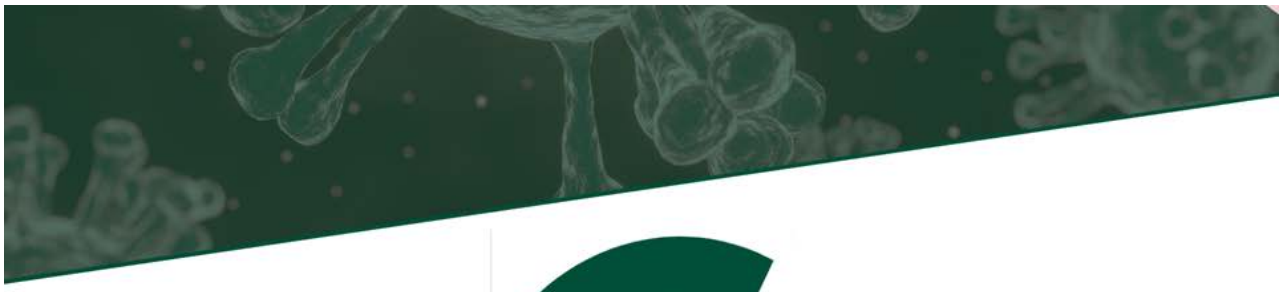
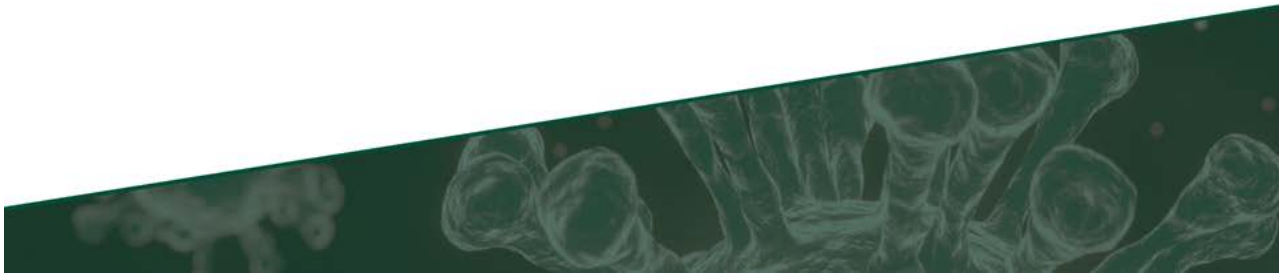


Microbiology and Molecular Genetics

Undergraduate Research Showcase 2023

Abstract Booklet



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1) Utilizing Crystallography to Find the Binding Site of Antitubercular Drugs

Maude Antol, Bilal Aleiwi, Edmund Ellsworth, Robert B. Abramovitch, and Sean Crosson
Department of Microbiology and Molecular Genetics

DosS/DosR is a two-component regulatory system in which DosS, a heme-containing sensor histidine kinase, is regulated by oxygen binding to the heme cofactor. Upon phosphorylation, DosS transfers a phosphoryl group to DosR (1). DosR is a DNA-binding protein that controls the entry of Mycobacterium tuberculosis and other mycobacteria into a latent, dormant state (1). The dormant state refers to a physiologic state when Mycobacterium tuberculosis is able to resist antibiotics and the immune system. DosS is therefore a possible target for antimicrobial treatment of Tb. This research is aimed at elucidating the structural basis by which select drugs bind to DosS and function as a possible adjunct therapy to eliminate Mycobacterium tuberculosis reservoirs and shorten the length of therapy. My experiments are aimed at defining the exact binding site of experimental tuberculosis antibiotics. Through protein crystallography, we are working to image the drug bound to DosS so we can see exactly where it is bound to DosS and what conformational changes occur. There is spectroscopic data indicating that the binding site of these compounds is near the heme site, which is blocked when DosS is oxidized (1). To date, we have been able to successfully image DosS in its unstable, reduced form where a drug could be potentially bound. Our next steps are finding new methods of drug soaking to solve the structure(s) of the reduced DosS crystal while it is bound to experimental drug compounds. Finding this binding site will allow us to understand how DosS targeting drugs might be used to inhibit DosS and therefore decrease the pathogenicity of Mycobacterium tuberculosis.

