Vascular Endothelial Growth Factor Impacts Blood Brain Barrier Integrity During Group B Streptococcal Infection

Natalie Alexander[1], Alexis Rickenbacher[1], Bailey Bacher[1], Alex Meyer[1], Caroline Brook[1], Brandon Kim[1,2,3,4]
[1] Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, USA
[2] Department of Microbiology, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA
[3] Center for Convergent Biosciences and Medicine, University of Alabama, Tuscaloosa, AL, USA
[4] Alabama Life Research Institute, University of Alabama, Tuscaloosa, AL, USA

Bacterial meningitis is a severe infection of the central nervous system (CNS) that occurs when the opportunistic pathogen Group B Streptococcus (GBS) is able to cross the highly selective blood brain barrier (BBB). The BBB is comprised of specialized brain endothelial cells (BECs) that contribute to the barrier function. The mechanisms in which GBS invades the CNS are not completely known. Vascular endothelial growth factor (VEGF) is a secreted factor that is known to increase vascular permeability and disrupt the BBB. RNA sequencing data revealed that VEGF was upregulated during GBS infection in BECs. As confirmation we demonstrated that VEGF secretion is increased during GBS infection in a BEC cell line (CMEC/D3). We further investigated the role of VEGF in the infection of GBS through our induced pluripotent stem cell-derived brain-like endothelial cell (iBEC) model, using Western blot and qPCR techniques. We saw an abundance of VEGF secretion following GBS infection in both CMEC/D3s and iBECs in Western blots performed. Examination of mRNA by qPCR also demonstrated a significant increase of VEGF expression in both cell lines. Together these data demonstrate GBS induced VEGF secretion at the BBB acts as a significant mechanism that contributes to barrier dysfunction.

Characterization of Herpes Simplex Virus Type 1 Portal Protein Mutants

Allen, J.[1], Visalli, M.A[1]., and Visalli, R.J[1].
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Savannah, GA

The *Herpesviridae* family is comprised of nine ubiquitous viruses causing primary and latent infection in humans. Herpes simplex virus type 1 (HSV-1) is an alpha-herpesvirus that can manifest as herpes labialis, ocular infection and encephalitis. The HSV-1 capsid contains a dodecameric assembly of pUL6 monomers at one vertex forming a portal for DNA translocation into the capsid. The portal contains several potential functional domains (e.g. β-hairpin, etc.) that coordinate the DNA encapsidation process during viral replication. In this study, we examined the contribution(s) of these domains to DNA packaging by constructing a library of mutant UL6 genes containing (i) random in-frame insertions of 15 base-pairs or (ii) specific mutations (deletion, scramble or substitution) to the region encoding the pUL6 β-hairpin (amino acids 541-558). Mutations throughout UL6 were confirmed by DNA sequencing. Each mutant gene was cloned into the HSV-1 strain-17 bacterial artificial chromosome (BAC) using a heat-shock controlled, homologous recombination event in SW102 *E. coli*. Mutant BACs were transfected into African green monkey kidney (Vero) and 31 (HSV-1 UL6 complementing) cell lines to generate mutant virus stocks. Western blot analysis of virus infected cell lysates was performed to confirm expression of mutant pUL6 proteins. Studies are proposed to characterize the viral mutants including viral replication kinetics, DNA cleavage analysis via southern blot, and western blot analysis of purified capsids to determine if portals are incorporated at the capsid vertex. Identification of domains critical for portal function may guide the development of new antiviral drugs targeting a unique mechanism of action.

The role of *Caenorhabditis elegans* host susceptibility on *Acinetobacter baumannii* transmission

C. E. Alvarez[1], C. V. Bowman[2], N. M. Vega[2,3], P. N. Rather[4]
[1]Program in Population Biology, Ecology, and Evolution in the Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, Georgia
[2]Department of Biology, Emory University, Atlanta, Georgia
[3]Department of Physics, Emory University, Atlanta, Georgia
[4]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia

Acinetobacter baumannii (*A. baumannii*) is a gram-negative nosocomial pathogen known for its propensity for hospital-acquired transmission. Despite its clinical significance, the dynamics of *A. baumannii* transmission remain poorly understood. In this study, we investigate the role of host susceptibility on primary *A. baumannii* infection in host populations. To achieve this, we employ the *Caenorhabditis elegans* (*C. elegans*) model system to explore the distribution of host susceptibility to *A. baumannii* infection and the underlying genetic components within a population. A wild-type population of N2 Bristol *C. elegans* and mutant strains with impairments in innate immune function (*dbl-1*, TGF-β-like; *bli-3*, ROS production) or ingestion and defecation (*exp-1,* defecation cycle mutant; *phm-2*, defective pharyngeal grinder function) were exposed to GFP-tagged *A. baumannii* to determine the importance of specific attributes of the host-microbe interaction. Fluorescence measurements and gut colony-forming unit (CFU) counts were obtained from individual worms to capture any heterogeneity in the distribution of primary infections and compare distributions of infections among populations. We observed differences in colonization between some but not all mutant worm species and wild-type worms, emphasizing the specificity of response to *A. baumannii* infection and the importance of these specific mechanisms for determining distributions of susceptibility.

Multiplex Nanopore amplicon sequencing for *Salmonella* serotyping.

David Ayala-Velastegui, Amy T. Siceloff, Nikki W. Shariat.
Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA.

Salmonella enterica is a bacterial foodborne pathogen that causes ~1.35 million illnesses each year. This species is composed of approximately 2,500 serovars that are determined by the composition of antigenic determinants on their cell surface. Because serovars differ in their capacity to cause human illness and their colonization skills, rapid *Salmonella* serotyping is essential to understanding the dynamics of the pathogen. Molecular assays are considered powerful tools for *Salmonella* surveillance due to their high sensitivity and the improvements allowed to characterize serovars in mixed populations. CRISPR-SeroSeq is a molecular deep serotyping tool that has demonstrated the presence of multiple serovars in animal and environmental samples, even within the same sample. However, the analysis of the sequence data is time consuming and not straightforward and there is a need for a more rapid approach. The aim of this study was to provide a molecular technique that accurately differentiates the most important *Salmonella* serovars that are a burden to public health and which are commonly found in food animals. Gene alignments of two *Salmonella* virulence genes were performed using BLAST and showed that the combination of these two sequences could be used to differentiate 35 serovars *in silico*. To evaluate this, three different *Salmonella* serovars were cultured and analyzed individually, and mixed together pairwise in proportions up to 1:1,000. The two virulence genes were amplified by PCR and after agarose gel electrophoresis confirmation, sequencing libraries were barcoded and sequenced on the Oxford Nanopore Technologies minION sequencer. Bioinformatic software was used for trimming the barcodes and analyzing raw reads. We show that this approach can provide a quicker alternative to analyze *Salmonella* serovar populations than CRISPR-SeroSeq. Also, Nanopore is an accessible platform that can be incorporated into different research and diagnostic fields around the world, providing novel applications to low and middle-income countries.

Enhancing Antimicrobial Stewardship in Rural Healthcare: Impact of an Educational Intervention

Thomas W. Bagwell[1]*, Jack H. Lambert[1]*, Raybun Spelts[2], Shondia Evans[3], Rafael Ponce[4], Kenneth I. Onyedibe[1]

Affiliations
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA
[2]Georgia Department of Public Health, Atlanta, GA
[3]Georgia Hospital Association, Atlanta, GA
[4]Division of Infectious Diseases, Mercer University School of Medicine, Macon, GA
*Contributed equally

Antibiotic resistance is a major concern in rural healthcare, particularly in critical access hospitals (CAHs) where resources and specialized antimicrobial stewardship (AMS) training are limited. Thus, we conducted an educational conference focusing on AMS for healthcare practitioners in CAHs. The educational program featured speakers in infectious disease who conducted didactic lectures, interactive sessions, and case-based learning involving antibiograms. A survey was administered before and after the conference to attendees to measure antibiotic related knowledge. Thirty participants completed the pre-survey while 19 completed the post-survey. About 47% of respondents were pharmacists, 33% infection control specialists, and 83% had roles in AMS or infection control. Comparative analysis of the pre- and post- surveys revealed significant differences. After education, fewer respondents recommended antibiotics for conditions such as all middle ear infections (decreasing from 50% to 36.84%), and mpox (from 20% to 15.79%). In response to case-based learning questions such as if knowing the most common cause of urinary tract infections (UTI) would help in selecting an empiric antibiotic, “yes" responses increased from 83% pre-survey to 95% post-survey. Additionally, respondents who agreed that assessing cumulative local susceptibility rates of common UTI bacteria increased from 90% pre-survey to 100% post-survey. After education, there was a significant ($p=0.029$) increase of 31% (27% to 58%) in respondents correctly choosing “empiric” therapy as the type guided by an antibiogram. Similarly, after education, confidence in interpreting an antibiogram rose from 67% to 100% ($p=0.005$). When asked about the minimum number of isolates required to create an antibiogram, 23% (7/30) in pre-survey and 95% (18/19) in post-survey answered correctly ($p < 0.001$). Our findings demonstrate that similar educational interventions in partnership with infectious disease and AMS experts can significantly enhance knowledge and confidence in antibiogram use. Such low-cost strategies can play a crucial role in combatting antimicrobial resistance.

The Effect of Putrescine on Phage Activation in *Pseudomonas aeruginosa* Communities

Amelia Balsizer[1], Breanna Eidson[1] & Sophie E Darch[1]
[1]Department of Molecular Medicine, University of South Florida, Tampa USA

Pseudomonas aeruginosa (Pa) primarily utilizes the communication system Quorum Sensing (QS) to regulate growth, dissemination, and virulence. Recently, it has been shown that in addition to QS, *Pa* are also able to use intrinsic phage (prophage) to sense and mediate responses to changes in environmental conditions like nutrient availability. Here, we have examined the activation of prophage in both planktonic and aggregate cultures of Pa.
Using traditional plaque assays, shotgun metagenomics, qPCR, metabolic panels, and TEM to characterize the regulation of prophage in Pa cells, we identified significant differences between planktonic and aggregate cultures. Specifically – the types of phages released and the metabolism profiles of the polyamine putrescine. Polyamines like putrescine are used metabolically but can also function as QS molecules. At high concentrations, putrescine signals the presence of lytic phage, triggering intracellular polyamine accumulation to prevent phage replication. Here we examine the effect of putrescine on phage activation both in planktonic and aggregate communities of Pa to determine if this response is only triggered in aggregate communities. We also examined this response when putrescine uptake and catabolism pathways are inhibited and predict this mutation will result in cells suspectable to lytic phage. Using TEM we saw that planktonic released filamentous-tailed phages whereas the SCFM aggregates released tailless phages. Shotgun metagenomics supported this imaging noting planktonic cultures were abundant with pf1 but aggregate cultures had far less. TEM imaging of phage from planktonic cultures with putrescine still had an abundance of tailed pf1-like phage but showed an increase in the tailless phage typical from aggregates. The phage from SCFM cultures with putrescine had markedly more pf1 and tailed phage present. This pattern of tailless phage in planktonic cultures with putrescine was seen again in the partial putrescine uptake mutant. QPCR from the cultures showed increased levels of pf1 in SCFM cultures with putrescine compared to those without.

Associations between *Staphylococcus aureus* lineages and infection of diabetic patients

Alex Banul

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading pathogen in patients with type 2 diabetes, resulting in severe infection and negative health outcomes. MRSA genetic lineages are known to have differing epidemiological and virulence profiles, resulting in a wide variety of infection presentations. While research exists on the ways MRSA regulates its metabolic pathway in response to different carbon sources, the impact of patient blood sugar concentrations on the transmission, colonization, and virulence of MRSA infections is not well understood. Virulence factors, such as superoxide dismutase A (*SodA*), have fitness impacts that are dependent on the glucose environment. This research sought to draw a connection between genetic lineage of MRSA and adaptive responses to glucose levels. This was done by comparing the growth rates of isolates of MRSA cultured in both normal and high glucose conditions. These MRSA strains were selected for testing from diverse strain backgrounds and taken from both diabetic and non-diabetic patients in order to find correlations between fitness and phylogeny. We then used a colorimetric enzyme activity assay to quantitatively observe *SodA* activity in these isolates and evaluated that in the context of host source and lineage. We found that across five distinct lineages of MRSA, those in clonal complex 8 exhibited higher growth rates than those of other complexes. We also found that carbon sources have a significant effect on SOD enzymatic activity in *S. aureus*. By understanding MRSA metabolic activity with regard to both phylogeny and virulence, this research will help to close a gap in our understanding of lineage effects on infection severity, particularly among diabetic patients.

Preliminary Analysis of Tennessee River Bloom at Water Treatment Plant

Natalie Yates[1], Brett Harris[1], and Eric Becraft[1]
[1]Department of Biology, University of North Alabama, Florence, AL, USA

Understanding the bacterial and algal populations of our local rivers is important to the health of the local environment. Occasionally, freshwater microbial blooms will occur due to the high input of organic nutrients, potentially complicating industrial processes and releasing dangerous toxins. In the early summer of 2023, the Tennessee River that separates Florence and Muscle Shoals, Alabama experienced a bloom complicating the water filtration process at the Muscle Shoals water treatment plant. Uniquely, the organism causing the bloom was also observed in high abundance post-filtration. The following are the preliminary efforts to identify the cyanobacterial or algal contributors to the freshwater bloom which will help direct future management efforts. Samples were collected from the Tennessee River and filtered water at the treatment plant and immediately processed through a 0.2 um filter and stored at -20°C. DNA was extracted and amplified at the 16s ribosomal RNA V3-V4 region and sequences were classified. Multiple cyanobacteria and algal species were identified at greater than 1% of the population in both the river and post-filtration samples. No cyanobacterial or algal species were in high abundance as the bloom had subsided by August; however, some of the species identified are known to contribute to freshwater blooms. Future experiments will consist of targeted qPCR analyses, seasonal sampling, and direct bloom sampling if the organism increases in abundance again in early summer 2024.

Deciphering the Role of Sumoylation During Epstein-Barr Virus Replication

Jessica Jenkins, Sheetal Tadiparty, Kristy E. Jones, and Gretchen L. Bentz
Mercer University School of Medicine, Macon, GA

Epstein Barr Virus (EBV), a ubiquitous gamma-herpesvirus, infection results in the establishment of viral latency in B lymphocytes. EBV latency is associated with numerous lymphomas, and the principal viral oncogene in the development of these lymphomas is latent membrane protein 1 (LMP1). We documented that LMP1dysregulates cellular sumoylation processes in lymphoma tissues, contributing to the viral protein's ability to modulate innate immune response and maintain viral latency. Sumoylation is a dynamic post-translational modification that is vital for cellular processes including: immune response, DNA damage repair sensing, cell cycle progression, resistance to apoptosis, and metastasis. Several cancers display dysregulation of the sumoylation process, making the SUMO machinery a sufficient target for anti-cancer therapies. However, the specific function of sumoylation in EBV replication remains undocumented. Here, we investigated three synthetically engineered small molecule inhibitors (ML-792, 2-D08, and TAK-981) along with a well-known natural extract inhibitor, Ginkgolic Acid (GA). Each inhibitor interferes at different stages of the sumoylation process. Results showed that these inhibitors differed in their ability to decrease global levels of sumoylated proteins, induce spontaneous EBV reactivation in latently infected B-cells, modulate viral replication, and effect virion structure. Data revealed that treatment with 2-D08 yielded the best anti-viral properties of the tested inhibitors. Therefore, we propose that 2-D08 may be the best potential therapeutic drug to aid the treatment of EBV-associated malignancies due to its ability to modulate the EBV life-cycle.

Evidence for transmissible colistin resistance in goose fecal microbiome

Elizabeth Bernate[1], Ezabelle Franck[2], Hayden Allman[2], Sophia Vizoso[1], Terence Crofts[2]
[1]Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA
[2]Department of Biomedical Sciences, Florida State University, Tallahassee, FL, USA

Bacterial resistance to antibiotics is an ever-expanding concern, especially antibiotics of last resort such as colistin. Once thought to be too toxic for clinical use, colistin, or polymyxin E, was introduced due to its ability to inhibit Gram-negative bacterial pathogens with little bacterial resistance. The widespread use of colistin and other broad-spectrum antibiotics (*e.g.,* as prophylactic treatments in animal husbandry) has selected for new mechanisms of resistance by which bacteria can survive antibiotic treatment. Recently, mobilizable colistin resistance genes (*mcr* genes) have been found to render colistin ineffective, with new homologs still being discovered, suggesting an increase in the level of dissemination of resistance. We used a functional metagenomic selection to search for novel colistin resistance genes in a goose fecal microbiome based on the hypothesis that migratory birds can be significant mediators of antibiotic resistance gene dispersal across environments. Here, we report evidence that *Acinetobacter* sp. *eptA* genes can confer colistin resistance. Furthermore, we show evidence that some of these genes are mobilized in environmental *Acinetobacter* strains, marking them as potential *mcr* precursors. Identification and discovery of novel resistance genes helps surveillance monitoring and is vital for tracking antibiotic resistance gene dissemination.

Bacteriophage when antibiotics fail: lessons learned from clinical *Staphylococcus aureus* treatment failure

Brandon A. Berryhill[1,2], Danielle B. Steed[3], Andrew P. Smith[1], Gavin H. Harris[4], Stephanie M. Pouch[5], Colleen S. Kraft[5,6], Bruce R. Levin[1]
[1]Department of Biological, Emory University, Atlanta, Georgia, USA
[2]Program in Microbiology and Molecular Genetics, Graduate Division of Biological and Biomedical Sciences, Laney Graduate School, Emory University, Atlanta, Georgia, USA
[3]Division of Hospital Medicine, Emory University School of Medicine, Atlanta, GA, USA
[4]Department of Medicine, School of Medicine, Emory University, Atlanta, GA, USA
[5]Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, USA
[6]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA

As a result of the increasing frequency of antibiotic resistance, there has been a resurrection of interest in the use of bacteriophages (phages) to treat bacterial infections. This recent surge in the therapeutic application of phages is not humanity's first attempt at phage therapy. In the early 20th century, phages were successfully employed to treat many common infections including *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Yersinia pestis.* Despite these reports of successes, the development of phage as a widely accepted therapeutic was abated in 1934 by an article pointing to how poor the science was around phage therapy. More specifically, the authors point out that there was an abundance of reports of successes and little consideration given to failures, and there was also a lack of mechanistic studies of how and why phages fail to treat certain infections. Phage therapy, as applied currently, is repeating many of these mistakes. None of the criteria as to what makes a good phage consider the population and evolutionary processes associated with using viruses as therapy and instead treat these viruses the same as antibiotics. Moreover, these standards also do not take into account that phage therapy is almost always administered concurrently with antibiotics. Here, we describe a case where a patient with a multi-drug resistant *S. aureus* was selected for phage therapy and the lessons we learned from this event, such as how to pick a good candidate phage and how to process the phage for intravenous administration, as well as how to robustly use these viruses to treat infections.

