture serum bactericidal assay, *N. gonorrhoeae* is allowed to colonize human cells, and is then challenged with normal human serum, a source of complement. We observe a significant decrease in bacterial survival when the complement regulatory proteins, CD46, CD55, or CD59 are knocked down in human cells by shRNA treatment. Finally, we have begun to characterize a triple knockout epithelial cell line (that lacks CD46, CD55, and CD59) for use in *N. gonorrhoeae* survival assays. With a better understanding of how *N. gonorrhoeae* evades the immune response we hope to inform the development of new therapeutics that help combat infections by this pathogen.

BOARD 54

Assessing phenotypes of *Thioclava Electrophica*

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One of the main challenges of understanding microbial function in environmental systems is the lack of genetic techniques that allow us to assess phenotypes. This has been especially true in chemolithoautotrophs which are often challenging to grow in the lab. To this aim we have been developing techniques for transferring genetic information into the facultative chemolithoautotroph, *Thioclava electrotrophica* ElOx9. Having developed a media compatible for growth with both *E. coli JG190* and *T. electrotrophic*, we have successfully developed a conjugation protocol that has allowed us to transform *T. electrotrophica* with both a fluorescent marker and introduce the minMar transposon. In this work we will present the mating efficacy for this process. In order to assess compatibility with more additional genetic techniques like CRISPR CAS-9 mediated genome editing, we have also been investigating the potential for electroporation, stemming from recent work published for *Shewanella.* Our current work suggested that *Thioclava* are capable of withstanding the electroporation conditions, which hold promise for the potential of this approach to yield genetic transformation. Our future work will entail using these techniques to construct a library of gene

deletion mutants using transposon mutagenesis that can be used to assess the genes involved in chemolithoautotrophic growth of *T. electrotrophica* on elementals sulfur and electron uptake from electrodes, to further study the potential genes involved in these processes.

BOARD 55

Antagonistic Activity of *Pseudomonas* Strains Isolated from Netherland Water Samples Against Multi-Drug Resistant Pathogens

Julia Horter* and Hans Wildschutte
Bowling Green State University, Department of Biological Sciences

The overuse and misuse of antibiotics has led to a global crisis whereby pathogens have evolved multi-drug resistance. In addition, large pharmaceutical companies have dropped drug discovery resulting in a decrease of new antibiotics to treat infections. Although antibiotics have traditionally been discovered from soil bacteria, recent work has suggested that water derived bacteria are a source of novel compounds. Pseudomonads represent a diverse group of bacteria that persist in soil and freshwater habitats and encode a wide range of secondary metabolites that have proven benefits in the pharmaceutical, agriculture, and industrial sectors. Here, we sought to investigate if *Pseudomonas* strains isolated from a Netherlands water habitat have the ability to inhibit multi-drug resistant (MDR) pathogens isolated from cystic fibrosis patients. We collected the environmental sample in March 2018 from a freshwater canal in Amsterdam using filtration. Strains were cultured on cetrimide media, steaked for isolation, and the genomic DNA was extracted for PCR and Sanger sequencing in order to create a phylogenetic tree. Antagonistic assays were performed using 196 environmental strains against a panel of 29 MDR CF-derived pathogens. Ninety-seven isolates were able to inhibit 21 pathogens. To identify genes involved in activity, we screened antagonistic isolates for their ability to undergo transposon mutagenesis and are optimizing mutagenesis to perform a large-scale mutant hunt. Arbitrary PCR and whole genome sequencing will be used to identify genes involved in secondary metabolite production. This workflow has the potential to identify novel antibiotics that are effective against MDR pathogens.

BOARD 56

Antagonistic interactions of two members of the gut microbiota

Samantha Edwards*[1], Daniel Savala[2], Clarissa Nobile[2] and Ruth GonzalezRios[1]
[1]Ashland University; [2]University of California Merced

The human gut microbiota – the collection of microbes that inhabit the digestive tract – is a complex ecosystem comprised of trillions of microbes. Bacteria, archaea, and fungi are the main members of this ecosystem. Changes to the microbiota can be caused by many factors, such as diet, changes in pH, and the use of broad-spectrum antibiotics. Such alterations affect the balance of the various members of the microbiota, oftentimes leading to infections, which are increased in immunocompromised individuals. Medical treatments, however, are commonly targeted to single microbial members, mainly because most microbes that inhabit or invade human tissue have been studied in isolation. Thus, it is important to understand healthy and disease states in their true context by studying the human microbiota as an ecosystem.