1. Huiqing Zheng, John T. Williams, Bilal Aleiwi, Edmund Ellsworth, and Robert B. Abramovitch. ACS Chemical Biology 2020 15 (1), 52-62 DOI: 10.1021

2) The bacterial outer membrane as a target for antimicrobial drug discovery

Anna Barker and Victor J. DiRita
Department of Microbiology and Molecular Genetics

Enterobacter hormaechei is a Gram-negative bacterium, from the *Enterobacter cloacae* complex *E. hormaechei* is a nosocomial, opportunistic pathogen that can cause infection in young children and elderly adults. With increasing resistance to many antibiotic drugs, including carbapenem, *Enterobacter* is becoming an increasing challenge in hospital settings. New antimicrobials are need to combat this pathogen. Because the outer membrane is a barrier to many antimicrobial therapeutics, one approach to developing new molecules against Gram-negative pathogens is to include an adjuvant to act on the outer membrane. Further, the outer membrane per se is a good target for antimicrobial discovery. We are using transposon mutagenesis to identify *E.*

hormaechei mutants with weakened outer membranes. The anticipated phenotype of such mutants is generalized envelope stress, which we will be able to monitor on selective and differential media, enabling us to identify mutants using high-throughput screens. Upon isolating mutants with envelope-stress phenotypes we will carry out further genomic analysis to map the transposon insertion and identify the gene(s) whose disruption led to the envelope stress phenotype. Once characterized, these genes will serve as targets for drug discovery approaches for molecules that potentiate the effect of other antimicrobials or that themselves kill *E. Hormaechei*.

3) Investigation of Antibiotic Resistance and Virulence Genes using Whole-Genome Sequencing of Human Shiga toxin-producing *Escherichia coli* Isolates from Wyoming, 2002-2020

Bailey Bowcutt[1], Wanda Manley[2], Joel Sevinsky[2], Robert Petit III[2], Jim Mildenberger[2], Karla Vasco[1], Noah Hull[2], and Shannon D. Manning[1]
[1] Department of Microbiology and Molecular Genetics Department, [2] Wyoming Department of Health, Public Health Laboratories

Shiga toxin-producing *Escherichia coli* (STEC) are linked to ~265,000 illnesses per year in the United States. STEC infections can cause enteric symptoms and, in some cases, hemolytic uremic syndrome (HUS), especially in vulnerable populations like children under 5 years of age and the elderly. Unlike with other enteric infections, antibiotic treatment is not recommended because it is associated with increased risk of HUS. Antibiotics can enhance the production of Shiga toxins by stressing the bacterial cells, resulting in the activation and spread of bacteriophages incorporated in the STEC genome that encode for Shiga toxin production. Despite the guidance against antibiotic treatment, antibiotic resistance has been reported in STEC strains, which is likely due to the presence of resistance genes acquired via natural selection. Therefore, it is important to enhance understanding of circulating antibiotic resistance genes in clinical STEC strains and define factors that increase the likelihood of infection. Herein, we used whole-genome sequencing and genomic processing pipeline Bactopia to analyze 260 strains from Wyoming patients collected between 2002-2020. All patient data have been extracted and genomes have been quality checked, assembled, and annotated to detect resistance and virulence genes as well as genes that dictate the serotype. An analysis of gene frequencies and trends over time is ongoing, while future analyses will examine trends by geographic location and cattle density, as cattle are an important STEC reservoir and ranching/farm work is a major industry in Wyoming.

4) Evaluating Epidemiological Associations and the Relationship between Antibiotic Resistance Phenotypes and Resistance Genes in Non-typhoidal *Salmonella*

Veona Cutinho, Samantha Carbonell, Karla A. Vasco, Heather Blankenship, Sanjana Mukherjee, and Shannon D. Manning
Department of Microbiology and Molecular Genetics