Investigation of biofilm formation by *Streptococcus equi* subspecies *equi* with clinical isolates

Hannah Blanton[1], Annalie Harris [1], DeLacy Rhodes[1]
[1] Department of Biology, Berry College, Mount Berry, GA

Streptococcus equi subspecies are globally important pathogens of animals. *S. equi* subspecies *equi* (SEE) is the causative agent of the equine disease strangles, an upper respiratory infection characterized by swollen lymph nodes that are able to abscess and disrupt breathing. Research has shown that another *S, equi* subspecies, *S. equi zooepidemicus* (SEZ) is able to form biofilms, though the clinical relevance of biofilms during SEE infection has not been investigated. Research from our lab has shown SEE (ATCC 33398) to also form biofilms but only when grown under conditions containing 5% CO2, suggesting that biofilm formation may take place inside the horse respiratory track. In order to understand the role of biofilm formation by SEE during disease, clinical isolates were donated from a veterinary diagnostic lab in Montana and their ability to form biofilms has been tested using an *in vitro* biofilm assay. Three clinical isolates were assessed for their ability to form biofilms under the media conditions of BHI (Brain Heart Infusion broth) supplemented with 6% sucrose and incubated at 37°C with 5% CO2. Biofilm mass was quantified through microtiter plate reading and visualized through crystal violet staining. Biofilm assay results demonstrated that clinical isolates form biofilms under the same conditions and at similar levels as SEE (ATCC 33398). Future research will perform biofilm assays with additional clinical isolates and will co-culture SEE and SEZ with mammalian cells to investigate the potential for biofilm formation during infection.

Comparing Growth Rates of *Acinetobacter baumannii* Phenotypes in Various Carbon Sources

C. V. Bowman[1], C. E. Alvarez[2], N. M. Vega[2,3]
[1]Emory College of Arts and Sciences, Atlanta, Georgia
[2]Emory University, Population Biology, Ecology, and Evolution, Atlanta, Georgia
[3]Emory University, Department of Physics, Atlanta, Georgia

Acinetobacter baumannii (*A. baumannii*) is a gram-negative, pathogenic bacterium that is responsible for over 10% of all hospital-acquired infections in the United States. It is capable of switching between two phenotypes - a more virulent state that predominates during *in vivo* infections (VIR-O), and a less virulent state that utilizes a wider range of carbon sources and demonstrates higher biofilm formation (AV-T). It has been found that the VIR-O phenotype shows attenuated growth when provided with common carbon sources, whereas AV-T can survive on a wide range of options. However, it remains unclear what selective advantage each phenotype will have under different growth conditions and with different nutrient sources. As such, I have sought to further explore the effects of various carbon sources on *A. baumannii* replication through the use of growth curves with various concentrations of tricarboxylic acid cycle (TCA) intermediates and common carbon sources. The results of these experiments showed that both AV-T and VIR-O colonies grew when provided with TCA intermediates as carbon sources; the results also identified an ideal concentration of each nutrient source for each phenotype. This study allows for the impact of environmental passage on the ratio of AV-T to VIR-O to be studied further, and opens the door for in-depth future experimentation on *A. baumannii* phenotypes, which will provide a greater understanding of the specific mechanisms behind this bacterium's hospital-specific infectivity.

A flow cytometric approach to the immunological characterization of *Periplaneta Americana*

Faith Boyer[1], Arthur Appel[2], Zakee Sabree[3], Elizabeth Hiltbold-Schwartz[1]
[1] Department of Biological Sciences, Auburn University, Auburn, AL
[2] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL
[3] Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH

The American cockroach (*Periplaneta americana*) is a globally distributed pest with a gut microbiome similar to humans, primarily composed of Bacteroides and Firmicutes. In mammalian systems, the impact of the gut microbiome on immunological composition and function has been well-investigated; however, an insect system provides a unique platform for investigating this interaction with the innate immune system. Insect immune cells, called hemocytes, have been investigated primarily through microscopy, leaving a knowledge gap in relative abundance and specific functionality of these different cell types. To address this knowledge gap, we employ flow cytometry, cell sorting and functional assays to isolate and characterize hemocyte populations found in *P. americana.* Our flow cytometric data reveals distinct hemocyte populations based on lectin-binding capabilities. Based on these parameters, we can sort isolated cell populations and investigate individual population activity through functional assays (i.e., ROS production, antibacterial capabilities). These data provide a foundation for developing a more robust characterization of hemocyte abundance and function. For future studies, we are annotating the hemocyte transcriptome and cultivating a colony of germ-free *P. americana* for investigation of the role of the gut microbiome in composition and function of the innate immune system.

Characterization of Murine Cytomegalovirus M72 Interactions with the CCR4-NOT Complex.

Olivia Brahms[1], Sandy Gopal Ph.D.[2], Jason Upton Ph.D.[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL
[2]Department of Microbiology, University of Texas at Austin, Austin, TX

Human cytomegalovirus (HCMV) is the largest of the eight known human herpesviruses and many of its conserved genes remain uncharacterized. The beta herpesvirus conserved core gene UL72 and M72, the murine cytomegalovirus (MCMV) homolog, were originally designated as the 2′ deoxyuridine 5′ triphosphate pyrophosphatase (dUTPase) homolog. However, previous studies have shown both UL72 and M72 lack dUTPase activity; therefore, they currently have no defined function. Through immunoprecipitation and mass spectrometry, we determined a list of potential functional interactors with M72. We identified several subunits of the carbon catabolite repression 4 (CCR4)–negative on TATA-less (NOT) complex as possible functional interactors with M72. The CCR4-NOT complex is a multiprotein, multifunctional complex that is regarded as the main deadenylase in eukaryotic cells. M72's interaction with the CCR4-NOT complex was confirmed in both isolation and during infection. We show that M72 strongly binds with the regions of CNOT1, the scaffolding protein of the complex, that are responsible for binding the deadenylase subunits of the complex. We also reveal that M72 and CNOT1 are both necessary for normal viral replication and suggest a genetic connection between the two. These results suggest a novel function for M72 in modulating the CCR4-NOT complex and pro-viral role for CNOT1 in CMV pathogenesis.

Detection of antibodies reacting with *Rickettsia* antigens in sera of healthy individuals from Georgia, USA

Kay'La Brennon[1] and Marina E. Eremeeva[1]
[1]Jiann-Ping Hsu College of Public Health, Georgia Southern University

Spotted fever group rickettsioses (SFGR) are caused by a group of tick-borne *Rickettsia*. Cases of these diseases have been increasing nationally and globally; however, relatively few studies have examined the exposure of Georgia, USA residents to tick-borne pathogens. In this rural state, *Amblyomma americanum*, the Lone Star tick, is common. It is frequently infected with *Rickettsia amblyommatis*, which is thought to be avirulent. Our goal was to determine if exposure to *Rickettsia amblyommatis* accounts for most of the human antibody responses detected by SFGR diagnostic tests in Georgia.The indirect microimmunofluorescence assay (IFA) was utilized to determine the end-point titers of IgG-type antibodies reacting with cell culture grown antigen of *Rickettsia rickettsii.* Positive sera were tested by western blotting to determine their reactivity with antigens of *R. rickettsii* and *R. amblyommatis.* Purified antigens of *R. rickettsii* and *R. amblyommatis* were denatured at 56^{o}C for 30 min and separated by 10% SDS-polyacrylamide gel electrophoresis. Resolved antigens were transferred to nitrocellulose membrane. Membrane was blocked with 5% non-fat dry milk Tris-saline buffer, incubated with 1/150 serum dilution, then with horse-radish peroxidase-labeled secondary antibody, and finally with 4-chloro-1-naphthol substrate. One-hundred and eleven sera tested IFA-positive with *R. rickettsii* antigen including 85% sera with an IgG antibody end-point titer of 1/256 or higher. All sera reacted with similar intensity to the high molecular weight protein bands of *R. rickettsii* and *R. amblyommatis* surface protein antigens (SPA). Some sera also reacted at 25-50 kDa where *Rickettsia* lipopolysaccharide (LPS) antigen migrates. Sera of healthy donors from Georgia, USA contain IgG-type antibodies reactive to SPA and LPS of SFG *Rickettsia*. Additional cross absorption experiments are required to determine whether *R. rickettsii* or *R. amblyommatis* is responsible for eliciting these antibodies.

Correlation of Nitrification and Cell enumeration of *Nitrosomonas europaea*

Christopher Bryan[1], DeLacy Rhodes[1]
[1] Department of Biology, Berry College, Mount Berry, GA

Nitrogen is an integral element in the production of proteins and nucleic acids in all living things, but for an organism to use it, it must be in a form that the organism can assimilate. Certain bacteria can gain energy by converting ammonia (NH_3) into nitrite (NO_2^-), where another species, such as *Nitrobacter* or *Nitrospira,* then convert this nitrite into nitrate (NO_3^-), which can be readily assimilated by plants. These nitrates are often a limiting factor in areas where plants have the potential to grow. This process is also used in biofiltration of toxins in wastewater treatment plants, as ammonia and nitrite are highly toxic to aquatic life at low concentrations. The purpose of this study was to gather preliminary data using the ammonia oxidizing bacteria *Nitrosomonas europaea*, specifically our strain's rate of growth, nitrification, and media acidification. Colorimetric assays were used to quantify nitrite production for *N. europaea* over time, while an improved Neubauer chamber was used with fluorescence microscopy for cell enumeration. Cell density and rate of nitrite production increased with culture age at a rate that was directly proportional. Future studies will focus on understanding the relationship between culture age and nitrification, along with studying the effects on nitrite production of *N. europaea* when a nitrogen fixing cyanobacteria species is added to the experiment.

Dispersion dynamics of *Pseudomonas aeruginosa a*ggregates in synthetic cystic fibrosis sputum media

Jessica R. Burns[1], Sophie E. Darch[1]
[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, USA

A highly opportunistic pathogen with the ability to persist in clinical and community settings, *Pseudomonas aeruginosa* (*Pa*) exhibits actively evolving multi-drug resistance and virulence capabilities. Forming robust biofilms and aggregates, *Pa* is known to establish long-term infections in those diagnosed with cystic fibrosis (CF). Understanding aggregate topography and dispersion dynamics is of particular interest in order to better characterize qualities that promote the persistence of these infections. For this study, we aim to quantitively analyze dispersal of *Pa* aggregates grown in a synthetic CF sputum media (SCFM2)- a model designed to mimic *in vivo* environmental and nutritional conditions. Mucin, a glycoprotein found at high abundance in sputum, was utilized to represent the increasing viscosity typically observed whilst a patient ages. Confocal microscopy was utilized to analyze planktonic cell dispersion over time for *Pa* wild type, PAO1, and compared against three known dispersal mutants- D*dipA*, D*nicD*, and D*sagS*. Isolates were imaged in SCFM2 with three different concentrations of mucin. The complex topology and spatial structure of aggregates were imaged over time and compared against planktonic cell count with an emphasis on volume over time, utilizing R Studio. Confocal microscopy provided an in-depth analysis of dispersion dynamics and the differences between PAO1 wild type and three dispersion mutants. In low viscosity, dense fields of planktonic cells were present with minimal aggregation. As the viscosity increased, all isolates experienced significant decreases in planktonic cell count and for some, entropic stacking was present. The differences in planktonic cell density suggests a lack of spatial availability in high viscosity environments, impeding efficient dispersal. Specifically, quorum sensing may become hindered due to increasing viscosity in the environment. With this, the data provides a high-resolution perspective of the impact evolving environmental conditions has on aggregate spatial organization and any potential consequences regarding pathogenicity.

Variability in *Salmonella* prevalence and serovar presence through turkey production highlights food safety challenges

Emily E. Cason[1], Anna V. Carlson[2], and Nikki W. Shariat[1]
[1]Department of Population Health, University of Georgia, Athens, Georgia
[2]Cargill, Inc.

Despite extensive *Salmonella* mitigation strategies used during turkey processing, 5.9% of human salmonellosis cases are linked to the consumption of contaminated turkey. This study sought to determine *Salmonella* prevalence and serovar diversity through turkey live-production and processing by sampling 22 flocks on the farm and during different stages of processing. Bootsocks (n=22) were collected on the farm immediately after load-out. At processing, pre-scald wingtips (n=6 composites of 10 per flock), pre-chill wingtips (n=6 composites of 10 per flock), mechanically separated turkey (MST; n=6 bins per flock) and ground turkey (n=6 bins per flock) samples were collected. *Salmonella* prevalence was determined using a commercial PCR assay and then confirmed by selective enrichment and culture. Deep serotyping by CRISPR-SeroSeq was performed on *Salmonella*-positive samples (n=167). At the farm, nine flocks were *Salmonella* positive, compared to 21 flocks that were positive in at least one location during processing. *Salmonella* prevalence decreased from pre-scald (56.1%) to pre-chill (18.2%) ($p<0.05$, non-parametric Mann-Whitney U test) then increased in MST (65.2%; $p<0.05$) and ground turkey (48.5%; $p<0.05$). In total, 34.7% of samples contained multiple serovars and deep serotyping detected one or more serovars in products that were absent before chilling in 15 flocks. In total, 19 serovars were detected; Uganda (28.4%), Hadar (24.3%), and Typhimurium (24.3%) were most prevalent. Almost half of samples containing serovar Typhimurium (20/42) corresponded to a live-attenuated Typhimurium vaccine. This study demonstrates that within the same flock, different serovars are detected in barn samples from final product samples, underscoring the complexity of tracking serovars in turkey production. This ultimately limits the applicability of serovar-specific controls such as vaccination. Additionally, this study shows a disconnect between *Salmonella* detected at the farm and downstream throughout processing plant steps. This highlights the challenge of identifying on-farm surveillance samples that accurately represent *Salmonella* in final products.

Symbiont-elicited shifts in host immunity govern a strong priority effect in the assembly of an insect-microbe symbiosis

Jason Chen[1,2], Anthony Junker[2], Nic Vega[1,2], Nicole Gerardo[1,2]
[1]Population Biology, Ecology, and Evolution Graduate Program, Emory University, Atlanta, Georgia
[2]Department of Biology, Emory University, Atlanta, Georgia

Many host-associated microbial communities are subject to priority effects, in which the trajectory of community assembly is dictated by the order in which different taxa colonize available niches. Thus, variation in colonization order can be a source of variation in microbial community composition between different hosts. However, the mechanisms by which these priority effects are imposed are diverse, and in host-associated microbiomes in particular, can stem from complex interactions between host and microbe. Here, we demonstrate strong and rapid priority effects in beneficial symbiont colonization of the squash bug *Anasa tristis*, using pairs of strains that are known to strongly compete during host colonization, as well as strains that are isogenic and thus functionally identical. By introducing strains into individual bugs in a sequential manner, we show that within-host populations established by the first strain are extremely stable, regardless of strain identity and competitive interactions. By treating bugs with antibiotics, we further show that colonization success by the second symbiont is still diminished even when space in the symbiotic organ is available and physically accessible for colonization. We propose that host immunity or physiology shifts rapidly in response to symbiont colonization in a manner that excludes subsequent symbiont infections.

Evaluation of Recombinant *Flavobacterium covae* Protein Vaccines in Channel Catfish *(Ictalurus punctatus)*

Emily M. Churchman[1], Miles D. Lange[2], Nithin Muliya Sankappa[2,3], Megan Justice[2], Jason Abernathy[2], Mark R. Liles[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL, USA
[2]United States Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, Auburn, AL, USA
[3]Oak Ridge Institute for Science and Education (ORISE), ARS Research Participation Program, Oak Ridge, TN, USA

Columnaris disease is one of the leading causes of mortality in production of channel catfish (*Ictalurus punctatus*). One of the etiological agents of columnaris, *Flavobacterium covae*, has shown to be highly prevalent and virulent in channel catfish compared to other *Flavobacterium* species. As food fish production continues to increase, the frequency of columnaris disease will continue to be a major problem for the US aquaculture industry. Previous work has shown that several proteins are upregulated during biofilm formation in *F. covae.* Here, we test the immunogenicity and efficacy of several biofilm-associated proteins to be used as recombinant protein vaccines. The genes encoding several *F. covae* predicted proteins were cloned into expression vector pET-28a(+) with a C-terminal His-tag. After transformation into *Escherichia coli* strain BL21 (DE3), protein expression was induced with 1 mM IPTG and purified under native conditions using HisPur Ni-NTA resin. Channel catfish were injected intraperitoneally with purified protein (20 ug/mL), and peripheral blood was collected 30 days post-vaccination. Preliminary data shows vaccinated fish exhibited sera IgM antibody specificity to the respective antigens when blotted to the reduced proteins. There was a significant increase in IgM antibody titers with vaccinated fish compared to the control group. In our next trial, groups of fish (n=540) were immunized by bath immersion with the recombinant protein(s) (1 ug/ml) or sham immunized. There was no significant mucosal IgM antibody production among the vaccinated groups, however each vaccinated group showed significant survival when challenged with *F. covae* (>30% compared to the control group) at nine weeks post-immunization. Differential gene expression was identified between the adjuvant only control and vaccine groups and these results will be discussed. These results lay the groundwork for potential vaccine candidates for use as a multi-valent subunit vaccine to protect farmed fish against columnaris disease during the production cycle.

Idiopathic Epidural and Multifocal Extremity Abscesses

William Reid Clark, MS2 and James E. McGrory, MD

Sepsis resulting from multifocal musculoskeletal infections involving the spinal column is a rare but life-threatening event. Timely treatment is necessary for prevention of irreversible sequelae or death. Predisposing factors associated with these multifocal infections include HIV, rheumatoid arthritis, diabetes, and immunosuppressive therapy. Spinal epidural abscesses usually result from hematogenous spread of pathogens from other infectious sites. The most prevalent causative agent is *Staphylococcus aureus*. The aim of our study was to evaluate the clinical course and outcome of a patient following the sequential surgical treatment of all infectious foci. A 65-year-old male with rheumatoid arthritis was seen with a one-week history of severe lower back pain radiating to both lower extremities and weakness. Lumbar MRI demonstrated an epidural abscess at L3-4 extending into the left psoas muscle. Following aspiration of the spinal region, the patient was started on broad-spectrum antibiotics. Cultures grew methicillin-sensitive *S. aureus*. The patient underwent three sequential posterior spinal debridement surgeries. He remained septic until two additional pyogenic foci involving the left foot and right hand were identified and surgically debrided. Following surgical drainage and open packing of infectious foci in the hand and foot, the sepsis resolved, and he was discharged on long-term antibiotics. We present a rare case of multifocal infections of the musculoskeletal system that also involved the spine and was complicated by sepsis. Detecting secondary abscesses in the deeper muscle layers of the extremities is complicated by unspecific and inconsistent local clinical symptoms. However, eradication of all septic foci is necessary.

Identification of a *Stenotrophomonas maltophilia* contact-dependent antibacterial toxin

Cristian V. Crisan[1,2], Joanna B. Goldberg[1,2]
[1]Department of Pediatrics, Division of Pulmonary, Asthma, Cystic Fibrosis, and Sleep, Emory University School of Medicine, Atlanta, Georgia, USA
[2]Emory+Children's Center for Cystic Fibrosis and Airway Disease Research, Emory University School of Medicine, Atlanta, Georgia, USA

Stenotrophomonas maltophilia is an emerging, multidrug-resistant Gram-negative bacterial pathogen ubiquitously found in natural and hospital environments. *S. maltophilia* can infect the lungs, blood, heart, brain, skin, eyes, and gastrointestinal system. Infections are commonly reported in immunocompromised, cancer, cystic fibrosis, and COVID-19 patients. Bacteremia can have mortality rates higher than 60%. When living in natural environments and during infections, *S. maltophilia* must survive in bacterial communities where it competes with other microbes for resources and space. Contact-dependent inhibition (CDI) is a common mechanism used by Gram-negative bacteria to eliminate competitor cells. Cells that deploy a CDI can deliver antibacterial proteins to intoxicate adjacent target cells. CDI systems are composed of three proteins: CdiA, CdiB, and CdiI. CdiA is a large, filamentous protein that contains a highly-variable C-terminal toxic domain (CdiA-CT). CdiA binds to receptors on target cells and transports its toxic CdiA-CT domain into the target cell's cytoplasm. The export of CdiA is facilitated by CdiB, which adopts a beta-barrel fold that threads CdiA through its interior lumen. The toxicity of CdiA-CT domains is neutralized by CdiI proteins, which prevent cellular self-intoxication. We discovered that many *S. maltophilia* strains from environmental and patient sources encode CDI loci. We demonstrated that a clinical *S. maltophilia* strain (named CFBR-615) transcribes CDI genes as an operon under standard laboratory conditions. To further study this locus, we showed that the C-terminal region of the *S. maltophilia* CFBR-615 CdiA is toxic when expressed inside *Escherichia coli* cells and has potent antibacterial properties. A CdiI cognate immunity protein encoded downstream of *cdiA* neutralizes its toxicity. We introduced alanine substitutions in putative *S. maltophilia* CdiA active site amino acids using site-directed mutagenesis and observed that these mutations abrogate CdiA toxicity. This study uncovers a new contact-dependent antibacterial toxin that might contribute to the ability of *S. maltophilia* to survive in diverse polymicrobial environments and to compete with other bacteria. The identification of proteins like CdiA that inhibit bacterial growth could lead to the development of novel antibacterial therapeutics against resistant strains.