This project studies the interactions of two members of the human gut, *Candida albicans*, the most common fungi pathogen to humans, and *K. pneumoniae*, a gram-negative bacterium, each of which have been studied in isolation and are individually known to play roles in human health and disease.

The microbial communities in the gut are mainly found in biofilm form, the preferred mode of arrangement in the human body and nature. Microbial interactions of mixed species biofilms were studied using confocal microscopy, optical density and cell proliferation assays. Morphological properties such as aggregate formation was also examined in planktonic cultures. This study has identified an important antagonistic effect of K. pneumonia towards *C. albicans*, inhibiting not only aggregate formation of *C. albicans* in planktonic cultures but also biofilm formation. Proteomics analysis will be performed to further evaluate secreted proteins involved in this pattern of inhibition.

The present study represents an important step towards understanding how these microbes interact and has the potential to uncover new information on how they shape the human gut microbiota.

BOARD 57

Identification of an Ampicillin Regulation Gene in *Stenotrophomonas maltophilia* OR02

Kaylea Dillon*, Michelle Ricchiuti and Jonathan Caguiat
Youngstown **State University**

Strenotrophomonas maltophilia OR02 (*S. maltophilia* 02) is a Gram-negative bacterium that was isolated from East Fork Poplar Creek, a heavy metal contaminated stream in Oak Ridge, TN. This microbe is resistant to salts of cadmium, chromium, copper, gold, lead, mercury, selenium and ampicillin. Transposon mutagenesis generated a *S. maltophilia* 02 mutant, AJ22, that was sensitive to 100 µg/ml ampicillin on LB agar medium. Liquid culture medium experiments showed that the ampicillin minimal inhibitory concentration (MIC) was 300 µg/ml for AJ22 and above 1000 µg/ml for the wild type strain. We hypothesized that the mutated gene would encode a protein for beta-lactamase, which inactivates penicillin. However, gene rescue, DNA sequencing and Basic Local Alignment Search Tool (BLAST) analysis experiments showed that the mutated gene encoded the protein, AmpG. This cytoplasmic membrane protein senses damage to the cell wall and signals regulatory proteins to induce the expression of penicillin resistance. Other strains of *S. maltophilia* cause disease in immunosuppressed individuals and penicillin-resistance makes it difficult to treat infected patients. The development of drugs that inactivate AmpG may restore the efficacy of penicillin against these resistant bacteria.

BOARD 58

Oil-Degrading Microbial Communities

Conor Dolson* and Annette Bollmann
Miami University

Diverse bacterial communities degrade the majority of the 600,000 metric tons of crude oil that spills or leaks into the environment each year. These communities behave much differently than pure cultures of oil-degrading bacteria. In microbial communities, bacteria interact with their environment and each other in unique processes including syntrophic metabolism, metabolic coupling, and biofilm formation. Understanding the structure of these communities and their assembly processes is important for utilizing, supplementing, and maintaining these communities in areas affected by anthropogenic oil spills. This study aimed to characterize microbial communities grown in laboratory enrichment cultures under different conditions. Cultures were grown in medium containing mineral salts and either 0.01% or 0.1% canola oil as a carbon source, and with or without 0.01% yeast extract as additional nutrient supplementation. The cultures were transferred to fresh medium once per week for ten weeks and samples for DNA isolation were taken at the same time. Communities were characterized by 16S amplicon sequencing and subsequent data analysis was performed using the

QIIME2 bioinformatics platform. Overall community analysis indicated higher oil concentration and addition of yeast extract foster greater diversity in oil-degrading communities. *Pseudomonas* and *Azospirillum* species were present in all enrichment cultures, and their abundance changed over time until reaching a peak after ten weeks of incubation. In addition, many notable differences in community composition were observed between cultures that received nutrient supplementation in the form of yeast extract versus those that did not.