Non-typhoidal *Salmonella* (NTS) is a leading cause of gastroenteritis, resulting in an estimated annual 153 million infections and 57,000 deaths globally. In the United States alone, NTS antibiotic resistance is on the rise with 16% of strains resistant to at least one essential antibiotic, causing approximately 212,500 infections each year. As a result, it is essential to identify epidemiological associations with antibiotic resistant NTS infections to mitigate risk in populations and develop rapid approaches for detecting resistance. In collaboration with the Michigan Department of Health and Human Services (MDHHS), we obtained 198 NTS isolates from 4 Michigan hospitals between the years 2011 and 2014. These 198 isolates were recovered from patients ranging in age from 2 months to 91 years. Over 35 serogroups were identified with Enteritidis (36%), Typhimurium (19%), and Newport (9.6%) predominating; 30 (15.1%) had phenotypic resistance to at least one of the 24 antibiotics tested. Whole genome sequencing (WGS) will be used to determine whether there is a correlation between resistance phenotypes and genotypes. A random subset of these isolates (n=177) was sequenced with Illumina (2x250 bp), and FastQC and Trimmomatic were used for sequence quality control and filtering, respectively. Sequences will be assembled and annotated to identify the resistance genes for comparison to the phenotypic data. Together, these data highlight the importance of surveillance studies, which can define frequencies of specific pathogen traits for a particular geographic location and for informing public health practices and interventions.

5) Toward the prevalence of a DNA variant associated with cleft palate in Finnish descendants from Michigan's Sauna Belt region

Josie Kleve[1], Elison Daher[1], Madison Patrus[3], Paula Somohano[1], and Brian C. Schutte, PhD[1,2,3]

[1] Microbiology and Molecular Genetics, [2]Department of Pediatrics, [3]College of Osteopathic Medicine

Unlike the rest of the world, Finland has a uniquely higher frequency of cleft palate only (CPO) compared to cleft lip and palate. Interestingly, a DNA variant associated with a higher risk of CPO was discovered in the Finnish population. This variant correlates with the regional distribution of CPO across Finland and is associated with altered expression of the IRF6 gene, which is essential for typical palate development. This association suggests that the variant contributes to the higher frequency of CPO seen in Finland. Significantly, 1% of the Finnish population carries this variant, but it has not been identified in any other part of the world. The region of Michigan along the southern coast of Lake Superior, known as the “Sauna Belt”, is home to a significant number of individuals with Finnish ancestry. We hypothesize that this genetic variant will also be found in descendants of Finnish immigrants living in the “Sauna Belt.” To test our hypothesis, we utilized the Michigan Neonatal Biobank to isolate DNA from 25 blood samples from babies of Finnish descent born with cleft palate in the Sauna Belt from 1987-present. Based on the penetrance of the DNA variant in Finland, we expect about 10% of these samples to carry

the variant. The results of this research will contribute to a better understanding of cleft palate in Michigan and help genetic testing and counseling services better provide support for individuals of Finnish descent born with cleft palate, as well as aid the discovery of novel therapies.

6) High Throughput Transposon Mutagenesis to Identify Genes Required for Desiccation Tolerance in Carbapenem-Resistant Enterobacter

Dylan S. Luce, Elizabeth N. Ottosen, and Victor J. DiRita
Department of Microbiology and Molecular Genetics

Enterobacter hormaechei is a Gram-negative, opportunistic pathogen that is ubiquitous in the environment and a common member of the gastrointestinal microbiota. Carbapenem-resistant Enterobacter pose a serious threat to human health due to increasing levels of antibiotic resistance, therefore requiring new methods of fighting infections. Frequently the cause of hospital associated infections, E. hormaechei can be transmitted between patients through contaminated hospital surfaces. To survive on fomites, it must overcome the stress of desiccation as well as treatment with disinfectants. We demonstrated that Enterobacter remains viable after up to 140 days of desiccation, significantly longer than a non-pathogenic laboratory strain of Escherichia coli. This suggests that persistence on hospital surfaces could facilitate Enterobacter transmission between patients. To control for outside variables impacting our results, we developed a desiccation chamber to stabilize humidity using Drierite desiccant and investigated the best methods of resuspending desiccated E. hormaechei cells. We will build on our preliminary results by screening a transposon mutant library of E. hormaechei to identify mutants unable to resist desiccation like wild type. Our mutagenesis approach (TnSeq) allows rapid identification of genes essential for desiccation resistance of E. hormaechei. This work has the potential for identifying novel targets to fight the spread of clinical infections and improve decontamination strategies in hospital settings.