Functional metagenomic discovery of novel chlorhexidine efflux pumps from across environments

Shuai, W[1], Shen, J[1], Franck, E[2], Allman, H[2], Hartmann E[1], and Crofts, TS[2]
[1]Northwestern University, Evanston, IL
[2]Florida State University, Tallahassee, FL

Antibiotic resistance is a growing concern. While overuse of antibiotics in healthcare and agriculture is a known contributor to this threat, less appreciated is the potential role played by disinfectants and antiseptics. Widespread use of these compounds selects for their own resistance and, concerningly, potentially for cross-resistance to antibiotics. The diversity of resistance mechanisms to disinfectants and antiseptics, and their ability to confer cross-resistance to antibiotics, is understudied. Here, we set out to apply functional metagenomic selections to survey environments at the interface of built and natural environments for resistance to the disinfectant chlorhexidine. Our samples included biofilms from the Shedd aquarium, mouse fecal pellets from a laboratory, and host-associated and pathogenic bacteria. We developed a new method of functional metagenomic library preparation called BarTa (Barcoded Tagmentation) assembly that allowed us to pool, and later demultiplex, dozens of samples from a given environment into a single functional metagenomic library, allowing for cheaper and higher-throughput library preparation and selection. We found that all three environments harbor dozens to hundreds of chlorhexidine resistance genes, with efflux pumps being the dominant mechanism. We link chlorhexidine resistance to multiple novel efflux pump classes and found evidence for antibiotic cross-resistance and multidrug resistance potential. These results reveal that chlorhexidine resistance in microbiomes at the human-nature interface is deep and can potentially mediate the spread of antibiotic resistance.

CRISPR interference (CRISPRi) Technology to Investigate Group B Streptococcal Pathogenesis

Cody Cutts[1], Audrey Sweten[1,2], Thomas A. Hooven[3], Brandon J. Kim[1]
[1]University of Alabama; [2]University of Arizona, [3]University of Pittsburgh of

Streptococcus agalactiae, also known as Group B Streptococcus (GBS), is a Gram-positive bacterium and the leading cause of neonatal meningitis. GBS can be vertically transmitted (mother to neonate) and there is currently no vaccine available on the market. There remains an urgent need to better understand GBS pathogenesis along with the desire to advance current classical mutagenesis techniques which can be laborious and time consuming. Our approach is to use CRISPR interference (CRISPRi) technology to develop a way to quickly and flexibly screen for phenotypes.

“Moonlighting”: Expanding the Proteome in Reduced Genome Organisms

James M. Daubenspeck[1] and Prescott Atkinson[1]
[1]University of Alabama at Birmingham

Moonlighting proteins, a protein that has acquired a secondary function, have been identified in all domains of life. These multi-function proteins are essential for genome reduced organisms like JCVI-syn3A to expand their proteome. One of the more common examples is the enzyme enolase which catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate in glycolysis. In the bacterial cytoplasm enolase is a homo-octamer, but a small percentage is transported to the surface as a monomer where it can no longer dimerize and is thought to function as a receptor. Our data suggests that Mycoplasma species initiate the export of the moonlighting protein by attaching a rhamnose to the protein. In the case of enolase this occurs at the dimerization interface. The rhamnose residue is linked through a phospholipid and anchored to the membrane. Phospholipase D, an enzyme that cleaves the rhamnophospholipid linking proteins to the membrane surface, releases proteins from JCVI-syn3A suggesting that this system is like the one identified in other Mycoplasma species. Our analysis of post-translational modifications in JCVI-syn3A have identified moonlighting proteins that are modified by a single hexose. This hexosylation modification is limited to surface exposed proteins in Mycoplasma species. These data suggest that the moonlighting system is active in JCVI-syn3A and may be essential for growth.

Generating small molecule sensors using nascent regulatory peptides

Alexis O. Delgado-Castillo and Luis R. Cruz-Vera[1]
[1]Department of Biological Science, University of Alabama in Huntsville, Huntsville Alabama 35899.

Bacteria contain several small open reading frames (smORF) that are involved in regulating the expression of operons. These smORFs produced peptides named regulatory nascent peptides (RNP) that interact with the ribosome exit tunnel components generating binding sites for small molecules such as antibiotics, amino acids, or metabolites. Once these small molecules interact with their binding sites (ribosome + RNP), they affect the translation of the smORF changing downstream mRNA synthesis or RNA secondary structures. These last effects modify the expression of downstream genes. We are interested in finding other small molecules that TnaC and the ribosome can detect besides L-Trp. Using bioinformatics and *in vitro* assays that test ribosome capacity to translate, we investigate how changes in L-Trp affect its recognition by TnaC and the ribosome. We observed that the L-Trp binding might depend on the interactions present between the indole ring of L-Trp with the sidechain of the TnaC aspartic residue at position 16th; since L-phenylalanine (L-Phe) and L-tyrosine (L-Tyr) are smaller of L-Trp, those are not sense by TnaC and the ribosome. Certain analogs of L-Trp can compete with L-Trp for the binding site, affecting L-Trp induction to stop translation of the TnaC smORF. Such analogs lack the carboxyl group of L-Trp, and others are methylated in the indole group. Our results suggest that L-Trp binding relies on the indole group, when its function in blocking ribosome translation depends on its total structure. For future experiments, we are expecting to create a modified version of L-Phe or L-Tyr that can be detected by TnaC and the ribosome-blocking translation.

Molecular Microbial Source Tracking to Identify Major Contributors to Dog River, Alabama

Penny Demetriades, Cassie Bates, Cade Kistler, Dakota Bilbrey, Ruth H. Carmichael, Brandi Kiel Reese, Sinéad M. Ní Chadhain

Sources of pathogens to aquatic systems can be human (e.g., wastewater treatment plants, septic systems) and non-human (e.g., livestock, wildlife, domesticated pets) in origin. Pathogens pose a significant threat to coastal resources, human health, and coastal economies, and there is a need to define sources of pathogens for management. Molecular microbial source tracking (MST) methods, such as quantitative PCR (qPCR), can identify sources of fecal contamination by targeting host-associated *Bacteroidales* and identify targets for mitigation of fecal pollution. The Dog River watershed is home to and provides recreation for many local residents and visitors, yet has historically experienced high levels of fecal indicator bacteria (FIB), especially after rainfall events. We partnered with Mobile Baykeeper, a local non-profit conservation organization, to investigate FIB sources from nine sites of tributary convergence within the Dog River. Water samples were collected following dry and wet events in September and October 2022, to determine FIB abundance and sources. Basic water chemistry data were collected along with *Enterococcus* abundance (via traditional plate counts) and qPCR of various host associated *Bacteroidales*. We targeted human, feral hog, dog, and cow associated *Bacteroidales* for source identification, as well as general *Bacteroidales* gut bacteria. Both traditional and molecular methods showed higher enterococcal and *Bacteroidales* abundance following rainfall. In addition, both traditional and qPCR methods found that upstream sites (e.g., MDDR2, NRBB) exhibited higher bacterial loads than downstream sites. Molecular MST analysis revealed that human associated markers were the largest contributor to the Dog River in both wet and dry conditions, with feral hog, dog, and cow markers making minor contributions. Our study shows that surface runoff from significant rainfall events can supply considerable bacterial loads to the Dog River, as has been seen in previous studies. We identified humans as a major contributor of microbial contamination to the Dog River. These results highlight the importance of molecular MST methods to identify sources and concentrations of fecal contamination, which can inform management and allow for appropriate interventions to make Alabama's waterways safer.

Evaluating *Bacillus velenzensis* isolates and culture conditions for inhibition of *Pythium insidiosum*

Georgia Denzik[1], Mark Liles[2], Priscilla Barger[1]
[1]Department of Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL, USA
[2]Department of Biological Sciences, College of Sciences and Mathematics, Auburn University, Auburn, AL, USA

Pythium insidiosum, Paralagenidium karlingii, and *Lagenidium giganteum* forma *caninum* are oomycete pathogens that cause severe cutaneous and disseminated disease in a wide range of animals and are of particular concern in companion animals. Current treatment protocols include aggressive surgical resection followed by prolonged antifungal and corticosteroid therapies. Even with these treatments, the prognosis is extremely guarded. To provide more targeted therapies against these pathogens, anti-oomycete medications are urgently needed. *Bacillus velezensis* is a ubiquitous, non-pathogenic bacterial species that produces a host of secondary metabolites. Metabolites of rhizosphere-associated *B. velenzensis* have been shown to be effective in plant growth promotion and in the control of plant-associated *Pythium* species. To determine if these same isolates have a similar effect on animal pathogenic oomycetes, an established *B. velezensis* library was screened against *Pythium insidiosum* on multiple media types, and zones of inhibition were measured. The results of this pilot study identified that multiple *B. velenzensis* isolates were capable of inhibiting *P. insidiosum* growth *in vitro* and indicated the importance of media composition in expression of *B. velezensis* bioactive secondary metabolites that inhibited oomycete growth. These research results support the potential use of *B. velezensis* in the treatment or control of oomycete pathogens in animal systems. Research is ongoing to screen this library against *P. karglingii* and *L. giganteum* forma *caninum*, and to determine the secondary metabolites responsible for oomycete growth inhibition.

The impact of butyrate on Group B *Streptococcus* intestinal pathogenesis

Kristen Dominguez[1], April K. Lindon[1], Alexia N. Pearah[1], Sophie E. Darch[1], Tara M. Randis[1,2]
[1]Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL
[2]Pediatrics, Division of Neonatology, University of South Florida, Tampa, FL

Group B *Streptococcus* (*Streptococcus agalactiae*; GBS) is a leading cause of neonatal sepsis worldwide. GBS is a pathobiont of the intestinal tract capable of translocating via the paracellular and transcellular routes leading to invasive disease. Neonatal susceptibility to invasive disease stems from immature intestinal barriers. We have previously shown that GBS intestinal colonization induces major transcriptomic changes in the colonic epithelium, specifically pertaining to intestinal barrier function and immune regulation. Butyrate, a microbial metabolite produced by fermentation of dietary fiber, bolsters intestinal barrier function and immune responses to enteric pathogens. Furthermore, maternal diet primes the neonatal intestinal environment and, more specifically, maternal butyrate treatment (mButyrate) fortifies intestinal barrier integrity in offspring. Therefore, we postulated that mButyrate would decrease GBS intestinal pathogenesis via enhanced intestinal barrier function. We used GBS COH-1 serotype III ST-17 in all experiments. First, we used human intestinal epithelial cells (IEC) lines (CACO-2 and T-84) to evaluate how butyrate impacts GBS-induced loss of cell viability, GBS adhesion and invasion of IEC, and epithelial monolayer permeability. Then, we used an established mouse model for post-natal GBS acquisition to evaluate the effects of mButyrate on offspring. Briefly, pregnant dams were either administered butyrate in their drinking water throughout pregnancy and weaning or not given butyrate (control). mButyrate (n=12) and control (n=9) pups were orally gavaged with GBS on P10. Intestines were harvested on P14 and processed for determination of bacterial burden. Weights were recorded 3 times weekly. In IEC, we found that butyrate significantly restores cell viability, reduces GBS invasion, and decreases monolayer permeability. mButyrate pups had decreased GBS colonization in the small intestine and restored weight gain post-infection. Based on these findings, butyrate offsets adverse effects of GBS *in vitro* and has the potential to be used as a maternal intervention for neonatal GBS sepsis.

From Commensal to Opportunist: A Case of *Trueperella bernardiae*-Associated Cellulitis and Septic Shock

Andrew Ensley[1], Sonny Patel[1], Muhammad S Khan[1,2], Henna Iqbal[1]
[1]Mercer University School of Medicine, Macon, GA
[2]Atrium Health-Navicent Hospital, Macon, GA

We hereby report a case of a male in his 60s who presented with altered mental status, hypotension, and a 12 cm air-filled subcutaneous abscess on his back upon physical examination. He had a history of morbid obesity, aortic valve replacement, coronary artery disease, congestive heart failure and insulin-dependent diabetes mellitus. He was suspected of developing septic shock, and prompt surgical drainage of the abscess was performed, followed by intravenous (I/V) administration of vancomycin, cefepime, metronidazole, and piperacillin/tazobactam. MALDI-TOF culture analysis of abscess drainage revealed isolated growth of *Trueperella bernardiae*. The patient's given antibiotics were continued, and he recovered successfully without long-term complications. T. *bernardiae* is considered a non-pathogenic commensal on human skin, oropharynx, and genitourinary tract. However, recently increasing reports of *T. bernardiae* infection have raised the need to consider it as an opportunistic bacterium that can cause a multitude of infections. This specific case emphasizes *T. bernardiae* induced cellulitis and septic shock in the presence of underlying immunocompromise and reports the course of infection, the precise identification technique, and management that led to improvement of his infection.

Neisseria subflava drives colonization resistance against *Pseudomonas aeruginosa*

Kelly Eick[1,2], Jennifer Farrell[1,2], Sam Brown[1,2]
[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA
[2]Center for Microbial Dynamics and Infection, Georgia Institute of Technology, Atlanta, GA, USA

Pseudomonas aeruginosa is a major pathogen in people with cystic fibrosis (CF) and causes significant morbidity and mortality. People with CF also have microbiomes of commensal bacteria in their lungs, which we hypothesized could help prevent the establishment of *P. aeruginosa* infection. In initial work, we inoculated various strains of *P. aeruginosa* at low doses into a synthetic community of commensal organisms *(Streptococcus mitis, Neisseria subflava, and Prevotella melanogenica)* to examine the potential of colonization resistance in the community. We found that the growth of *P. aeruginosa* was reduced in all tested strains by the presence of the community. This prompted us to look closer at how individual community members interact with *P. aeruginosa*. To study the individual interactions within our 3-species community, *P. aeruginosa* was grown with each of the members of the community, alone and in combination, in a synthetic CF sputum medium. We found that *Neisseria subflava*, alone and in pairs, was the primary driver of *P. aeruginosa* growth suppression.

Bactericidal efficacy of diverse R-pyocins against MDR and XDR *Pseudomonas aeruginosa*

Isaac Estrada[1], Stephen P. Diggle[2]
[1]Department of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Pseudomonas aeruginosa (*Pa*), a notorious opportunistic pathogen, poses a significant threat to human health due to its intrinsic resistance mechanisms and the emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains. With the dwindling effectiveness of conventional antibiotics, alternative therapeutic strategies are urgently needed. To address this problem, we explored the potential of R-pyocins, protein-based antimicrobial substances produced by *Pa*, as a novel antimicrobial weapon against these insidious pathogens. R-pyocins produced by both laboratory and clinical strains of *Pa* show bactericidal activity against MDR and XDR high-risk *Pa* strains. To understand the host-range and diverse potency of R-pyocins sourced from clinical strains of *Pa*, we investigated the prevalence of R-pyocin genes in a panel of 100 high-risk *Pa* clinical strains sourced from the Walter Reed Army Institute of Research, characterized as part of the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN). This panel included six of the top ten widespread high-risk sequence types ST235, ST211, ST244, ST357, ST175, and ST654. Genomic typing revealed 60% of these high-risk strains carry genes for at least one subtype of R-pyocins, with significant genetic variation within the R-pyocin subtype sequences. The *in vitro* bactericidal activity of 52 identified R-pyocins was evaluated using spot assays against the 100 high-risk strains and all strains showed susceptibility to at least one R-pyocin. The thermostability of eight extracted R-pyocins was also evaluated and showed structural stability up to 60^{o}C. Lastly, the cytotoxicity of the same eight R-pyocins was investigated using murine Bone Marrow Dendritic cells and did not show any cytotoxic effects. Based on these analyses, we demonstrate that R-pyocins sourced from clinical strains of *Pa* have diverse efficacy and potent antimicrobial activity against high-risk MDR/XDR *Pa* strains. This work highlights the potential of these proteinaceous antimicrobials as an alternative therapeutic option against antibiotic resistant bacterial infections.

Environmental Effects on Polymicrobial Interactions between *A. actinomycetemcomitans* and *S. gordonii*

Gina R. Lewin[1], Emma R. Evans[1], Marvin Whiteley[1]
[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA

Aggregatibacter actinomycetemcomitans (Aa) is a gram-negative bacterium and oral pathogen that can cause gum disease. During infection, it is known to associate itself with the gram-positive oral commensal *Streptococcus gordonii* (Sg), likely in order to cross-feed off of L-lactate that Sg produces. However, Sg is known to produce large amounts of hydrogen peroxide (H_2O_2). To combat this, Aa has mechanisms to detoxify H_2O_2 such as catalase and quinol peroxidase. Here, we investigate the role of these interactions on growth and survival by utilizing a ΔspxB mutant of Sg, which produces significantly less H_2O_2 than wildtype, and a ΔlctD mutant of Aa, which cannot metabolize lactate. We cocultured Sg and Aa in an in-vitro biofilm model on Chemically Defined Medium for Oral Microbes (CDM) under various conditions that they are likely to encounter during human infection. After 24 hours of incubating, the biofilms were harvested and plated for CFUs on selective media. Our results showed that under aerobic conditions, Sg^{WT} growth was improved in coculture with Aa, while Aa tended to perform worse with Sg^{WT} than by itself. By contrast, coculture between $Sg^{\Delta spxB}$ and Aa performed similarly to their respective monocultures. This suggests that the production and detoxification of hydrogen peroxide plays a key role in the interactions between the two bacteria. Additionally, Aa^{WT} outperformed $Aa^{\Delta lctD}$ under several conditions, but Sg grew similarly regardless of which strain it was grown with, implying that cross-feeding lactate has little effect on Sg but is beneficial for Aa. Overall, this study demonstrates a relationship between these bacteria which is both mutualistic and competitive. Sg supplies Aa with additional metabolites but inhibits its growth, and Aa detoxifies H_2O_2 sufficiently to protect Sg from self-inhibiting.