BOARD 59

Determining HIV Infectivity Through Mutation Delta32 Interaction

Kyle Patton*[1,2], Amnah Ali[1,2] , Leéna Boone[1,2] ,Virginia Ford[1,3] , Kiana Williams[1,2] , H. W. Kestler[1]
1 Lorain County Community College Department of Biology
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The Human Immunodeficiency Virus (HIV) targets and weakens the body's immune system which can develop into Acquired Immunodeficiency Syndrome (AIDS). Infection begins when HIV interacts with the primary receptor, CD4, and one of two co-receptors, CXCR4 or CCR5. When CCR5-Delta32 is present, CCR5 is down-modulated. Since HIV is unable to infect Delta32 homozygotes, Delta32 may affect CXCR4 surface expression. This research will determine the effects of CCR5-Delta32 on CXCR4 cell-surface expression..

In order to distinctively see the effects of CCR5-Delta32, CXCR4 must be overexpressed on the T-cell. *cxcr4* was isolated from H9 human T-cell line by PCR. After isolation, the *cxcr4* fragment was blunt-end ligated using HpaI restriction enzyme into pLXSN, a retroviral shuttle vector. This ligation of pLXSN-*cxcr4* was transformed into competent *E. coli* cells. The DNA was extracted and restriction endonuclease maped to verify pLXSN-*cxcr4*.

Once a successful clone of pLXSN-*cxcr4* is accomplished, it will then be transfected into packaging cell line PT67 to produce retroviral particles. This process will also be carried out with various *ccr5* alleles including Delta32 mutation; wild type *ccr5*; and TG5, a novel point mutation isolated from a possibly HIV-resistant subject. Once these retroviral particles are produced, they will then be transfected into tumor cells like CEMX174.

HIV-resistance will be tested by exposing cells to HIV that are expressing *cxcr4* and various *ccr5* alleles.

Abstracts of OBASM Podium Presentations

Saturday, April 13
3:00 – 4:00 pm

Session A

3:00 – 3:15 pm

Methanogenesis from glycine betaine proceeds through a corrinoid-dependent methyltransferase pathway

Adam Creighbaum*[1], Tomislav Ticak[2], Shrameeta Shinde[1], Xin Wang[1], D.J. Ferguson Jr.[1,3,]
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The enzymatic pathways involved with utilization of quaternary amines (QAs) are relatively uncharacterized in the spectrum of one-carbon metabolism. Previous studies have shown select strains in the archaeal genus *Methanococcoides* grow using tetramethylammonium (QMA), glycine betaine (GB), and choline but the physiological pathways for this metabolism were not elucidated. We recently described the first strain in the archaeal genus *Methanolobus* that catabolizes GB to methane. We hypothesized that this process is performed through a corrinoid-dependent GB:coenzyme M methyl transfer pathway. By using trimethylamine (TMA) dependent methanogenesis as a model, which involves the rare amino acid *L*-pyrrolysine (Pyl)-containing enzyme (MttB), our previous work demonstrated a Pyl-lacking MttB homolog (DSY3156) from *Desulfitobacterium hafniense* Y51 initiates GB metabolism through a methyl transfer pathway and was designated 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. Draft genome sequences of *Methanolobus vulcani* B1d revealed genes encoding a predicted non-Pyl MttB homolog enzymes (MV1727). MV1727 both catalyzes GB dependent methylation of free cob(I)alamin indicating it is a MtgBenzyme. Further analysis of the *Ml. vulcani* B1d genome revealed the presence of a methylcorrinoid:CoM methyltransferase (MV2112) a corrinoid binding protein (MV1728) and a corrinoid reductive activation enzyme (MV2107). Through proteomic analysis we were able to determine that MV1727, MV1728, MV2107, and MV2112 were in high abundance when *Ml. vulcani* B1d was grown on GB relative to growth on TMA or methanol. Recombinant MV2112 catalyzes methylcob(III)alamin dependent methylation of CoM. MV1727 and MV2112 were then combined with MV1728 and MV2107 *in vitro* to successfully reconstitute a GB:CoM pathway from *Ml. vulcani* B1d. To our knowledge, this is the first demonstration of reconstitution of this pathway *in vitro* using recombinant enzymes.