7) Identifying ATP-insensitive avcD mutants

Aubree C. Muethel, Micah J. Ferrell and Christopher M. Waters
Department of Microbiology and Molecular Genetics

AvcID is a novel type III toxin-antitoxin system encoded on the VSP-1 island of V. cholerae that functions as an anti-phage defense system with homologous systems found in many medically important bacteria. AvcD (Antiviral Cytidine Deaminase) is an enzyme that deaminates deoxycytidine while AvcI (AvcD Inhibitor) is a small regulatory RNA that inhibits deamination. During infection AvcI is lost, releasing AvcD to deplete dCTP pools starving phages of nucleotides necessary for replication. There are two domains present in AvcD, a C-terminal deoxycytidylate deaminase (DCD) domain and an N-terminal P-loop

NTPase (PLN) of unknown function. Purified AvcD was found to be inhibited by high concentrations of ATP while AvcD expressed in E. coli did not exhibit toxicity until the stationary phase when cellular energy levels decline. We hypothesize that AvcD deaminase activity is regulated by the cell's energy state through ATP binding to the PLN domain. Our goal is to identify ATP-insensitive AvcD mutants exhibiting growth arrest in the exponential phase consistent with activity in the presence of high ATP levels. Identified mutant enzymes are expressed and characterized by dCTP deamination assay in the presence and absence of ATP. Assessing mutations in the domains of AvcD will further understanding of phage defense by AvcID toxin-antitoxin systems necessary for effective use of phages as therapeutics.

8) Spotted-Wing Drosophila Females Exhibit Ovipositional Preferences

Marion Parshall, Juan Huang, and Julianna Wilson
Department of Entomology

Spotted wing drosophila (SWD), *Drosophila suzukii*, is a devastating insect pest whose ability to infest intact fruit has had significant negative impacts on cherry and berry yields globally. Abiotic and biotic factors can be indicators for SWD to alter their decisions about where and if they should lay their eggs. Therefore, the modification of the ovipositional behavior of SWD based on its surrounding environment and the accessibility of preferable conditions was explored. The first factor investigated was the impact of the number of tart cherries available for oviposition, where the number of tart cherries given to each SWD pair varied from 1 to 8. When more cherries were offered, the SWD female did display a preference for dispersing her eggs amongst available cherries. The second factor examined was to see if an SWD female exhibited ovipositional preference in cherries with no previous egg infestation. Each SWD pair was given one previously infested cherry with SWD eggs and 0 to 7 clean cherries without eggs in a container. When 1 infested and 1 un-infested cherry was offered to the pair, females laid more eggs in un-infested cherries than those previously infested ones. Knowledge about the ovipositional behavior of SWD can be integrated into the current understanding of this invasive species and utilized to improve current pest management strategies for SWD.

9) Potential drought-induced immune response in Arabidopsis thaliana

Madison Putmon, Pai Li and Brad Day
Department of Plant, Soil, and Microbial Sciences

Potential drought induced immune response in Arabidopsis thaliana Plant immunity is triggered by pathogen invasion and aims to counteract pathogens, and in some cases, abiotic stresses can also trigger an immune response. This was demonstrated in other studies, where a cold treatment was observed to trigger salicylic acid (SA)-mediated immune response which was accomplished by deactivating calmodulin-binding

transcription activator (CAMTA) transcription factors. CAMTA transcription factors repress immune genes so by deactivating them, an immune response can be produced and regulated. Although a cold-induced immune response was observed, it is undetermined if other abiotic stresses can produce an immune response and if they can produce an SA-mediated immune response. I will be investigating a possible drought induced immune response by Arabidopsis which was suggested by other studies. I will accomplish this by performing RT-qPCR to measure SA signaling marker genes PR1 and CBP60g on a variety of drought-treated plants. By studying this transcriptional regulation, we can obtain a better understanding of the relationship between drought and immunity response. In the future, this research could be expanded by studying other abiotic stresses such as osmotic pressure to determine if there is an immune response triggered.