Persistence of host-associated bacterial and mitochondrial DNA markers in sediment microcosms

Yucheng Feng[1] and Wenjing Ren[1]
[1]Department of Crop, Soil and Environmental Sciences, Auburn University, AL, 36849, USA

Monitoring microbial water quality traditionally focuses on measuring microbial parameters in the water column. Sediment, however, has been shown to be a reservoir for fecal bacteria in aquatic environments, and the decay of fecal bacteria and host-associated genetic markers in sediment is understudied. Here, we utilized laboratory microcosms set up in sentinel chambers to examine the persistence of bacterial and mitochondrial DNA (mtDNA) markers associated with humans (HF183 and HcytB), cattle (CowM3 and QMIBo), and chickens (LA35 and Chicken-ND5), as well as the general *Bacteroidales* marker AllBac, in freshwater sediments, using quantitative polymerase chain reactions (qPCR). The microcosms containing freshwater sediment were spiked with sewage, cattle feces, and poultry litter and were buried in saturated sediment. The microcosms were held for a 40-day period, and samples were collected from the sentinel chambers at various time intervals. The results showed that all host-associated bacterial markers decayed faster than mtDNA markers. Cattle-associated markers exhibited prolonged persistence compared with human- and chicken-associated bacterial and mtDNA markers. Additionally, the abundance of the general *Bacteroidales* marker AllBac remained stable throughout the experimental period and showed greater persistence than host-associated bacterial markers. The time required to inactivate 90% of cultivable *E. coli* was significantly longer in microcosms inoculated with cattle feces and poultry litter than in sewage-inoculated microcosms. These findings provide valuable insights into the fate of bacterial and mtDNA markers in sediment. Knowledge of the variability in inactivation rates of *E. coli* from different fecal sources will lead to the development of more reliable predictive models for microbial risk assessment.

Development of a FRET System to Observe Ribosomal Movement over mRNA

Megan Fitzgerald[1], Zheren Ou[1], Alexey Petrov[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL, USA

Ribosomes sequentially read mRNA during translation. During translation initiation, a 40S subunit moves along mRNA searching for a start codon in a process called scanning. Then, in the elongation phase of protein synthesis, ribosomes step precisely by three nucleotides toward the 3′ end of mRNA upon reading each codon. The molecular mechanisms of scanning, translocation, and ribosome movement during these steps are not fully understood. Molecular scale distances can be measured using a Förster resonance energy transfer (FRET). Here, we site-specifically labeled 40S subunits at the RPS26A gene. Using *S. cerevisiae* as a model system, we appended the RPS26A gene with a C-terminal ybbr tag in the strain lacking the RPS26B homolog. The cells were viable, with RPS26-ybbr being the sole source of the protein. Sfp phosphopantetheinyl transferase catalyzes transfer of a dye moiety from CoA-dye substrates to a ybbr tag. The mutant eukaryotic ribosomes were site specifically labeled at the ybbr tag. Preliminary experiments demonstrated FRET between 40S ribosomal subunits and mRNA. The FRET signal is sensitive to 80S ribosome formation. We are planning to use this system for studying scanning initiation, elongation, and frameshifting during translation.

Water quality field test kits turned diagnostic tool for asymptomatic bacteriuria

R. Fleck[1], J.A. Stocki[2], I. Nguyen[1], H.L.T. Mobley[3], and A.E. Shea[1]
[1]University of South Alabama, College of Medicine, Department of Microbiology and Immunology, 5851 USA Drive N, Mobile, Alabama, 36688
[2]Tufts University School of Medicine, 145 Harrison Ave, Boston, MA 02111
[3]University of Michigan Medical School, Department of Microbiology and Immunology, 5641 West Medical Center Drive, Ann Arbor, Michigan 48109

Between 2-10% of pregnant women unknowingly contract asymptomatic bacteriuria (ASB), defined as $>10^5$ colony forming units (CFU) per mL of urine. ASB increases the risk of adverse pregnancy outcomes such as pyelonephritis, preterm labor, and low birth weight infants. While pregnant women in the US are routinely screened for ASB, those in areas with limited resources and funding are without a mechanism for ASB detection. Aquagenx© water quality test kits detect *E. coli,* the most common causative agent of ASB, and total coliform bacteria in drinking water via colorimetric and fluorescent indicators. We evaluated the Aquagenx© system for compatibility with human urine, then proceeded to develop an ASB screening protocol with Aquagenx© using disposable inoculating loops. We diagnosed artificial ASB- samples (10^4 CFU/mL) with a false positive (FP) rate of 33% and ASB+ (10^5 CFU/mL) with a false negative (FN) rate of 6% (n=18). The typical perineal flora present in clean catch urine from healthy volunteers (n=5) did not induce color change or UV indicators. Hospital-acquired patient samples (n=10) confirmed clinical diagnoses with Aquagenx© (FP=0%, FN=0%). We found that Aquagenx© technology is compatible with human urine, the color indicator is specific to *E. coli*, and the UV function allows accurate detection of other coliform contaminants. With a carefully designed urine dilution protocol using plastic inoculating loops, these kits can be used as a diagnostic ASB screening tool in low-resource countries, boasting only a 6% false negative rate in in vitro studies.

When *Klebsiella* takes over: the battle of bacteriophages against *Klebsiella pneumoniae* in a fecal microbiota transplant recipient

Sarah Forsstrom[1], Brandon Berryhill[1], Joshua Manuel[1], Michael Woodworth[2], Bruce Levin[1]
[1] Department of Biology, Emory University; Atlanta, Georgia, 30322, USA.
[2] Department of Medicine, Emory University; Atlanta, Georgia, 30322, USA.

Fecal Microbiota Transplantations (FMTs) are a potential effective treatment for gut microbiome dysbiosis and have been shown to alter the microbial gut community. In addition to the bacteria, these communities include an abundance of bacteriophages (phages). In a recent case study, prior to the administration of the FMT, a metagenomic analysis revealed that a patient's enteric microbiome was found via to be dominated by *Klebsiella pneumoniae.* Following the FMT treatment, all Klebsiella species disappeared. The mechanisms responsible for this loss have yet to be determined. We postulate that phages in the FMT contributed to the treatment efficacy of FMTs. To test this hypothesis, we isolated *K. pneumoniae* and Klebsiella phages from the patient's microbiome and the FMT used for treatment. We are exploring the population dynamics of *K. pneumoniae* and the isolated phages in the patients' gut microbiome prior to the FMT and comparing how the alteration in the patient's gut microbiome after the FTM treatment affect these dynamics. We anticipate that the pre-existing phages in the patient's gut microbiome are able to contribute to the alteration in bacterial composition once the FTM treatment occurred, and we are currently testing this hypothesis.

Effects of gene deletion on Escherichia coli cell growth

Lea Freeman, Kieffer Haehnlein and Luis R. Cruz-Vera[1]
Department of Biological Science, University of Alabama in Huntsville, Huntsville Alabama 35899.

We have seen that expression of several genes were affected by a mutation in ribosomes that generate macrolide resistance. These changes are accompanied by early entrance to the stationary phase during cell growth in rich media. We would like to know which affected gene(s) were causing these effects in cell growth and gene expression. Cell growth was monitored using *E. coli* bacterial cells containing individual deletions of selected genes. To test gene expression, we performed a retro-transcription couple to PCR (RT-PCR) assay in these mutant bacteria. We analyzed bacteria containing deletions of the *cadC_cadBA* operon, which regulates bacterial cell acidification, and the *tnaA* gene that regulates alkalinity in bacterial cells. Both sets of genes were highly affected by the macrolide resistant mutation in ribosomes. Our results show that the *cadBA* operon does not affect cell growth in rich media. However, in absence of a functional *tnaA* gene, bacteria go into stationary phase faster than bacteria with a *tnaA* functional gene. Interestingly, in the absence of the *tnaA* gene activity we did not observe changes in the expression of the *cadC_cadBA* operon as well as other affected genes previously observed in the macrolide resistant bacteria. These last results indicate that the growth phenotype produced by the macrolide resistant bacteria is due to a unique change in the expression of the *tnaA* gene. But changes previously observed in the expression of the *cadC_cadBA* operon is not linked with this growth phenotype.

A FLOW CYTOMETRIC APPROACH TO ANALYSING PHAGOCYTOSIS IN THE AMERICAN COCKROACH

Chad Hamm[1], Elizabeth Schwartz[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL, USA

Much of the previous literature on phagocytosis in insects has been done using microscopy. These studies have utilized fluorescently labeled antigens to study phagocytosis and rely on cells' morphology before and after an antigen's uptake to classify those cells with phagocytic qualities. To add to the scientific knowledge of innate immunity, we are using flow cytometry to quantifiably classify those phagocytic cells by size and complexity into separate populations from the greater whole of the insect hemolymph. Using flow cytometry we used fluorescently labeled latex beads, zymosan particles, and pH-sensitive bacteria to measure phagocytosis activity. We should be able to see not only which populations can phagocytize foreign particles, but also if those cells transport the particles to lysosomes or if there is a selectivity to specific surface antigens. Lastly, we looked at the rate of degradation of these particles as they move through the lysosome. These experiments also lend themselves to future studies using methods like Fluorescence Activated Cell Sorting (FACS) to separate phagocytes from the rest of the population of hemolymph for other functional studies.

Mechanisms of Aggregate Formation in *Pseudomonas aeruginosa*

Alexa D. Gannon[1], Jenet Matlack[2], Sophie E. Darch[1]
University of South Florida, Tampa, Florida, USA[1]
Moffit Cancer Center, Tampa, Florida, USA[2]

Pseudomonas aeruginosa (*Pa*) is an opportunistic pathogen that causes chronic infections in people with cystic fibrosis (CF) and is a major cause of morbidity and mortality. In the CF lung, *Pa* biofilms form as dense, free-floating aggregates of ~10-1,000 cells. This architecture is distinct from the well-recognized "mushroom" shaped biofilms that are observed in classical *in vitro* models. There is a significant gap in our understanding of *Pa* aggregate biology and ecology due to the lack of *in vitro* experimental systems that replicate this spatial organization and its related gene expression and behaviors relevant to *in vivo* conditions. Here, we sought to identify genes important for aggregate formation, with a goal that inhibiting these genes could have therapeutic potential by preventing or disrupting biofilms. We cultured *Pa* in synthetic cystic fibrosis media (SCFM2), which closely recapitulates *in vivo* aggregate size and morphology. Using time-course RNAseq and bioinformatic analyses, we define gene expression patterns over the first 8 hours of aggregate formation, specific to the sputum-like environment. Using confocal laser scanning microscopy (CSLM) and computational pipelines we quantify aggregation impairment in single-gene transposon mutants, and we propose functions for several previously unannotated hypothetical genes in the *Pa* genome. In this study, we demonstrate the importance of using *in vitro* models that more closely replicate human infection sites and therefore bacterial behaviors *in vivo*. Here, we use a multi-disciplinary approach to identify novel genes and mechanisms important for *Pa* aggregate formation. Future studies will focus on methods to disrupt the function of identified genes and may be useful in preventing aggregate formation and spread *in vivo*.

COBRA HA vaccination elicits long-live protective immune responses against pre-pandemic H2, H5, and H7 influenza subtype viruses

Pan Ge [1,2] and Ted M. Ross [1,2,3,4]
[1]Center for Vaccines and Immunology, University of Georgia, Athens, GA. USA
[2]Florida Research and Innovation Center, Cleveland Clinic, Port Saint Lucie, FL, USA
[3]Department of Infectious Diseases. University of Georgia, Athens, GA. USA
[4]Department of Infectious Biology, Lehner Research Institute,
Cleveland Clinic, Cleveland, OH, USA

Highly pathogenic avian influenza (HPAI) viruses remain a great threat to both poultry industry and human public health and continues to spread worldwide. Our laboratory previously demonstrated that adjuvanted pre-pandemic (H2, H5, and H7) HA-based computationally optimized broadly reactive antigen (COBRA) vaccination significantly protects against lethal challenge against both A/Mallard/Minnesota/2008 (H2N3) and A/Sichuan/26221/2014 (H5N6, clade 2.3.4.4) viruses in immunologically naïve mice, as well as mice with pre-existing anti-influenza immunity due to infection with historical H1N1 and H3N2 influenza viruses. In contrast, mock vaccinated mice were not protected against both morbidity and mortality. In this study, mice were vaccinated with COBRA designed for H2, H5, H7 hemagglutinin (HA) and two neuraminidase (NA) HA proteins, N1 and N2. Vaccinated mice were fully protected from lethal H5N6 influenza virus challenge. Following vaccination, collected sera had cross-reactive IgG antibodies against a panel of wide-type H2, H5, and H7 HA and N1 and N2 NA antigens. Mice with pre-existing H1N1 and H3N2 influenza viruses and then vaccinated with these vaccines also had enhanced HA stem antibodies compared to vaccinated naïve mice. In addition, elicited antisera had hemagglutinin-inhibiting (HAI) receptor-blocking activity a panel of H2Nx, H5Nx, and H7Nx influenza viruses. These protective antibodies were maintained up to 4 months post-vaccination. Overall, prior immune responses elicited by historical seasonal influenza infections elicit broader antibodies that recognize a broader number of cross-reactive antibodies elicited against the HA and NA proteins on H2Nx, H5Nx, and H7Nx viral subtypes compared to immunologically naïve hosts.

Evolved heteroresistance following long-term exposure to bacteriostatic antibiotics

Teresa Gil-Gil[1], Brandon A. Berryhill[1], Joshua A. Manuel[1], Andrew P. Smith[1], Fernando Baquero[2], Bruce R. Levin[1]

[1] Department of Biology, Emory University; Atlanta, Georgia, 30322, USA.

[2] Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria, and Centro de Investigación Médica en Red, Epidemiología y Salud Pública (CIBERESP) Madrid, Spain.

Antibiotic heteroresistance (HR) is typically defined by the presence of one or more sub-populations at a frequency of at least 10^{-7} with a resistance level that crosses the breakpoint at or greater than 8 times the susceptible main population. A heteroresistant population appears susceptible to a drug, but upon exposure to the drug resistant sub-populations ascend. However, when the antibiotics is removed, the heteroresistant populations once again appears susceptible to the treating antibiotic. This inherent instability of resistance makes HR clinically problematic. Heteroresistant bacteria have primarily been identified via screening clinical isolates in a retrospective manner. In this study, we explore the pharmaco-, population, and evolutionary dynamics of an *Escherichia coli* strain (MG1655) which has no pre-existing heteroresistance. We exposed this *E. coli* to two bacteriostatic antibiotics, chloramphenicol and azithromycin, for thirty days. The results of our experiments provide evidence suggesting bacteria exposed to these drugs replicate, evolve, and generate a sub-population of small colony variants (SCVs). These SCVs, apart from presenting a substantial increase in the minimum inhibitory concentration (MIC) to the drug they were exposed to, rapidly revert back to normal colony variants which have a lower MIC. Hence, the pressure of these drugs selects for the evolution of HR in populations previously lacking this trait.

Landfills shape the native microbial community and selection for antimicrobial resistance.

Anuradha Goswami[1], J. Jeffrey Morris[1]
[1]Department of Biology, University of Alabama at Birmingham, AL, USA

Antibiotic overuse has resulted in the threat of antimicrobial resistance (AMR). Through surveys and genetic analysis, current research seeks to intervene in the causes of AMR. Soil samples were collected from the open dump at North Birmingham landfill, superfund sites, the city center, and neighboring residential areas in Birmingham, AL. Samples were characterized through 16S rRNA sequencing. The co-occurrence species was identified through network analysis, and the functional traits were imported into the network to find the hub species and metagenome (functional annotation) in the occurrence of AMR. The research outline two specific hypotheses were:
Hypothesis 1: Microbial interaction between landfill microbiomes could support microbial survival and potentially shape the microbial community.
Hypothesis 2: Microbes can develop antibiotic resistance by affecting fundamental non-AMR pathways strongly linked to AMR genes.
The study shows landfill sites could be a vault of AMR emergence. It also states that metal pollution affects soil diversity and induces cross-resistant AMR species. The landfill core microbiome comprises 11 distinct taxa from Acidobacteria, Actinobacteria, Chloroflexi, and Proteobacteria phylum. These microbes were estimated to correlate positively with 456 other microbes in the microbial network. Moreover, three distinct landfill microbes belonging to Blastocatellaceae, Vicinamibacterales and Xanthobacteraceae were found to be mutually exclusive or in competition with 266 microbes in the community. The resistance of AMR genes related to antibiotic targets such as tetracycline- tetB, vancomycin- van RB, RC, and SC were significantly higher in landfill and superfund samples. Network analysis predicted that AMR genes in landfill microbiomes are closely associated and may interact with essential pathways, such as metabolism, signal transduction, the two-component system, transporters, and transcription factors. The study commenced a debate stating that the landfill sites are a rich source of emerging new tolerant species and, therefore, can no longer be neglected when discussing AMR.

Determining the role of the gut microbiome in *B. germanica* fipronil resistance

Samantha Grady[1], Arthur Appel[2], Elizabeth Hiltbold Schwartz[1]
[1] Department of Biological Sciences, Auburn University, Auburn, AL
[2] Department of Entomology and Plant Pathology, Auburn University, Auburn, AL
[3] Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH

The German cockroach (*Blattella germanica*) is a eusocial urban pest that has developed resistance to a variety of insecticides since their introduction in the 1960's. Resistance to fipronil, a phenylpyrazole compound, has previously been associated with increased cytochrome P450 expression in *B. germanica*. Recent studies have suggested that the *B. germanica* gut microbiome, such as *Pseudomonas* and *Enterobacter spp*., may contribute to this resistance through metabolic degradation for use as a carbon or nitrogen source. Fipronil metabolizing bacteria have primarily been isolated from field-caught *B. germanica*, which may have been exposed to multiple insecticide classes, thus specific resistance to this insecticide has not been selected for. Thus, the bacteria and the metabolic pathways associated with fipronil degradation are poorly understood. To address this, we have used two approaches, first we have characterized the gut microbiome of a fipronil resistant lab strain of *B. germanica* and compared it to a susceptible strain using 16S rRNA sequencing. We have also used fipronil selection to 1) isolate bacteria from whole gut homogenates that are capable of using fipronil as a carbon source and 2) culture *B. germanica* on low doses of fipronil in drinking water. We will then compare the microbiome of the selected animals to untreated controls. This data provides a greater understanding of the microbial mechanisms underlying fipronil metabolism in the German cockroach gut.

Describing the effects of antibiotic-resistance ribosomes on gene expression in Escherichia coli

Kieffer Haehnlein[1] and Luis R. Cruz-Vera[1Ŧ]
[1]Department of Biological Science, University of Alabama in Huntsville, Huntsville, Alabama 35899.

The ribosome is the cellular machinery responsible for protein synthesis and it is the target of antibiotics such as macrolides. All newly synthesized peptides leave the ribosome through a cavity called the exit tunnel, which is composed of the ribosomal proteins uL4, uL22, uL23 and uL24, and nucleotide of a large ribosomal RNA (23S rRNA). The lysine-dependent acid-resistance gene *cadB*, for example, is affected by the presence of a mutant ribosomal protein uL22(K90D). This project aimed to record how *cadB* expression is affected by the presence of the uL22(K90D) mutant protein, as well as by the presence of a mutation in the 23S rRNA located at the same tunnel region (named 23S rRNA +A751ins). Bacterial strains containing either mutant genes, uL22(K90D) or 23S rRNA +A751ins, were transformed with two types of plasmids carrying a green fluorescent protein (GFP) reporter gene. One plasmid contains a transcriptional fusion between *cadB* and GFP, whereas the other plasmid contains a protein fusion with both genes. Cellular growth curves were performed in neutral and in acidic condition under rich media to determine the time for maximum expression of the reporter genes. Detection of GFP was achieved using confocal microscopy. Results showed a decrease in transcriptional activity among bacteria with the K90D mutation compared to the wild type cells. A difference was also observed between the wild type and mutant cells carrying the protein fusion plasmid; however, these results were less obvious than the results for transcriptional activity. No observable significant difference was detected in transcriptional nor translational activity between wild type and 23S rRNA +A751ins mutant cells. Our results suggest that changes at the uL22 protein residues at the constriction region have more effects in gene expression than changes in the 23S rRNA.