3:15 – 3:30 pm

Back from the dead: How changes in LPS structure alter its transport by $LptB_2FG$

Emily Lundstedt*, Brent Simpson, Rebecca Davis, Natividad Ruiz
The Ohio State University

The cell envelope of gram-negative bacteria contains both an inner membrane (IM) and outer membrane (OM). The OM provides a unique permeability barrier against small hydrophobic antibiotics. This barrier function is due to the presence of the glycolipid lipopolysaccharide (LPS) in the outer leaflet of the OM. LPS is synthesized in the IM and shuttled across the periplasm to the OM by the lipopolysaccharide transport (Lpt) machinery. The Lpt complex is made up of seven different proteins that form a bridge between the two membranes, three of which form the ATP-binding cassette (ABC) transporter, $LptB_2FG$, which uses ATP binding and hydrolysis to power the extraction of LPS from the IM and its transport to the outer leaflet of the OM. Like other ABC transporters, $LptB_2FG$ has two nucleotide-binding domains (NBDs) that bind and hydrolyze ATP, and two transmembrane domains (TMD) that interact with the substrate. To better understand how the movement of the $LptB_2$ NBDs is coupled to the extraction of LPS performed by the LptFG TMDs, we conducted structure-function analysis of residues along the interface between the NBDs and TMDs, and identified a novel residue in LptB, Glu86, that is essential for function. Suppression analysis revealed that a non-functional LptB(E86A) variant could be suppressed by loss-of-function mutations in the LPS biosynthesis gene *lpxM*. Since loss of LpxM function results in an altered LPS structure, our results indicate that interaction of the LPS substrate with the LptFG TMDs influences the activity of the $LptB_2$ NBDs. Furthermore, our data also suggest that residue Glu86 in LptB participates in conferring conformational changes that drive transport of LPS by LptFG.

3:30 – 3:45 pm

***Histoplasma* relies on gluconeogenic carbon substrates to proliferate in macrophages**

Qian Shen*, Stephanie Ray, and Chad Rappleye
Department of Microbiology, Ohio State University

Microbial pathogens rely on exploiting host nutrients to proliferate during infection. Intracellular pathogens, particularly those exclusively living in the phagosome such as *Histoplasma capsulatum*, must adapt and acquire nutrients from the intracellular environment. In this study, we investigated what host nutrients could be utilized by *Histoplasma* as the major carbon source to proliferate in macrophages. In liquid culture, *Histoplasma* yeasts can grow on hexoses and amino acids but not fatty acids as the sole carbon source. Metabolic gene expression profiling showed that *Histoplasma* yeasts down-regulated glycolysis when growing in macrophages. Consistently, depletion of hexose kinase/glucokinase in *Histoplasma* did not affect *Histoplasma* virulence in macrophages or mice. In contrast, *Histoplasma* yeasts up-regulated gluconeogenesis during intramacrophage growth. This is further supported by the fact that lack of Pck1 which catalyzes the committed step of gluconeogenesis resulted in severe attenuation both in macrophages and mice. Our results demonstrated that *Histoplasma* yeasts rely on gluconeogenic substrates (i.e., amino acids) but not hexoses to proliferate in macrophages.

3:45 – 4:00 pm

Tracking Diel Metabolism of the 2014 Lake Erie Toxic Microcystis Bloom Through Metatranscriptomics

Michelle Neudeck*[1], Paul Matson[1], R. Michael McKay[1,2], George S. Bullerjahn[1]
[1]Department of Biological Sciences, Bowling Green State University
[2]GLIER, University of Windsor

The cyanobacterial Harmful Algal Bloom of 2014 in Lake Erie made national news when the water supply to the city of Toledo was disrupted for three days. The increasing frequency of these blooms makes it imperative to study how these organisms react to nutrient loading versus circadian rhythyms. A 48-h Lagrangian survey was conducted in August of 2014. RNA was extracted from the water samples and sequenced. The metatranscriptome was analyzed using RNASeq and three strains of Microcystis from Lake St. Claire and the western basin of Lake Erie. Photosystem I was primarily transcribed during the night and Photosystem II was primarily transcribed during the day. The genes from the toxin, microcystin, are transcribed during the afternoon.

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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