10) Role of neurogenin 3a in zebrafish enteric nervous system development

Victoria Reinbold, Ann E Davidson, Kristen Loundsbury, and Julia Ganz
Department of Integrative Biology

The enteric nervous system (ENS) is the largest part of the peripheral nervous system and consists of a network of neurons and glial cells embedded in the walls of the gut. The ENS is vital in its role for gut motility, blood flow, immune response, as well as many other functions. Zebrafish, Danio rerio, are an ideal model for studying ENS development due to their easy genetic manipulation, transparent larvae, fully sequenced genome, and fast development. A set of key genes are known to regulate ENS development, but identifying which genes regulate ENS development in specific areas of the gut remains to be determined. Previous work has identified a mutant allele sa211 that results in an early stop codon in the neurogenin 3a (ngn3a) gene. Preliminary data suggest that ngn3a mutant zebrafish larvae lack ENS neurons in the midgut. Thus, we hypothesize that ngn3a controls neuronal differentiation in a specific gut area. To test this hypothesis, we first established a genotyping assay for ngn3asa211. We then identified heterozygous carriers of the sa211 mutant allele. Currently, we are crossing heterozygous carriers to generate homozygous mutant embryos and are performing immunohistochemistry with the pan-neuronal markers Elavl and acetylated Tubulin to label ENS neurons. We will then compare ENS neuron numbers in homozygous ngn3asa211 mutant larval guts to their wildtype siblings along the entire length of the gut. This work will contribute to a better understanding of the genes that regulate ENS development in zebrafish.

11)

Brianna Smith, Tyler Verburg, Sarah Roosa, Yasser Aldhamen, and Andrea Amalfitano
Department of Microbiology and Molecular Genetics

12) Investigating Deep Subsurface Microbial Communities in Gas-rich Rock-Hosted Aquifers: Relationship between microbial biomass and depth

Nicole Smith and Matthew Schrenk
Departments of Earth & Environmental Sciences, and Microbiology & Molecular Genetics

The deep subsurface biosphere is sometimes referred to as the "Dark Energy Biosphere", due to dependence of microbial communities on chemical energy to fuel their growth rather than light. High pressures and temperatures combined with low permeability and nutrient availability challenge subsurface microbes with extreme conditions deep within the Earth's crust, leading to questions concerning how deeply these organisms permeate into subsurface environments (Sahu 2021). In this study, drill cuttings from a hydrogen exploration well drilled to 11,000 feet in Nebraska were utilized to uncover the relationship between depth, geochemistry, and the abundance and biodiversity of microbial communities. Total genomic DNA was extracted and purified from 10g samples; the DNA extracts were quantified fluorometrically before being tested by PCR amplification using bacterial specific primers. Through the quantified DNA (ng of DNA per gram of sample), it was shown that as depth increases, there is a decrease in the abundance of biomass and microorganisms. These data are being used to identify correlations between the geological composition of the subsurface and microbial diversity and cell density. These data will help to understand nutrient and gas utilization by subsurface microbes within the deepest levels of the biosphere.

13) Exploration of Coulter counter methods suggests overestimates of RBC abundance

James Suggitt (MMG), Madeline Peters (MMG), Nina Wale (MMG, EEB, IB)
Departments of Microbiology & Molecular Genetics, Ecology, Evolution, & Behavior, and Integrative Biology

The invasion of red blood cells by Plasmodium parasites causes malaria, a disease which affects over 220 million people annually. Quantifying the number and type of red blood cells present over the course of a malaria infection is crucial to understanding within-host infection dynamics and how resource limitation may limit infection severity. Currently, the in vivo murine model for Plasmodium spp. infection relies on Coulter counting to measure the daily variability in blood cell composition; however, apparent disparities between the Coulter counter and complete blood counts call this method into question. By comparing whole and hemolyzed blood processed by a Coulter counter, the degree of error in the Coulter method of counting can be determined. Current data indicate that up to one-third of objects previously considered red blood cells using the Coulter method are not, in fact, red blood cells. Ultimately, this study utilized healthy host blood, meaning that future studies considering active malarial infections are necessary to determine how these discrepancies play out in infection, e.g., with host immunity and parasite activity.