4-Ethoxybenzoic Acid-Containing Hydrogels Reduce Biofilm Formation by *Staphylococcus aureus*

Muhammad A. Haider[1], Eric S. Gilbert[1]
[1]Georgia State University

Biofilm-associated infections, frequently attributed to microorganisms such as *Staphylococcus aureus*, represent a critical public health concern, particularly within healthcare environments. In preliminary work, 4-ethoxybenzoic acid (4EB) was identified as an anti-biofilm, anti-pathogenic compound, inhibiting biofilm formation by nearly 90 percent with minimal impact on planktonic growth. In this research, the potential of 4EB to control *S. aureus* biofilm formation when incorporated into a hydrogel coating was investigated. *S. aureus* was cultured on 1.5 percent agarose, a representative hydrogel, with or without included 4EB. Amyloid protein concentrations in the biofilm extracellular matrix were measured using the thioflavin T assay and identified an 85 percent reduction in fluorescence in agarose containing 0.8 mg/ml 4EB relative to untreated controls. Crystal violet staining of biofilms to measure accumulated biomass demonstrated up to 70 percent reduction in biofilm formation compared to untreated controls on agarose containing 1 mg/ml 4EB. Conversely, incorporation of 4EB into a hydrophobic polymer coating resulted in an inconsistent, concentration-independent impact on biofilm formation. These collective findings provide support for the hypothesis that 4EB's anti-biofilm activity within the hydrogel stems from its diffusion into the aqueous phase, suggesting promising avenues for further exploration in mitigating biofilm-related infections.

Biofilm formation of *Streptococcus equi* ssp. *equi* alters antibiotic susceptibility *in vitro*

Annalie Harris[1], Bryce Thomas[1] DeLacy Rhodes[1]
[1]Department of Biology, Berry College, Rome, GA, USA

Streptococcus equi subspecies *equi* (SEE) is the causative agent of strangles, a highly contagious disease affecting the upper respiratory tract of equines. Strangles is characterized by sudden-onset fever, purulent nasal discharge, and large abscesses in the lymph nodes that can rupture, causing respiratory distress. Recent work in the Rhodes lab has established that SEE has the ability to form biofilms *in vitro* when the bacteria are cultured with 5% CO2. The purpose of this study was to determine if growth in a biofilm affects the susceptibility of SEE to the common antibiotics penicillin, amoxicillin, tetracycline, and azithromycin. To assess antibiotic susceptibility, antibiotic assays were performed with SEE grown both planktonically and in a biofilm, and the minimum inhibitory concentration (MIC) of each compound was determined. Following 24 hours of antibiotic exposure, resazurin was used to detect living cells and absorbance was measured. Data showed that SEE living in a biofilm requires significantly higher concentrations of antibiotics to achieve the same level of growth inhibition as determined with planktonic cultures. As current antibiotic treatment of strangles has been developed without the consideration of biofilm formation, treatment protocols may be incomplete in clearing an SEE infection. Future studies are warranted to fully elucidate the role of biofilms within an equine host and to determine the best protocol for antibiotic treatment for strangles.

Uncovering the interplay between patient variables and uropathogen biology

Sicily Hardy[1], Rachel Fleck[1], Olena Gordonya[1], Juleigh Jeffreys[1], Ryan Walde[#], and **A.E. Shea[1]**
[1]Department of Microbiology and Immunology, University of South Alabama Medical School, 5851 USA Drive N, Mobile, AL 36688
[#]Department of Pathology, University of South Alabama Medical School, 2451 University Hospital Drive, Mobile, AL 36617

Urinary tract infections (UTIs) affect a broad patient population and inflict a substantial financial burden on the U.S. healthcare system: they are the second most frequent infectious disease worldwide. Nationally, over 70-80% of UTIs are caused by uropathogenic *Escherichia coli* (UPEC), which is often contracted via autoinoculation from the gut microbiota. Interestingly, an analysis of patient data from our University Healthcare System serving the Gulf Coast region of Alabama determined that out of 3366 confirmed cases, only 47.33% were caused by UPEC. This is a major deviation from the national reported average. We also observed increased numbers of cases where the causative agents were *Klebsiella spp*. and *Proteus spp*., which are traditionally considered to be associated with complicated UTIs. We are therefore poised to perform a unique study. Our overarching goal is to define the pathogen- and patient-associated variables underlying these observed regional differences in the Gulf Coast. Based on data collected thus far, patient BMI to infectious etiology revealed a higher median BMI in patients with *Enterobacter*-derived UTI. When determining preliminary odds ratios for the likelihood of females versus males contracting specific UTI pathogens, we observed that *Proteus mirabilis* skews at a ratio of 17:1 towards infecting biological females. Beyond the correlative analyses, we will dive into the pathogen biology and virulence factors associated with unique patient variables and their respective disease outcomes. Overall, with this large clinical study involving diverse patient and pathogen populations, we will produce a more holistic view of uropathogenesis.

Patient and Pathogen Diversity in Urinary Tract Infections

Sicily Hardy[1]
[1]Department of Microbiology, University of South Alabama, Mobile, AL, USA

A urinary tract infection (UTI) occurs when bacteria enter the urethra and infect the urinary tract. In some cases, UTIs progress to severe outcomes such as urosepsis and pyelonephritis. UTIs are the second most frequent infectious disease worldwide, affecting a large patient population resulting in significant healthcare costs. Nationally, epidemiologic data demonstrates that UPEC accounts for 70-80% of UTI cases. Mobile, AL has greater racial and socioeconomic diversity compared to the national average. Non-biological factors such as socioeconomic status are known to influence disease outcomes. I hypothesized that because of Mobile's differing demographics, the incidence of UPEC will not reflect the national averages. My goal was to see if there was any predictive correlation between patient variables and the severity of disease outcomes. Colony PCR was used to screen for the DNA genome of clinical strains of UPEC (n= 24) patients to determine the toxigenicity. Furthermore, to assess the ability of UPEC strains to move via flagella, I completed three trials of motility assays. Results showed that the number of toxins carried in the genome correlated with patient sex and sepsis outcomes. Strains with a greater number of toxins in their genome were also determined to more frequently cause sepsis. Polymicrobial infections were associated with pregnant patients and/or increased body mass. These data suggest that further studies using an increased sample number are required to fully define the pathogenic profile and bacterial variance associated with diverse patient cohorts. Based on these findings, we can use pathogen/strain biology to predict patient risk and disease outcomes.

Potential efflux pump inhibitor Ber-C3 interacts with MexY residue 175 in *P. aeruginosa* MexXY-OprM

Shraddha M. Hariharan[1], Logan G. Kavanaugh[1,2], Graeme L. Conn[1,3]

[1] Department of Biochemistry, Emory University School of Medicine, Atlanta, GA
[2] Graduate Program in Microbiology and Molecular Genetics, Emory University, Atlanta, GA
[3] Emory Antibiotic Resistance Center, Emory University, Atlanta, GA

Antibiotic export through efflux is a prominent resistance mechanism in pathogenic bacteria. For example, the MexXY-OprM system of *Pseudomonas aeruginosa* plays a critical role in clinical resistance development to aminoglycoside antibiotics. MexXY-OprM, a member of the Resistance-Nodulation-Division (RND) efflux pump superfamily, is a tripartite complex comprising an inner membrane transporter (MexY), periplasmic adaptor (MexX), and outer membrane protein (OprM). MexY is the primary site for recognition and efflux of substrates such as aminoglycoside antibiotics, and understanding the selective binding mechanism may lay the foundation for developing efflux pump inhibitors (EPIs). Our lab previously identified a di-berberine conjugate, Ber-C3, as a specific inhibitor of MexXY-OprM. Ber-C3 synergized with the aminoglycoside gentamicin (Gen), reducing this antibiotic's minimum inhibitory concentration (MIC) in *P. aeruginosa* strains PAO1, PA7, and PA14; however, Ber-C3 showed decreased efficacy in PA14. We hypothesize that the observed difference is correlated to a residue variation in the PA7/PAO1 (E175) and PA14 (Q175) MexY distal binding pockets containing the drug binding site. In this study, we aim to elucidate the role of residue 175 in the binding affinity of substrate antibiotics (e.g., Gen) and efflux pump inhibitors (e.g., Ber-C3). Binding interactions of $MexY^{E175}$ and $MexY^{Q175}$ with Gen and Ber-C3 will be assessed *in-vitro* via isothermal titration calorimetry (ITC) and *in-vivo* via MIC assays. Thus far, we have established an expression system and purification procedures for preparing large quantities of MexY for binding assays. Additionally, MIC assays suggest that the switch from glutamic acid to glutamine at position 175 may be important for Ber-C3 conformation and binding interactions, but additional potential contributors to decreased Ber-C3 efficacy in PA14 remain uncovered. This study reveals possible binding mechanisms of Ber-C3 in the MexY transporter and furthers our broader investigations of Ber-C3 as a possible lead for EPI development to combat efflux-based antibiotic resistance.

Phenotypic characterization of *Lactobacillaceae* strains isolated from human urinary microbiome

Rayan Haque, Layla Jeries, Rachel McCullars, Wren Jenkins, Tatyana Sysoeva
University of Alabama in Huntsville

Study of the relationship between urinary microbiome composition and urinary conditions such as urinary tract infections (UTIs) and kidney stones could lead to the development of more effective treatments and prevention methods. Multiple species of *Lactobacillaceae* are found in the urinary microbiome. The goal of this research was to phenotypically assess two strains of lactobacilli in terms of their relationship with these two common conditions. An *L. gasseri* 5006-2 isolate obtained from a healthy, female patient and an *L. delbrueckii* 5010-2 isolate obtained from a female patient diagnosed with recurrent UTI were studied. The two strains were assessed for their inhibition of common uropathogenic *E. coli* growth. In agar plates, wells in the middle of seeded *E. coli* lawns on an agar plate were filled with lactobacilli liquid culture and assessed for lawn formation upon 48 hours incubation. Zones of inhibition formed on plates with the wild type *E. coli* strain SVS 1144-WT and a mutant strain SVS 1144-K90D with an altered acid stress response were compared to ascertain whether lactobacilli inhibit *E. coli* growth via acidification. No significant difference in Inhibition mutant and wild type *E. coli* strains were observed, suggesting that lowered environmental pH is not the main method by which lactobacilli inhibit *E. coli* growth and prevent UTIs. Lactobacilli growth in the presence of oxalate was also studied as oxalate-rich urinary microbiomes are predisposed to kidney stone development. Lactobacilli streaks were able to grow on oxalate-rich agar plates. Lactobacilli liquid culture was grown in a 96-well-plate with 10 mM sodium oxalate solution and growth curves over 24 hours were assessed via OD readings taken by a plate reader. Preliminary results suggest that both strains are inhibited by oxalate. Further characterization of lactobacilli can guide the development of improved treatment and prevention plans for common urinary conditions.

Harnessing Bacterial Activity for Sustainable Decolorization of Textile Dyes and Pollution Mitigation.

Hayden

With global concerns over water pollution intensifying due to industrial effluent discharge, the textile sector emerges as a major contributor to environmental deterioration. This study delves into a novel approach using laboratory bacteria, namely Micrococcus luteus, Bacillus subtilis, Pseudomonas sp, and Enterobacter, to effectively decolorize and biodegrade textile dyes. In contrast to conventional methods, this research investigates the potential of bacterial-driven bio-treatment to eliminate undesired color and toxicity from textile effluents. Through spectrophotometric analysis, the decolorization efficacy of pure bacterial cultures was evaluated over incubation periods of 3, 5, and 7 days. The dyes under examination included Congo Red, Acid Yellow 23 (Tartrazine), Orange II (sodium salt), and Acid Black dyes, commonly employed in the textile industry. Notably, all selected bacterial strains exhibited robust decolorization activity, with the most pronounced results observed after a 7-day incubation, accompanied by minor variations. This investigation underscores the capability of some selective bacterial species to efficiently metabolize dyes frequently utilized in textile manufacturing, thus highlighting their potential significance in industrial effluent treatment. By embracing bacterial-mediated decolorization, this study proposes a sustainable avenue to alleviate the pollution burden stemming from textile wastewater, paving the way for eco-friendly and effective remediation strategies.

Analysis of Interbacterial Competition Amongst Lactobacilli Isolated from the Human Urinary Tract

Kaylie Hintze[1], Tatyana Sysoeva[1]
[1]Department of Biological Sciences, The University of Alabama Huntsville, Huntsville, AL, USA

The rise of antibiotic resistant urinary tract infections (UTIs) has led to an increased interest in alternative treatment and prevention methods that do not rely on traditional antibiotic applications. Our prior study showed that most of the tested urinary lactobacilli have the ability to inhibit uropathogens and therefore can be furthered studied as a clinical application. However, female urinary microbiome is usually populated with one or few species of lactobacilli already. It is not known how the commensal urinary lactobacilli interact amongst themselves and how the introduction of a better uropathogen-competing strain into the bladder will affect urinary microbiome or UTI development. To determine possible competitive relationships between the lactobacilli species, a series of well-diffusion assay experiments were performed. Using this technique, we identified 5 urinary isolates of *Lacticaseibacillus rhamnosus*. *L. rhamnosus* has a strong ability to inhibit uropathogenic growth and is a promising candidate for preventative UTI treatment. We tested the ability of *L. rhamnosus* to inhibit isolates of 4 other species of urinary lactobacilli: *Lactobacillus gasseri*, *Lactobacillus delbrueckii*, *Lactobacillus animalis*, and *Lactobacillus johnsonii*. Both, *L. rhamnosus* cells and spent medium, were tested to determine whether observed inhibition was cell-dependent. The results showed that all tested strains of *L. rhamnosus* could significantly inhibit *L. delbrueckii* species in a cell-dependent manner but could minimally or could not inhibit the other lactobacillus. This inhibition was present amongst all tested strains of available urinary isolate *L. delbrueckii*. To follow up on this interlactobacilli inhibition, we established draft genomes of the used urinary lactobacilli for further analysis. Early genetic analysis of the strains indicates that the *L. delbrueckii* strains show the presence of a lysogenic phage most similar to *L. delbrueckii phage* JCL 1032 which could cause *L. delbrueckii* to be less capable of competing with strains such as *L. rhamnosus.*

Alternative Mechanisms of Prokaryotic Translation Initiation

Brielle Sorkin[1], Tianhan Huang[1], Alexey Petrov[1]
**B.S. and T.H contributed equally*
1 Department of Biological Sciences, Auburn University, Auburn, AL, USA

In a textbook view of prokaryotic protein synthesis, translation initiation is mediated by the Shine-Dalgarno (SD) sequence in mRNA, located 7-11 nucleotides upstream of the start codon. The SD sequence recruits mRNA to the ribosome by base pairing it with a complimentary anti-SD sequence in 16S rRNA, resulting in interactions that determine the strength, efficiency, and fidelity of initiation. Initiation Factor 2 (IF2), in complex with GTP, recruits initiator tRNA to the 30S ribosomal subunit, while IF1 and IF3 enhance the fidelity of the process. Following start codon recognition, the 50S subunit is recruited and joins in a partial-rotated conformation. Upon GTP hydrolysis by IF2, the ribosome then changes to the non-rotated conformation. However, only about 70% of mRNA in *E. coli* carry the SD sequence, while the remaining 30% initiate via alternative mechanisms. Within the realm of non-canonical mRNAs lie "leaderless" mRNAs, which lack a 5′ UTR and, consequently, SD sequence. Translation of leaderless mRNAs is part of a stress response mechanism to unfavorable conditions, remodeling the bacterial proteome and allowing bacteria to rapidly adapt to changing environments. Little is known about the mechanism of leaderless translation, despite the commonality of these mRNAs in Eubacteria and Archaea. To define the mechanism of leaderless initiation, we developed a novel system that allows for the following of translation at the single-molecule level. Single-molecule fluorescence methods permit the direct observation of initiation using fluorescently-labeled ribosomal subunits and mRNA to follow mRNA recruitment, initiator tRNA binding, formation of the complete 70S ribosome, and ribosome conformation throughout initiation. The results reported here suggest that leaderless initiation occurs through two different pathways. The ratio of these pathways is influenced by buffer condition, initiation factors, the S1 protein, and 50S and mRNA concentrations. The aim of this work is to delineate leaderless translation, thus paving the way to understanding bacterial physiology, gene expression, stress adaptation, and the evolution of protein synthesis.

Failed Lumbar Fusions in Patients with Renal Insufficiency Undergoing Transforaminal Lumbar Interbody Surgery. A Retrospective Study

M. Grace Hurley, MS [1], Chad Evans, MD [2]
[1] Mercer University School of Medicine, 1633 1St Avenue, Columbus, Georgia, 31901
[2]The Hughston Foundation, 6262 Veteran's Parkway, Columbus, Georgia, 31909

Introduction: Renal insufficiency is a medical condition in which the kidneys do not adequately filter waste products from the blood and may result from reduced blood flow, genetic factors, or chronic urinary tract infections. Patients with reduced renal function often have complicated comorbidities including susceptibility to infection and osteoporosis. Patients with chronic kidney disease undergoing spinal fusion surgeries have an in-hospital elevated mortality risk. This study investigates patients with renal insufficiency undergoing transforaminal lumbar interbody fusion (TLIF) surgeries and analyses rates of fusion failures as evidenced by pedicle screw loosening, pseudarthrosis, implant migration or implant subsidence.
Methods and Materials: This is a retrospective single center analysis of all patients with renal insufficiency who underwent a TLIF between 2017 and 2022. 2639 patients were identified and studied. Comorbidities studied including Body Mass Index (BMI), surgical complications, operative time, tobacco use, diabetes mellitus, dyslipidemia, autoimmunity, and implant types.
Results: The retrospective analysis reviewed data from 2639 TLIF patients; 61 patients were diagnosed with renal insufficiency prior to their TLIF procedure. At last follow-up, 2 of the 61 patients had pseudarthrosis of the fusion site, and 1 had a surgical site infection. This represents a 3.28% (2/61) pseudarthrosis rate and a 4.91% (3/61) adverse event occurrence rate. There were no in-hospital deaths.
Discussion: Transforaminal lumbar interbody fusion surgery is a common surgical procedure. Fusion rates have been reported to range from 72.7% to 97.4% depending upon the type of graft, surgical exposure, and length of follow up [1,2]. In this review, TLIF patients with renal insufficiency has a similar fusion rate and complications when compared to patients in large studies with normal renal function. The Hughston Clinic TLIF fusion rate was over 90%.
Conclusion: Our study reveals no significant association between renal insufficiency and reduced rates of spinal fusion in TLIF patients. Surgeons should be vigilant about the heightened risk of pseudarthrosis in renal insufficiency patients, which could significantly impact outcomes. Further prospective investigations are required to validate these findings conclusively.

An innovative approach to teach bacterial classification in pre-clinical microbiology education

Henna Iqbal[1], Kenneth Onyedibe[1]
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA, USA

Bacteria form an intense component of reading and learning for students enrolled in microbiology education. Moreover, the foundational topic of bacterial classification represents a significant portion of introductory bacteriology coursework. The purpose of our study was to analyze whether bacterial classification can be taught with a different approach which might be more engaging and beneficial to students, especially in pre-clinical programs. The participants of this study were students enrolled in a two-semester medical school bridge program that offers a Master's degree in Pre-clinical Sciences. To implement our study, we presented two equally timed sessions in classroom. While the first session was based on the traditionally accepted method of bacterial classification using Gram staining and morphology, the other session presented a unique approach of understanding bacterial origin and classification in the light of evolution. We used a phylogenetic tree to signify clinically relevant groups of bacteria and highlight characteristics of individual species using the knowledge of evolutionary relationships. An online survey was distributed to the students to collect their feedback after the two lecture sessions. The results from the survey showed that 74% of participants would prefer to learn bacterial classification using a combined approach which includes both Gram-staining and morphology as well as the phylogenetic tree. When asked if the study of bacterial classification through an evolutionary tree diagram is a clear and concise way of understanding bacteria, 79% of the students either agreed or strongly agreed to this statement. Additionally, 78% of students considered the alternative tree-based approach more engaging, and 71% responded that the new approach has the potential to expand their clinical knowledge of bacteria. Overall, our study strongly supports the use of phylogenetic tree-based classification as an additional method to improve the learning of medically important groups of bacteria at varying levels of education.

Mobile genetic elements bridge the gap between clades in *Pseudomonas aeruginosa*

Iris Irby[1], Eli Mehlferber[1], Hana O'Hagan[1], Anthony Candelmo[1], Sam Brown[1]
[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Pseudomonas aeruginosa is a gram-negative bacterial pathogen which causes a wide range of chronic and acute human infections. *Pseudomonas aeruginosa* is classified into three primary phylogenetic clades: clades A, B, and C, each with a unique phylogenetically structured core genome. Our work demonstrates that their accessory genomes exhibit a similar phylogenetic structure. This structure motivates our investigation into the connections between clades through mobile genetic elements (MGEs), specifically plasmids and prophages, through bioinformatic and experimental approaches. We identified 2,553 prophage regions in 476 closed *P. aeruginosa* genomes, along with 135 plasmids from NCBI. We then clustered these MGEs into similar groups using ANI. Using the dates of sample collection, we tracked the of the spread of the MGEs. We revealed that these MGE clusters are either generalists, infecting all three clades and with frequent intercladal transfer, or specialists, exclusively transmitting within a single clade. Experimentally, we assessed the activity of a generalist prophage found in two distinct clades, demonstrating its capacity to infect a diverse array of members from the opposing clade.
Both generalists and specialist MGEs carry dangerous gene cargo. We found that prophage generalists may harbor a small antibiotic resistance gene, while plasmid generalists can accommodate a substantial number of resistance genes. Prophage specialists found in small clusters often carry a high number of resistance genes, but exhibit limited horizontal transmission capability. This suggests dormancy of such prophages, raising the possibility of their evolution into pathogenicity islands. This study underscores the critical role of MGEs in shaping the genetic landscape of *P. aeruginosa* and identifies a potential evolutionary endpoint for prophages.

Outcome of Zaire *ebolavirus* infection linked to specific proteins in Sierra Leone

Ellen Jones[1], Kenneth Onyedibe[1]
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA

The presence of proteins in human viruses impacts the host response. Between May and June of 2014, 160 genomes of *ebolavirus* were analyzed from infected patients in Sierra Leonean hospitals. Study data was from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) database (https://www.bv-brc.org/). Of the 160 genomes, 77(48%) were fully coded. Fifty-four (70%) were from patients who died while twenty-three (30%) were discharged. In the 77 fully coded *ebolavirus* genomes, there were 11 different proteins and seven of these were found in each of the 77 strains irrespective of outcome. The other four proteins (large structural proteins; glycoprotein 1,2; virion spike glycoprotein; and RNA-directed polymerase large) were found in some but not others. Virion spike glycoprotein was found in five (6%) of the 77 fully coded strains and 80% (4/5) were in patients that died. The glycoprotein 1,2 was found in every other genome where there was no virion spike glycoprotein (72/77). Fifty (69%) were in patients that died, while twenty-two were discharged. RNA-directed polymerase was found in eight (10%) of the 77 genomes and were not seen in the strains with large structural protein. However, 88% (7/8) of patients with genomes containing RNA-directed polymerase died. The one discharged patient with RNA-directed polymerase was also the only other patient whose genome had virion spike glycoprotein that survived. We used a paired two-sample t-test based on outcomes (discharged vs died) to determine significance of results which yielded a t-critical value of 2.23 and p-value of 0.006. Thus, suggesting that presence of certain proteins and fatality outcome may be associated, although this was from a limited sample size. Further research into the association of the genome coding for virion spike glycoproteins and RNA-directed polymerase should be conducted as these strains were more likely to be fatal.

Methods of Manipulation- The Role EBV's LMP1 has on SUMO-activating Enzyme

Kristy E. Jones, T. Tyler Moore, John P. Cheek, Haaris Ahmed, Jessica Jenkins, Gretchen L. Bentz
Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, USA

Epstein-Barr virus, a ubiquitous herpesvirus, is classified as a group 1 carcinogen and is associated with 1.5% of all human cancers. The principal viral oncoprotein is latent membrane protein 1 (LMP1), a constitutively active member of the tumor necrosis factor receptor family that mimics CD40. LMP1 activates multiple signaling pathways that can be regulated by post-translational modifications, such as phosphorylation and ubiquitination. LMP1 carries out these signal transduction events through C-terminal activating regions (CTAR)-1 and CTAR2. We documented that LMP1's much less studied CTAR3 modulates a different post-translational modification, sumoylation, where the small ubiquitin-like modifier protein is covalently attached to a target protein, via interaction with the SUMO-conjugating enzyme, Ubc9. We documented that SUMOylated proteins accumulate in LMP1-positive lymphomas, and the oncoprotein targets multiple steps of the sumoylation process to promote this accumulation. Here, we investigated if LMP1 affected the SUMO-activating enzyme, a dimer of SAE1 and SAE2, which adenylates SUMO, activating it and allowing SUMO to be transferred to Ubc9. We hypothesized that LMP1 targets SAE1 and SAE2, which augments the increased protein sumoylation observed in EBV-associated malignancies. First, native immunoprecipitations were performed to investigate if LMP1 interacts with the SUMO-activating enzyme. Results showed that LMP1 hijacked the SUMO E1, which resulted in its increased interaction with SUMO, increased SUMO activation, and increased sumoylation of known target proteins. Second, immunofluorescence microscopy was performed, and data revealed that the presence of EBV and expression of LMP1 increased the cytoplasmic localization of the SUMO-activating enzyme. We concluded that EBV LMP1 does manipulate the SUMO-activating enzyme, altering its localization, and promoting the accumulation of SUMOylate proteins in cells. Because the dysregulation of sumoylation processes is associated with the numerous diseases, these findings help further elucidate the multipronged approach by which LMP1 manipulates a cellular process and contributes to viral pathogenesis.

Antimicrobial Effect of Lemon Pulp on Fish Spoilage Bacteria

Omolola C. Kafi[1], Mediha Aksoy[2], Burak Aksoy[1], Benjamin Beck[2]
[1]College of Forestry, Wildlife and Environment, Auburn University, Auburn, AL, USA
[2]USDA-ARS Aquatic Animal Health Research Unit, Auburn, AL, USA

Seafood is highly regarded for its high-quality proteins, n-3 polyunsaturated fatty acids, and other nutrients. However, seafood products are highly perishable, making up 35% of food wasted in the United States. Bacteria is the major cause of spoilage for most seafood products, making preservation to elongate the shelf life of utmost importance. The use of chemical food preservatives which have been called 'slow poisons', though effective, confer various adverse side effects. Substitution with natural or 'greener' alternatives, however, has been on the rise. This preliminary work investigates the effect of modified lemon pulp (LP) on inhibiting spoilage bacteria and increasing the shelf life of catfish fillets. Prominent spoilage bacteria (labeled 'D' and 'L') in rancid fillets were isolated and inoculated into growth media of varying LP concentrations. LP demonstrated a considerable bacteriostatic effect on isolate 'D', with no visible growth to $1.8x10^7$ CFU/mL on 0.2% and 0.1% LP concentrations respectively. However, LP had no significant effect on growth of isolate 'L'. The observed antimicrobial effect of LP on the prominent fish spoilage bacteria was tested using packaging coated with LP in comparison to control (uncoated packaging). In the control group, spoilage bacteria exhibited a 5-log increase in growth; however, no visible growth of bacteria was detected in the fish fillet stored in LP-coated paper after 5 days of refrigeration (4°C) storage. Following 11 days of storage, bacterial growth reached $6x10^6$ and $3x10^7$ CFU/mL for fillets stored in LP-coated paper and control fillets, respectively. Modified LP has potential as an alternative strategy to reduce synthetic preservatives and extend the shelf life of seafood and similar products with controlled environmental impact through innovative inclusion in food packaging.

Rapid adaptation of a pathogen population in response to climatic fluctuations

Amanpreet Kaur[1], Ivory Russell[1], Neha Potnis[1]
[1]Department of Entomology and Plant Pathology, Auburn University

Climatic fluctuations pose a growing threat of altered plant disease dynamics, with expanded host or geographical ranges. Disease resistance is the most effective and environmentally sound approach to manage diseases. However, we lack understanding of how climatic fluctuations may alter plant-pathogen interactions and what efficient ways would be to manage the disease. In this study, we assessed the efficacy of disease resistance in pepper against a leaf spot pathogen, *Xanthomonas perforans*, under elevated ozone in open-top chamber field conditions. The study design also involved use of two pathogen genotypes, mimicking co-occurrence of different pathogen genotypes in pepper fields. We observed a higher disease severity on the resistant cultivar under elevated ozone. Such compromised disease resistance may be due to alteration of host defense, pathogen fitness, associated microbiome, or their combined effect. Here, we focused on pathogen response given the observation of high variability in pathogen population on resistant cultivar under elevated ozone compared to the ambient conditions. While susceptible cultivar showed dominance of a single pathogen genotype regardless of the environment, resistant cultivar supported co-occurrence of both pathogen genotypes under ambient environment, indicative of maintenance of heterogeneity as a strategy for adaptation. However, such heterogeneity was only evident during end season under elevated ozone. In addition to the altered strain dynamics, higher mutation rate, and presence of de novo parallel mutations in the pathogen population under elevated ozone during single season are suggestive of a plastic eco-evo response of pathogen population to adapt to the resistant cultivar under altered climate.

Pseudogenes and host specificity in a bacterial plant pathogen

Navdeep Kaur[1], Neha Potnis[1] and Leonardo De La Fuente[1]
[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

Xylella fastidiosa is a bacterial plant pathogen that infects a wide range of economically important crops. Different *X. fastidiosa* strains vary in their host specificity, but the molecular mechanism behind this is still unclear. Pseudogenes in bacteria, which resemble genes structurally but do not code for any protein, may represent vestiges of functions no longer needed for fitness. Interestingly, pseudogenes in a bacterial human pathogen were hypothesized to be a hallmark of host specialists, as a sign of genome reduction for niche adaptation. The objective of this study was to identify pseudogenes in different strains of *X. fastidiosa* and relate their abundance with potential host specialist/generalist phenotypes. Using Pseudofinder, we analyzed pseudogenes on five different subspecies of *X. fastidiosa*, i.e., subsp. *fastidiosa, multiplex, pauca, sandyi and morus*. Results indicate that subsp. sandyi has the highest percentage of pseudogenes followed by subsp. *morus* and *pauca*, while subsp. *fastidiosa* and *multiplex* had the lowest percentages. Strains from the first three subspecies are known to have a narrower host range compared to the last two, therefore our observations support the hypothesis mentioned above. We then focused on strains known to infect blueberries either symptomatically or asymptomatically and compared pseudogenes among them. We hypothesize that genes pseudogenized only in asymptomatic strains may be important for symptom development in symptomatic strains. Using this criterion six gene sequences were identified that were shared among all blueberry-infecting strains. Five of those sequences were hypothetical proteins, and one was annotated as a protein with a GGDEF domain, possibly involved in regulatory functions via cdGMP. Future work will include mutational analysis of these selected genes and phenotypic characterization in vitro and in planta. Our studies aim to elucidate the basis of host specificity in this devastating plant pathogen.

Di-berberine conjugates are potent MexXY-OprM efflux inhibitors in *Pseudomonas aeruginosa*

Kavanaugh, LG.[1]*, Mahoney, A.[2]*, Dey. D.[1], Wuest, WM.[1,3], Conn, GL.[1,3]
[1] Department of Biochemistry, Emory University School of Medicine, Atlanta, GA
[2] Department of Chemistry, Emory University, Atlanta, GA
[3] Emory Antibiotic Resistance Center, Emory University, Atlanta, GA
* co-first authorship

The Resistance-Nodulation-Division (RND) efflux systems are tripartite complexes–comprising inner membrane transporter, periplasmic adaptor, and outer membrane proteins–that contribute extensively to clinical bacterial antibiotic resistance. The serious threat pathogen *Pseudomonas aeruginosa*, a chronic colonizer in cystic fibrosis (CF) patients, contains 12 RND efflux systems including MexXY-OprM which uniquely effluxes aminoglycoside antibiotics. To negate efflux-mediated antibiotic resistance, adjuvant efflux pump inhibitors (EPIs) are currently being developed. Based on the reported specific but weak MexXY-OprM EPI activity of berberine, a natural isoquinoline quaternary alkaloid, we used an *in-silico* high-throughput screen of berberine analogs (~10,000 compounds) against MexY to identify analogs with potentially enhanced activity. The top 100 compounds based on Schrödinger Glide SP docking score were k-means clustered based on chemical and scaffold similarity. The top four compounds were investigated using checkerboard synergy assays in *P. aeruginosa* PAO1, of which the di-berberine hexane conjugate (BerC6) showed increased synergy with aminoglycosides. To determine if lipophobicity and conformational freedom of dimeric berberine scaffold could increase efflux inhibition, we optimized the di-berberine conjugates using variable alkyl linker length and linker type. Resulting analogs were tested using checkerboard synergy assays, minimum inhibitory concentrations (MIC), and time-kill growth curves with multiple *P. aeruginosa* lab and pan-aminoglycoside-resistant clinical isolates. These assays revealed the di-berberine propane conjugate (BerC3) at 64 μg/mL is effective at reducing the MIC of tested aminoglycosides (2- to 16-fold) in PAO1, PA7, PA14, and pan-aminoglycoside resistant clinical isolates and is specific for MexXY-OprM. Interestingly, increasing the alkyl length of the di-berberine conjugate resulted in aminoglycoside-independent growth inhibition. These studies suggest the propane berberine di-berberine conjugate, BerC3, to be an effective and specific EPI for the MexXY-OprM efflux system in *P. aeruginosa.* Additionally, observations in inhibitor development may shed light on important mechanisms governing antibiotic recognition and efflux in RND-type systems.

Antibiotic Resistance and Biofilm Formation in *Acinetobacter baumannii* Clinical Isolates

Olivia Craig, Haley Channell, Lynsey Young, and Lauren B. King
Department of Biomedical Sciences, Mercer University School of Medicine, Columbus Campus

Acinetobacter baumannii is a gram-negative aerobic pathogen that opportunistically infects immunocompromised individuals in hospital settings. Classified as an ESKAPE pathogen, *A. baumannii* is known for its extensive antibiotic resistance, making effective treatment difficult. Antibiotic resistant clinical isolates have been studied, but research on biofilm formation and its contribution to antibiotic resistance, including the role of persister cells, is lacking. Our lab investigated the antibiotic resistance profiles and biofilm formation of eight *A. baumannii* isolates from St. Francis Hospital in Columbus, GA using standard minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and biofilm formation assays. Seven isolates were characterized as high biofilm formers and one isolate produced low amounts of biofilm. Three of these high biofilm formers were sensitive to all three antibiotics used while the one low biofilm former showed higher levels of resistance, suggesting a potential inverse relationship between biofilm forming capabilities and antibiotic resistance. Interestingly, while the growth of some strains was inhibited at clinically relevant concentrations, the bactericidal concentration of antibiotics was higher for cultures grown to logarithmic and stationary phases. Furthermore, preliminary data suggest that stationary phase bacteria require a higher concentration to achieve bactericidal effects, which suggests that persister cells may play a role in resistance and in subsequent treatment failures. More work is yet to be done to characterize the role of both biofilm formation and of persister cells in *A. baumannii*'s drug resistance, and both have significant clinical implications.

Use of a Chicken Embryo Assay to Investigate Virulence of *Salmonella* from Poultry Environmental Sources

Steven Ray Kitchens[1], Yagya Adhikari[2], Kenneth S. Macklin[2*], Stuart B. Price[1]
[1]Department of Pathobiology, Auburn University, Auburn, AL, USA
[2]Department of Poultry Science, Auburn University, Auburn, AL, USA
[*]Currently: Department of Poultry Science, Mississippi State University, Mississippi State, MS, USA

Salmonella is a zoonotic enteropathogen that causes significant disease in humans and livestock animals. It is important to understand this pathogen in host organisms and in the environment. Our research group is investigating prevalence of *Salmonella* in the poultry production chain from farm through processing. A *Salmonella* isolate lacking the O-antigens used for serotyping was isolated from a poultry farm environmental sample. This "rough" isolate was sequenced and determined to be most similar to *Salmonella* Infantis. To better understand this wild "rough" isolate, a chicken embryo lethality assay was used to assess virulence of this isolate. Specific-pathogen free (SPF) eggs were incubated until day 11 of development. SPF eggs were inoculated with the *Salmonella* rough isolate (10^2 CFU/egg) in allantoic fluid. Eggs were candled daily to monitor embryo mortality up to day 17 of development. SAS Studio v.3.81 (SAS Institute Inc., Cary, NC, USA), Kaplan–Meier curves and log-rank test were used to analyze data. The survival of chicken embryos was significantly different (p=0.0043 and p=0.0291) between eggs inoculated with *S.* Typhimurium versus the "rough" isolate. There was no significance found between the "rough" isolate and a monophasic *Salmonella* serovar 4,[5],12:i:-. Significance (p=0.0395) was found between *Salmonella* Infantis and the "rough" isolate, but significance (p=0.0942) was not found when eggs were inoculated with 10 times (10^3 CFU/egg) the regular dose of the "rough" isolate when compared to *Salmonella* Infantis. This work shows that the chicken embryo lethality assay is beneficial when investigating attenuation of virulence of *Salmonella* isolates without having to sacrifice laboratory animals.

Contribution of blood-brain barrier host cell signaling to efflux transport failure during *Streptococcus agalactiae* infection

Hallie E. Knight[1], Sarah F. Hathcock[1], Brandon J. Kim[1]
[1]Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA

The blood brain barrier (BBB) is a highly specialized network of brain endothelial cells (BECs) that facilitates the passage of molecules to and from the central nervous system, utilizing a barrier formed by complex tight junctions, greatly restricted endocytosis rates, and many protein nutrient influx and drug efflux transporters. P-glycoprotein (P-gp) is an efflux transporter that is highly expressed in the BBB and prevents the entry of many toxins and pathogens into the brain. One such pathogen, *Streptococcus agalactiae* is the leading cause of neonatal meningitis, and we hypothesize that a loss of BBB identity is critical to *S. agalactiae* invasion and the progression of *S. agalactiae*-induced meningitis. Modeling a human BBB using an induced pluripotent stem cell-derived brain-like endothelial cell (iBEC) model that possesses many characteristics of the BBB, including functional P-gp, we quantified P-gp activity by performing a fluorescent substrate accumulation assay. Comparison of percent fluorescence accumulation, as well as protein abundance and gene expression of P-gp in infected and uninfected cells, revealed an inhibitory effect caused by *S. agalactiae* on P-gp. Next, we wanted to discover which host cell signaling pathway *S. agalactiae* activated to cause a decrease in P-gp function. The mitogen-activated protein kinase (MAPK) pathway is known to impact P-gp in other disease states. Using various inhibitors of MAPKs, we did not observe a rescue effect by P-gp during combined *S. agalactiae* infection and signaling inhibition of the pathway, suggesting that an alternate pathway may be involved. Host cell signaling pathways between *S. agalactiae* and the BBB are a potential target for therapeutic intervention. By understanding which pathway(s) are involved in the loss of BBB identity and contributing to pathogenesis, in addition to increasing understanding of *S. agalactiae* virulence factors, further efforts can be concentrated on particular mechanisms of infection and treatment development.