14) Gene editing in CRISPR-Cas9 expressing transgenic tobacco reveals high frequencies of chimeric editing

Grace Urban and Guo-Qing Song
Department of Horticulture

Chimeric editing is often reported in gene editing. To assess the extent of chimeric editing, we created a transgenic tobacco line carrying a marker, beta-glucuronidase gene (gusA), introduced a CRISPR-Cas9 editing vector into the transgenic tobacco line to knock out gusA, and then investigated the gusA editing efficiencies in T0 and subsequent generations. The editing vector contained a Cas9gene, two guide RNAs, gRNA1 and gRNA2. The two gRNAs were designed to knock out a 42-nucleotide fragment of the coding region of gusA. The editing vector was transformed into the tobacco using Agrobacterium tumefaciens-mediated transformation and hygromycin selection. T0 transgenic lines were used to evaluate gusA-editing efficiencies through histochemical GUS assays, polymerase chain reactions (PCR), and next-generation sequencing of PCR amplicons. Profiles of targeted sequences of 94 T0 transgenic lines revealed that these lines were regenerated from non-edited cells where editing occurred and created chimeric cells in these lines during or after regeneration. Detailed analysis showed that on-target mutations at the AtU6-gRNA1 site and the AtU3-gRNA2 site were found in 4.3% and 77.7% of T0 transgenic lines, respectively. To overcome the issue of extremely low editing efficiencies in T0 lines, we conducted a second round of shoot induction from the chimeric line(s) to enhance the success of obtaining lines with all or most cells edited. The mutation profiles in T0 transgenic lines provide valuable information to understand gene editing in plant cells with constitutively expressed CRISPR-Cas9 and gRNA.

15) Engineered Fungal and Bacterial Living Materials

[1]Bryce Waller, [1]Nadia Shoenherr, [2]Tian Li, [2]Xiwie Shan, [1]Gregory Bonito
[1]Microbiology and Molecular Genetics Michigan State University, [2]School of Mechanical Engineering Purdue University

Engineered living materials are materials composed of inert substances containing living organisms that benefit from the organism's structure, metabolic processes, and other functional properties. This study aims to create new living materials with a focus on mutualistic relationships between fungi and bacteria to display increased strength, lifespan and self-healing capabilities, compared to their uninoculated counterparts. Different wood type samples and biocomposite blocks made from hemp hurd, straw, and inoculated grain spawn pressed in molds will be the base structures to create our living materials. Fungi were selected based on inoculation procedures (mycelial vs. spore-based), incubation times, strength, and growth conditions to yield 70% inoculation of the pore sapces in test wood blocks 2 weeks after inoculation. Bacterial-fungal inoculations will be inoculated into wood and biocomposite blocks individually. If bacterial-fungal

relationships prove ineffective, select microbe inoculation will follow. qPCR will be used to verify that cell densities of both organisms increase over time, while compound and electron microscopy will be carried out in conjunction with weight comparison to ensure that fungi and bacteria have reached 70% inoculation of wooden blocks. All materials will undergo tensile strength testing to determine the effectiveness of fungal and bacteria inoculations.

16) The Effect of SPY555 on Neurite Outgrowth

Ashley Ziemer, Bashar Jawich, and Kyle Miller
Department of Integrated Biology

The ability to observe how individual neurons grow is a fundamental step in understanding how the nervous system is wired during development. Given the importance of microtubules in providing structural support and as tracks for the long-distance delivery of intracellular cargoes how they are formed during neurite outgrowth has been a central question. For decades, the bright inorganic dye rhodamine tubulin has been the standard for fluorescent speckle microscopy tracking of microtubules. Its major limitation has been that because it cannot pass through the plasma membrane it needs to be microinjected into neurons. Here, we investigated the feasibility of the cell permeable inorganic, taxol-based dye, SPY555 for tracking the motion of microtubules within neurons. Using six different concentrations of SPY555, we found microtubule motion could be tracked without significantly impairing neurite outgrowth. This suggests that SPY55 is a promising label for tracking the motion of MTs during neuronal development.

Thank you to all the presenters and mentors that have participated in this Showcase, and to Dr. Vic DiRita and the MMG department for their continuing support!

Best wishes to the graduating seniors for a bright future!

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Molecular Genetics