The contribution of a *Flavobacterium covae* mannose-binding adhesin to pathogenesis in channel catfish (*Ictalurus punctatus*)

Stacey LaFrentz[1], Hannah Smith[1], Emily M. Churchman[1], Benjamin R. LaFrentz[2], Priscila da Silva Figueiredo Celestino Gomes[3], Mark Liles[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL, USA
[2]Aquatic Animal Health Research Unit, United States Department of Agriculture-Agricultural Research Services (USDA-ARS), Auburn, AL, USA
[3]Department of Physics, Auburn University, Auburn, AL, USA

Flavobacterium covae is pathogenic to many freshwater aquaculture species, especially to channel catfish (*Ictalurus punctatus*). The mechanisms that lead *F. covae* to be virulent in fish are not well understood. Adhesins are bacterial cell surface bound proteins that may bind carbohydrates on the host surface, while in some prokaryotes adhesins cause aggregation or biofilm formation. For instance, *Escherichia coli* mannose-specific fimbrae bind to the bladder epithelium and foster urinary tract infections. In previous gill adhesion and mucus chemotactic studies in fish, carbohydrates bound to the *Flavobacterium* cells blocked the ability of the bacteria to adhere to gills or response to mucus, respectively. In this study, when *F. covae* C#2 was grown in the presence of 5 mM to 50 mM mannose we observed cell aggregation at 24 hrs of incubation. We are trying to determine the mannose-binding adhesin(s) on the outer membrane of *F. covae* that are responsible for mannose-induced cell aggregation. Growth curves for this isolate using standard media supplemented with different carbohydrates were conducted. In the presence of 50 mM D-glucose, D-sucrose, D-galactose, N-acetyl-D-glucosamine or with standard media without yeast extract, *F. covae* C#2 reached stationary phase of OD_{600}= ~0.7-0.9 within 36 hrs. However, when grown with 50 mM D-mannose the stationary phase OD_{600} was only ~0.5 and the culture consisted primarily of small cell aggregates at 24 hr. Seven virulent strains of *F. covae* were grown in the presence of no carbohydrate source, 10 mM or 50 mM D-mannose. Three of the seven isolates aggregated in all mannose concentrations, while two were unaffected and the other two had intermediate aggregation. Ongoing experiments will explore the adhesin(s) responsible for mannose-induced aggregation using genomic, transcriptomic, and proteomic approaches to understand how the adhesin(s) participate in *F. covae* binding to catfish mucus and pathogenesis.

LeBleu

Urinary tract infections occurrence and recurrence is increased in patients who are administered a urinary catheter during hospital stays. About 1 million U.S. cases of catheter-associated urinary tract infections (CAUTI) are annually reported, accounting for almost a quarter of all hospital-acquired infections. Uropathogenic *E. coli* (UPEC) has been identified as the main causative agent of CAUTI and these pathogens become increasingly drug resistant. Cost efficient methods to reduce the CAUTI burden should be considered alongside current urinary catheter sterilization methods.

Cold atmospheric pressure plasma (CAPP) has well documented microbicidal properties. CAPP was strongly bactericidal against gram-negative bacteria, such as non-pathogenic *Escherichia coli*. Therefore, we thought to establish whether UPEC and drug resistant strains of *E. coli* are susceptible to the CAPP bactericidal action and to test the ability of CAPP to reduce bioburden in narrow flexible tubing mimicking urinary catheters. We used a jet source of argon CAPP to develop a quantitative method to measure plasma bactericidal action and showed that UPEC and drug-resistant strains of *E. coli* are similarly susceptible. Using a similar quantification approach, we established that CAPP can propagate through narrow tubing without full loss of bactericidal capacity. Taken together, our results show that CAPP might be a feasible method for sterilization of medical insertion devices.

Genome Wide Association Study of Natural Competence in Plant Pathogenic Bacterium *Xylella fastidiosa*

Ranlin Liu[1], Andreina I. Castillo[2], Luis F. Arias-Giraldo[3], María Pilar Velasco-Amo[3], Rodrigo P. P. Almeida[2], Blanca B. Landa[3], Neha Potnis[1], Leonardo De La Fuente[1]
[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA
[2]Department of Environmental Science, Policy and Management, University of California, Berkeley, Berkeley, California, USA
[3]Institute for Sustainable Agriculture, Consejo superior de Investigaciones Científicas (CSIC), Córdoba, Spain

Horizontal gene transfer (HGT) contributes to genetic diversity and can influence adaptation of plant pathogens to the environment. Natural competence is one of the mechanisms of HGT that relies on uptake and recombination of extracellular DNA. *Xylella fastidiosa* is an xylem-limited plant pathogenic bacterium, causing diseases on many economically important crops. Homologous recombination has been hypothesized to contribute to host shift of *X. fastidiosa* based on genetic analyses. To date, natural competence among *X. fastidiosa* has been described only in a few strains. Here we characterized the natural competence of 129 *X. fastidiosa* strains with diverse host and geographic origins by measuring recombination efficiency of plasmid DNA. Results showed great variability of recombination rates among tested strains, where most (88%) of subsp. *fastidiosa* strains were naturally competent, whereas only 16% subsp. *multiplex* strains were naturally competent. Genome wide association study identified putative genes associated with natural competence such as *hsf*, annotated as a surface protein, as well as type IV pili genes *pilY1-1* and *pilY1-3*. Their role in natural competence will be tested in the future. This study revealed diversity of natural competence among *X. fastidiosa* strains and will help understand mechanisms of natural competence.

Revealing complex *Salmonella* communities in beef cattle feces

Reese A. Lofgren[1], Aubrey C. Thompson-Smith[2], Travis Tennant[2], Ty Lawrence[2], Loni W. Lucherk[2], Amber K. Richards[1], Nikki W. Shariat[1]
[1]Department of Population Health, University of Georgia, Athens, GA, USA
[2]Department of Agricultural Sciences, West Texas A&M University, Canyon, TX, USA

Salmonella enterica can be divided into over 2,600 serovars, each with different phenotypes, including their ability to cause human and animal illness. Therefore, identifying *Salmonella* serovars is an important part of food safety risk assessment. Traditional serotyping, which has used a culture-based approach, is typically only practical to identify one or two dominant serovars in a single sample. CRISPR-SeroSeq is a deep serotyping method of amplifying and sequencing the native CRISPR spacers in *Salmonella* and using this information to assess the relative frequency of different serovars in individual samples. Previous work has shown that environmental samples collected in cattle feedlots harbor highly complex populations of *Salmonella* that consist of multiple serovars. It is unknown whether these complex populations are found in the animals at slaughter. To address this, we used CRISPR-SeroSeq to evaluate 92 *Salmonella* positive fecal samples collected from cattle at harvest. Across all samples, 82.6% contained more than one serovar. On average, samples contained 3.9 serovars per sample (range 1-8 serovars). The most frequently detected serovars were Montevideo (detected in 75.0% of samples), Muenchen (66.3%), Cerro (60.9%), Anatum (56.5%), and Mbandaka (56.5%). Both Montevideo and Muenchen are in the top 20 serovars listed by CDC as causing human illness. These results indicate that complex *Salmonella* populations are present in bovine fecal samples. Because more than four out of every five samples contained multiple serovars, this study highlights the importance of using methodology that can detect multiple co-occurring serovars. Future efforts will include comparison of fecal *Salmonella* populations to paired lymph node samples from the same animals. Collectively, these studies will allow us to explore the community dynamics as serovars shift spatially and temporally in beef production.

The Role of the Microbiome in Social Isolation Stress and Re-socialization Intervention in a Prairie Vole Model

Mackey, C.S PhDc[1], Chun E PhD[2], Brown, A.N. PhD[3], Washburn, B.K. PhD[3], Liu Y PhD[2], Wang Z PhD[2], Jones K.M PhD[1]

1. Florida State University, Department of Biological Sciences, Tallahassee Fl, USA
2. Florida State University, Department of Psychology, Tallahassee Fl, USA
3. Florida State University, Department of Biological Science Core Facilities, Tallahassee Fl, USA

Statement of Purpose: This study aims to investigate the effectiveness of re-socialization of isolated animals compared with access only to another animal's microbiota as interventions for prolonged social isolation stress in a prairie vole model.

Methods: Male voles, who had been pre-acclimated with a cage mate were subject to an 8-week intervention study where they began as either socially isolated or kept in a cohoused environment for an initial period of four weeks. After the initial four weeks, animals subject to isolation were then either returned to a cohoused environment with their previous cage mate, maintained in isolation while being provided fresh stool samples from the former cage mate, or maintained in isolation with no further manipulations. Behavioral data and stool samples were acquired at the 0-week, 4-week, and 8-week timepoints with brain tissue and blood samples being additionally taken at the 8-week period. Behavioral data was analyzed using either DeepLabCut for positional data or jwatcher for social data, and stool samples were prepared in triplicate for 16S amplicon sequencing of the V3-V4 rRNA gene region. Resulting behavioral data was analyzed using estimated marginal means predicted by linear modeling. Microbiome data was analyzed using a collection of alpha and beta diversity metrics, the rmcorr r package for repeat measures correlation, and a combination of whole group- and individual-based differential abundance measures.

Results and Conclusions: Analysis of the behavioral data showed the intervention methods were able to reliably rescue animal behavior related to locomotive measures, but not reliably rescue social behaviors. Surprisingly, animals that had been maintained in a cohoused social environment for the full 8-week paradigm displayed higher aggressive and anxiety-like behaviors in a novel social environment than the other groups in the post-intervention period. Correlation results between the 16S amplicon and behavioral data provided evidence for connections between the microbial structure of the gut and key significant behaviors. Notably, some bacterial taxa not only display significant correlations with behavioral data, but also appear as significant hits in other analysis streams such as the differential abundance analysis. For example, the *Bacteroidia* class had a significant negative correlation with locomotive activity in an open-field behavioral test as well as significant hits in the individual-based differential abundance analysis where the taxa group showed opposing log foldchange trends between the isolated and collective intervention animals. Additionally, significant results in the beta-diversity analysis at 8 weeks when comparing the isolated animals to the other treatment groups indicate larger changes happening to the gut microbiome composition. When paired with taxa-specific results such as the prior *Bacteroidia* example, these beta-diversity results evidence the ability of the intervention methods to either rescue or at least buffer the gut microbiome from the effects of social isolation.

Group B Streptococcal induced unfolded protein response aids blood-brain barrier disruption

Emily Maguire, Henry Mauser, Alyssa Arnett, Nadine Vollmuth, Nathan Correll, Brandon J. Kim

Bacterial meningitis is a serious life-threatening infection of the central nervous system (CNS) that occurs when blood-borne bacterial pathogens disrupt the blood-brain barrier (BBB) and enter the CNS. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and mechanisms of how the BBB fails to protect the CNS during infection remain unclear. The endoplasmic reticulum (ER) is a subcellular compartment that aids in protein folding and secretion. We have found that GBS induces ER stress that results in the upregulation of the protein chaperone GRP78 and the loss of tight junction abundance. Treatment of BECs with a chemical ER-stressor, tunicamycin, also recapitulates upregulation of GRP78 and loss of tight junction abundance. Either GBS infected BECs or tunicamycin treated BECs upregulate cytokines and chemokines such as IL-8, IL-6, CXCL1, and CXCL2 suggesting that ER stress contributes to activation of BECs. Examination of RNAseq transcriptomic response of BECs to GBS infection suggest that the ATF6 branch of the UPR is activated during GBS infection. Taken together, our findings suggest that the UPR is activated in BECs during GBS infection, and that the UPR may contribute to GBS mediated BBB disruption.

Dinoflagellate *Breviolum minutum* GIMAP gene expression in response to thermal stress

Sage N. Martin[1], Sara Stiers[1], Ariel N. Taylor[1], Angela Z. Poole[1]
[1]Department of Biology, Berry College, Mt. Berry, GA, USA

The diversity and productivity of coral reefs rely on the symbiotic relationship between cnidarians, such as corals and sea anemones, and photosynthetic, unicellular organisms called dinoflagellates. Many environmental factors related to climate change are threatening this symbiosis, leading to its breakdown called coral bleaching. Bleaching can ultimately result in the destruction of the entire coral reef ecosystem, impacting many species. Therefore, the goal of this research is to investigate a previously unexplored molecular aspect of cnidarian-dinoflagellate symbiosis. This project focuses on a protein group called GTPases of Immunity Associated Proteins (GIMAP) in the dinoflagellate symbiont *Breviolum minutum*. GIMAPs have been shown to be involved in bleaching related processes in cnidarians, but nothing is known about them in dinoflagellates. To begin an introductory exploration of dinoflagellate GIMAPs, expression of four GIMAP genes in *Breviolum minutum* was investigated as a function of symbiotic state and temperature stress. To do this, *in-hospite* (in the *Exaiptasia diaphana host*) and cultured *B. minutum* were exposed to control and elevated temperature treatments and sampled at 24 and 48 hr timepoints for RNA extractions. To measure GIMAP gene expression, cDNA was synthesized from RNA and used in qPCR. For the control temperature, *in-hospite B. minutum* had a higher baseline expression than cultured *B. minutum*. Additionally, cultured *B. minutum* had a stronger response to heat treatment, particularly at the 24 hr timepoint. These results indicate that dinoflagellate GIMAPs play a potential role in symbiosis and the stress response.

Quantitative Analysis of the melanization reaction in the American cockroach

Aaron Martin

Periplaneta americana (American cockroach) is a ubiquitous pest that can harbor a myriad of pathogenic organisms such as *E. coli* O157:H7, ultimately having profound effects on food security. Despite this arthropod becoming increasingly resistant against insecticides, its immunological landscape supported by effector cells called hemocytes has been inadequately explored. This premise extends to the process of melanization, an immunological hallmark existing in most insects. The crux of this process lies in synthesis of cytotoxic melanin, leading to the generation of reactive oxygen species that adversely affect organisms pathogenic to the arthropod. While a fundamental understanding of this process exists in other insects, few studies have examined its mechanism or kinetic characteristics within the American cockroach. Addressing this knowledge gap, we have extracted whole hemolymph and hemocytes for use in functional assays to observe variation between in the process of melanization. Our study explored this through the technique of spectrophotometry, recording the optical density of samples unstimulated or stimulated via PAMPs to demonstrate a differential effect of melanization. Through these experiments, we ultimately provide a basis to analyze one of the core immunological processes of *Periplaneta americana*.

Exploring β2-adrenoceptor signaling in *Neisseria meningitidis*, brain endothelial cell interactions

Henry Mauser[1], Leo Enders[2], Kelsey M. Gray[3], Simon Peters[2], Kimberly M. Stroka[3], and Alexandra Schubert-Unkmeir[2], Brandon J Kim[1,2]
[1]University of Alabama, Department of Biological Sciences
[2]Institute for Hygiene and Microbiology University of Würzburg Germany
[3]Fischell Department of Bioengineering, University of Maryland

Bacterial meningitis is a life-threatening infection of the central nervous system (CNS) that occurs when bacteria penetrate the blood-brain barrier (BBB) – a network of specialized brain endothelial cells (BECs) that restrict the traversal of circulating toxins and pathogens. *Neisseria meningitidis* (Nm) is a leading cause of meningitis, but many of the mechanisms that enable it to penetrate the BBB and invade the brain remain unknown. Recent research has identified two single nucleotide polymorphisms (SNPs) in the β2-adrenoceptor (B2AR) gene that predispose individuals to meningitis caused by Nm. Additionally, Nm attachment to BECs has been shown to facilitate B2AR recruitment, further implicating the role of the B2AR during Nm meningitis. Propranolol and carvedilol are two commonly administered drugs that inhibit B2AR signaling. By utilizing a novel induced pluripotent stem cell-derived brain endothelial cell model, we observed that B2AR inhibition with propranolol and carvedilol prevented Nm invasion of brain endothelial cells in a dose-dependent manner. Furthermore, quantification via TEER and continuity analysis of occludin, a critical tight junction protein of the BBB, show that propranolol rescues barrier integrity during infection. We also observed that Nm infection induces intracellular calcium signaling, a downstream effect of the B2AR canonical signaling pathway. Taken together, these results suggest that B2AR canonical signaling induces blood-brain barrier dysfunction and promotes *Neisseria meningitidis* invasion. These findings suggest a novel role for approved beta-antagonist drugs in the prevention of bacterial meningitis.

Localization of Tomato Spotted Wilt Virus (TSWV) proteins in SF9 insect cells

Michael Mayfield[1], Kathleen Martin[1], Yuting Chen[2], Anna Whitfield[2]
[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
[2]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Peanuts (*Arachis hypogaea*), are one of the most economically important crops for Alabama, with the state ranking as the second in the nation for peanut production. In 2022, the state was responsible for harvesting 558,900,000lbs of peanuts, an estimated $144,000,000 in value. Alabama's legume production faces a threat in the form of Tomato spotted wilt virus (TSWV), a member of the *Bunyaviridae* family. Orthotospoviruses are capable of inflicting yield losses of up to 100% under optimal conditions and is transmitted by thrips (*Thysanoptera*), specifically tobacco thrips (*Frankliniella fusca*) and western flower thrips (*Frankliniella occidentalis*) in a persistent propagative manner. This makes TSWV a member of one of the two viral families capable of replicating in both plant and insect cells. While TSWV proteins have been previously localized in plant cells, their expression in an insect vector remains unknown. This study identifies TSWV protein localization when expressed in insect cells. We utilized fluorescence microscopy to uncover differences in protein expression between insect and plant cells. TSWV's genome encompasses six open reading frames (ORFs): a polymerase (L), glycoproteins (Gn/Gc), non-structural movement protein (NSm), nucleocapsid protein (N), and non-structural silencing protein (NSs). We hypothesize that TSWV proteins display similar localization patterns in both cell types due to the virion structure, with differences for the movement and silencing proteins. We constructed ORF fusions with green fluorescent protein (GFP) for all TSWV proteins except the polymerase (due to size), transfected them into SF9 insect cells, and recorded localization patterns. Our findings revealed intriguing localization patterns for NSm, NSs, N, and Gn/Gc proteins. Our next experiments will identify potential host factors and promises innovative management strategies for virus control. Given TSWV's substantial economic impact on Alabama's peanut industry, our findings advance more effective management strategies.

Isolation of *Campylobacter* from bile sourced from poultry and association with cecal counts, gallbladder size, and bile color

Abigail McConnell[*GS], Madalyn Jennings[1], Matthew Hughes[1], Juan Figueroa[1], Marco Reina[1], Jeff Buhr[2], Dianna Bourassa[1]
[1]Department of Poultry Science, Auburn University, Auburn, AL
[2]US National Poultry Research Center, USDA-ARS, Athens, GA

Campylobacter is one of the top foodborne pathogens associated with poultry products. When birds are withdrawn from feed, research has shown that the gallbladder increases in size with the length of time the birds are off feed. This poses a risk for rupture during processing, and it has been suggested that leakage of bile may contribute to *Campylobacter* contamination. However, information on the potential contamination of *Campylobacter* from bile is lacking. This study sought to determine if *Campylobacter* can be isolated from bile in the gallbladder of broilers and if the level of *Campylobacter* present in ceca, the size of the gallbladder, or color of the bile is indicative of *Campylobacter* positive bile samples. Gallbladders and the corresponding paired ceca were collected from the evisceration line of commercial processing plant on three different days (feed withdrawn and from different flocks). For each of 3 repetitions, n=10 gall bladder/ceca pairs were collected for a total of 30 gallbladder and 30 ceca samples. Bile was extracted using a sterile needle and syringe and the cecal contents were extracted using manual expression. One gram of the ceca contents from each sample was added to 9 mL of phosphate buffered saline, vortexed, and diluted. Bile and cecal contents were plated for enumeration on Campy Cefex agar and allowed to incubate for 48 hours at 42°C in a microaerobic atmosphere. Bile samples were enriched with one part bile and one part 3M Campylobacter enrichment broth and then incubated. After incubation, the samples were confirmed positive or negative using the 3M Molecular Detection Assay. The relationship between ceca *Campylobacter* counts and positive bile samples were analyzed using Student's t-test and gallbladder size (mm^2) were analyzed with the ANOVA procedure of SAS. Statistical significance was considered at $P \leq 0.05$. There was no significant association between positive bile samples and cecal *Campylobacter* counts (P=0.3532) or between the size of the gallbladder and positive bile samples (P=0.3598). Through visual observation it appeared that there was a color difference between the positive and negative bile samples, most discernible after 48 hours of incubation in 3M *Campylobacter* enrichment broth with positive samples having a more yellow appearance. These data indicate that the likelihood of bile being positive for *Campylobacter* may not be related to levels of *Campylobacter* in the ceca or size of the gallbladder, however bile color may be indicative of the presence of *Campylobacter*, particularly following enrichment. The suggestion that leakage of bile has a significant impact on carcass *Campylobacter* contamination does not appear to be valid.

In silico identification of a novel *Bacillus* phage from the nunu microbiome

Daisy McGrath[1], Tsai-Tien Tseng[1]
[1]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw. GA

This study identified an uncharacterized phage as a new member of *Caudoviricetes* from a homemade dairy product called nunu, a West African fermented dairy beverage sold in open-air markets lacking standardized starter culture or pasteurization. Metagenomic data from next generation sequencing (NGS) with NCBI accession number PREJEB20873 was analyzed *in silico* by our workflow. After initial quality control and adapter trimming with fastp, one metagenome assembled genome (MAG) generated by MetaSPAdes consisted of 50,572 bp with a GC content of 43%. CheckV found the assembly of this MAG to be high quality, 100% complete, and error-free, along with the presence of direct-terminal repeats (DTR) as a verification. Within the Pharokka pipeline, Prodigal predicted 90 Open-reading frame (ORFs) from this particular MAG, further designated as dairy microbiome 2 (DM-2). After BLAST searches, these ORFs can be functionally assigned into the following modules: host cell lysis, nucleotide metabolism and replication, morphogenesis, including tail and capsid proteins, and DNA packaging. Constraint-based Multiple Alignment Tool (COBALT) from NCBI created multiple alignments and phylogenetic trees for selected ORFs. Overall, 57 ORFs were found to be similar to ones from *Bacillus* phage 1_ICo-2020, which belongs to the class of unclassified *Caudoviricetes* according to NCBI Taxonomy. A predicted large terminase at ORF26 showed a sequence similarity of 96% when compared to the one from *Bacillus* phage 1_ICo-2020. BLAST searches also revealed that this predicted terminase shares similarity with other *Bacillus* phages, including Andromeda, Anath, and Shbh1. Supported by the prediction from RaFAH within the iPHoP pipeline, the proposed host for this novel phage would belong to the *Bacillus* genus. Many species of *Bacillus* were found in the nunu microbiome, which also supports the proposed host specificity for DM-2. In conclusion, DM-2 is a new member under *Caudoviricetes*.

A summer research program across multiple campuses with both in-person and remote participants

Emily A. McMackin[1], Robert J. Visalli[2], Gretchen L. Bentz[3]
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Columbus, GA
[2]Department of Biomedical Sciences, Mercer University School of Medicine, Savannah, GA
[3]Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA

With the Step 1 exam switching to pass/fail, medical students are increasingly demanding research opportunities as a way to distinguish themselves while applying for residency, regardless of specialty. The most opportune time for the bulk of this research is the summer between their first and second years of medical school. In both 2022 and 2023, over 100 Mercer University School of Medicine (MUSM) students participated in summer research. Some of these students performed research in person, while others participated remotely. The fact that MUSM has three 4-year medical school campuses introduces an additional layer of complexity to managing equal research opportunities for all students. We have developed a six-week medical student summer research program that supports students who are interested in multiple types of research (academic/medical education, bench, clinical, and population health) and provides both remote and in-person participation options. This process includes matching students with faculty research projects, six professional development sessions, a summary of research due at the end of the summer, and an oral or poster presentation at the university-sponsored Joint Research Conference in the fall. We predict that this model can be adapted for other types of students with differing interests and time commitments during a summer research period.

Single-cell manipulation of *Pseudomonas aeruginosa* using a microfluidic platform

Caroline D. Miller[1], Sophie E. Darch[1]
[1]Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL

Microfluidic systems are robust and versatile tools that utilize the properties of fluid mechanics to govern fluid flow at the microscale. The precise and consistent control possible using droplet-based microfluidics allows for microbial applications such as particle encapsulation. Novel techniques such as this to encapsulate and further study the physiology and interactions between bacterial cells are needed to understand clinically relevant, opportunistic pathogens such as *Pseudomonas aeruginosa*. The interactions between individuals and groups of *P. aeruginosa* cells are of significant interest due to their highly virulent behavior and persistence in immunocompromised patients. Thus, droplet-based microfluidics presents an opportunity to isolate *P. aeruginosa* and observe the impact of environmental changes at the microscale. A flow-focusing microfluidic device was used to manipulate the relative discontinuous (deionized water or fluorescently labeled gelatin solution with *P. aeruginosa* cells) and continuous (mineral oil and surfactant solution) phase flow rates to control droplet generation and diameter (mm). Droplets were transferred to incubation chip microwells (e.g., 20 mL) and observed using confocal microscopy in the presence and absence of antibiotics (tobramycin and colistin). *P. aeruginosa* cells were then isolated, and single cell RNA-sequencing performed using the Chromium Next GEM Single Cell 3' RNA-sequencing platform to generate transcriptomic profiles of each droplet population. Differentially regulated genes were identified using bioinformatic approaches, both in the presence and absence of antibiotics. Among other high-resolution methodologies to encapsulate aggregates and single-cells, droplet-based microfluidics is a promising tool to isolate and observe the development of aggregates from single bacterial cells in designed spatial confirmations. Here we show how this microfluidic system can be used as a high-resolution tool that allows for *P. aeruginosa* isolation on a single-cell level in biocompatible materials and coupled with single cell genomics to understand bacterial response to antibiotic treatment.

Discovery of novel therapeutics for viral myocarditis utilizing human stem-cell derived cardiomyocytes

Lauren Mitterway, Elizabeth McCabe, Hunter Vogel, Jon Sin, Brandon Kim
Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, 35401

Myocarditis, an inflammation of the myocardium, oftentimes leads to sudden death due to the enlargement and weakening of the heart muscle. Coxsackievirus B (CVB3) is a common cause of myocarditis as it directly targets and damages the myocardium tissue during its infection. Previously, mouse or HL-1 cardiac cells were used to study CVB3 infection, but they lack human-specific properties and the ability to beat, which can greatly affect host-virus interaction. Recent work has developed a method for the derivation of human induced pluripotent stem cell (hiPSC) derived cardiomyocytes using small molecules known as the GSK inhibitor-Wnt inhibitor (GiWi) method. Using Gsk3 and Wnt inhibitors and other small molecules, we are able to derive actively beating cardiomyocytes of human origin. This method is advantageous as it does not require genetic modification, is specific to human cardiac muscle, and has been demonstrated to be effective in a variety of hiPSC lines. HiPSCCMs were differentiated and began beating on day 12 post differentiation initiation. Confirmation of successful differentiation was confirmed by immunostaining for Alpha Actinin on day 20. To determine if propranolol impacted CVB3 on hiPSCCMs, we infected hiPSCCMs with enhanced green fluorescent protein (EGFP) expressing CVB3. We observed that propranolol treated hiPSCCMs had overall lower EGFP signal when compared to vehicle treated cells. Additionally, propranolol reduced the live virus produced after infection by measuring plaque forming units (PFU) via plaque assay and reduced the level of a key viral protein VP1 as measured by western blot. From this, we have concluded that hiPSCCMs will be extremely useful in modeling human cardiomyocytes during CVB3 infection. Additionally, we have demonstrated that an already FDA-approved drug is effective at inhibiting CVB3 infection of human cardiomyocytes. Future work will determine how CVB3 impacts the physical beating of hiPSCCMs and investigate the impact on calcium signaling.

Capacity of diverse *Bacillus* species to control both oomycete and fungal pathogens

Francesco S. Moen[1], Oluwakemisola E. Olofintila[2], Jahangir Alam[3], Beatrice Severance[2], Zachary A. Noel[2], Douglas C. Goodwin[3], Mark R. Liles[1]
[1]Department of Biological Sciences, Auburn University, Auburn, AL, USA
[2]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
[3]Department of Chemistry and Biochemistry, Auburn University, Auburn, AL, USA

Fungal and oomycete pathogens cause billions of dollars in crop losses every year. Plant growth-promoting rhizobacteria (PGPR) have been studied as a biological alternative to chemically derived fertilizers and pesticides in agriculture for decades. *Bacillus velezensis* are particularly effective as PGPR strains due to their ability to form endospores and their safety when applied as a crop seed treatment. However, currently available biological agents are either ineffective at preventing diseases in commercial crops or are administered (i.e. sprayed) in ways that diminish potential disease biocontrol. There is therefore a need to enhance efficacy of *Bacillus* PGPR strains and to identify strains that produce bioactive metabolites that inhibit the viability of fungal and oomycete pathogens. We screened over 300 spore-forming *Bacillus* sp. from a unique PGPR collection and identified 156 strains that inhibited plant pathogenic oomycete species, while 90 of those could also inhibit select fungal species. *In plantae* assays were conducted to identify *Bacillus* strains that could protect soybean grown in field soil under growth chamber conditions, revealing that *B. velezensis* JJ334 inhibited disease due to the fungal pathogen *Fusarium oxysporum* and resulted in 256% greater root biomass relative to the infected control, whereas *B. velezensis* JM199 and JM907 inhibited disease due to the oomycete pathogen *Globisporangium ultimum* and resulted in 238% and 64% greater root biomass relative to the infected control, respectively. Twenty-nine strains of *Bacillus* with anti-fungal and/or anti-oomycete profiles were submitted for genome sequencing to predict biosynthetic gene clusters (BGCs) encoding secondary metabolites. The BGCs identified included lichenysin, fengycin, surfactin, and bacilysin. Current experiments are investigating how these PGPR strains can be enhanced in their biocontrol efficacy, including identifying streptomycin-resistant mutants with increased secondary metabolite expression, and by combining spores with a prebiotic formulation for use as a seed treatment.

Capsule serotype alters Phosphorylcholine and Choline binding protein A exposure on the bacterial surface impacting pneumococcal adhesion and invasion

Md. Mohasin[1], Hannah Majors[1], Eriel Martinez[1], Elaine I. Tuomanen[2] and Carlos J. Orihuela[1]
[1]Department of Microbiology, University of Alabama at Birmingham, USA
[2]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA

Rationale: The polysaccharide capsule that surrounds *Streptococcus pneumoniae* (*Spn*) is one of its most important virulence determinants. Capsule influences pneumococcal interactions with host cells. We hypothesized that the biochemical structure of capsule impacts the surface exposure of two key *Spn* adhesins: surface phosphorylcholine (PC) and choline binding protein (CbpA) that mediate *Spn* adhesion and invasion to vascular endothelial cells.
Methods. To assess surface exposure of PC and CbpA among 15 isogenic capsule-switch mutants created in TIGR4 genetic background, binding of monoclonal antibody against PC (TEPC-15) and laboratory-derived mouse antibody against CbpA, was examined using flow cytometry. CbpA levels were determined by immunoblot using whole cell lysates. Adhesion and invasion assays were performed using resting and TNFα-stimulated murine cardiac endothelial cell (MCEC).
Results: Among the panel of isogenic capsule-switch mutants, those carrying serotype 6A and 7F had the greatest surface exposure of PC and CbpA. TIGR4 producing capsule type 3, 6B, 11A, 23F, and 33A demonstrated moderate PC and CbpA exposure. TIGR4 producing serotypes 2, 6C, 6D, 19A and 19F had a low-PC and CbpA surface exposure. Adherence and invasion of *Spn* to MCEC was positively correlated with exposure of PC and CbpA. Accordingly, TIGR4 producing capsule type 6A, 7F and 19F had the highest rates of adhesion and invasion.
Conclusion: Our results demonstrate that surface exposure of PC and CbpA varies in serotype specific manner, and this directly influences the bacterium's ability to interact with host cells. This effect can be attributed to the biochemical features of the capsule, although the mechanism(s) remain to be elucidated.

Experimental evolution of simple phytoplankton communities

Jeffrey Morris

Microalgal phytoplankton form the base of the food web in many aquatic ecosystems and are responsible for as much as half of total global primary productivity. Laboratory experiments with phytoplankton cultures show that these organisms' responses are strongly affected by the presence of heterotrophic bacteria, but relatively little is known about the specific nature of the interactions between these fundamentally different classes of microbes. Here, we show that the bacterium *Alteromonas macleodii* EZ55, originally isolated from a culture of the picocyanobacterium *Prochlorococcus*, experienced rapid positive natural selection when grown in co-culture with eukaryotic phytoplankton for 500 generations. The genomes of EZ55 cultures grown alongside a diatom and a coccolithophore evolved dramatically differently than those grown with cyanobacteria, with several strong lines of evidence suggesting that specific, directional adaptation occurred as these bacteria shifted from cyanobacterial to eukaryotic algal partners. Genomic analysis revealed striking examples of convergent evolution, with genes related to environmental sensing, nutrient transport and acquisition, cofactor biosynthesis, and biofilm formation mutated much more often in co-cultures with eukaryotes than with cyanobacteria. Moreover, EZ55 cultures evolved with eukaryotes had significantly reduced ability to effectively partner with *Prochlorococcus*, their original host. These results support the paradigm that marine heterotrophic bacteria like *Alteromonas* spp. evolve specific partnerships with different functional categories of primary producers over short time scales with potentially important consequences for nutrient cycling and overall microbial community structure.

Evaluation of pathogen recovery efficiency using non-woven synthetic scrub samplers against cellulose sponges for environmental surface sampling

C. Nguyen[1], A. Lyons [1], J. Whitworth[1], M. Williams[1], J. Noble-Wang [1]
[1]Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion, Clinical and Environmental Microbiology Branch. Atlanta, GA, United States.

Contaminated healthcare surfaces play an important role in pathogen transmission and hospital-acquired infections. Optimizing healthcare environmental sampling is crucial in identifying infection transmission sources during epidemiologic investigations. Many nosocomial infections are caused by *Acinetobacter baumannii*, Carbapenemase-resistant *Klebsiella pneumoniae* (KPC+), *Clostridioides difficile,* methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE). Currently, the commonly used sampling device is a stick-mounted cellulose sponge. As new devices become available, assessing pathogen recovery efficiency against the cellulose sponge is imperative.
We compared pathogen recovery efficiency of two sampling tools, a cellulose sponge, and a multilayered non-woven composite scrub sampler, from stainless steel and plastic surfaces. Surfaces (50in^2 coupons) were inoculated with 500µL of 10^4 CFU/mL bacteria suspended in 20% artificial test soil (ATS) and evenly spread across the coupon. After sampling, sponges were processed using a stomacher and concentrated via centrifugation before culturing and quantifying. Percent recoveries from cellulose and synthetic sponges were compared using a two-tailed paired t-Test. Statistical significance was defined as $p<0.05$.
Cellulose sponge recovery efficiencies from stainless steel (ST) and plastic (PL) were significantly higher ($p<0.05$) than the scrub sampler for *C. difficile* spores (ST 66.5%; PL 75.18%) and VRE (ST 75.10%; PL 84.65%). MRSA recovery (56.6%) from steel using cellulose was significantly higher than the scrub sampler, but not for plastic. No significant differences were observed between the scrub sampler and cellulose sponge for *A. baumannii* recovery from steel or plastic. KPC+ recovery was significantly higher in the scrub sampler than the cellulose sponge, with average recoveries of 40.03% from steel and 30.0% from plastic. These data confirm that cellulose sponge recovery was significantly more efficient on stainless steel for 3 of 5 healthcare pathogens tested. Improved KPC+ recovery by the scrub sampler indicates that pathogen characteristics need to be considered when selecting an environmental sampling tool.

Evaluating *Campylobacter jejuni* and *Campylobacter coli* phylogeny

Connor G. Norris[1], Amy T. Siceloff[1],and Nikki W. Shariat[1]
[1]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA.

Campylobacter is a leading bacterial foodborne pathogen and *C. jejuni* and *C. coli* are significant causes of gastroenteritis. Regulatory agencies routinely conduct whole-genome sequencing of *Campylobacter*, resulting in extensive genomic data being publicly available on databases like Pathogen Detection on NCBI (~119,000 genomes). Our objective was to curate a manageable dataset to assess the overall genomic diversity of *C. jejuni* and *C. coli* and to explore their phylogenetic relationships without compromising representative genomic diversity or overemphasizing outbreak-related genomes. Within Pathogen Detection, genomes are categorized into SNP Clusters, wherein all genomes differ by up to fifty base pairs and each cluster is presented as an individual phylogenetic tree. SNP Clusters were selected for analysis if they contained a minimum of ten genomes meeting specific criteria: U.S. origin, Illumina sequencing platform, an NCBI Species TaxID 195 or 197, and an N50 >25,000. Phylogenetic trees from SNP Clusters meeting these criteria were evaluated using Phylogenetic Diversity Analyzer (PDA) to select representative genomes. A preliminary phylogenetic tree was built from 1,854 assemblies from Pathogen Detection using kSNP and FastTree, then run through PDA again to get a collection of 468 isolates representing the overall genetic diversity of *C. jejuni* (246 genomes) and *C. coli* (222 genomes). The short-read sequences for these genomes were assembled using SPAdes and annotated with Prokka. Genome alignments for each individual species were made using Roary and subsequent trees were generated using RAxML. The tree of *C. coli* shows a separation of swine/cattle samples from poultry samples and clonal expansion of poultry samples into two sub-clades. The tree of *C. jejuni* also shows two clades, with one clade split into two sub-clades. Future aims will be to link the clades identified with different metadata, including association with human illness or different animal sources.

Bioinformatic and PCR analysis of a *Rickettsia amblyommatis* variable gene encoded on its pMCE_2 plasmid

Chiamaka Ann-Marie Ogwara and Marina E. Eremeeva, Jiann-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA 30458

Background

Rickettsia amblyommatis is commonly detected in *Amblyomma americanum* ticks in eastern United States. This *Rickettsia* is associated with several disease presentations in humans, but its genetic variability is poorly known.

Purpose

To perform bioinformatic analysis of *Rickettsia amblyommatis* strain GAT-30V unique pMCE_2 plasmid encoded gene (MCE_8365) and evaluate its diversity in *R. amblyommatis* detected in ticks.

Methods

Nucleotide and protein BLAST was used to analyze sequence and functional domains of MCE_8365 protein and to identify its orthologues in other isolates of *Rickettsia*. Primers targeting the repeat region of the MCE_8365 gene were used to characterize this gene fragment in *R. amblyommatis* from *A. americanum* ticks collected from a local wildlife preserve.

Results

MCE_8365 gene of *R. amblyommatis* GAT-30V contains three 81 nucleotide tandem repeats (TR); it encodes for 1034 amino acid (aa) protein with two functional domains. The N-terminus contains a 300 aa conserved SpoT/RelA motif belonging to the (p)ppGpp synthase/hydrolases protein super family which coordinates many signal transduction mechanisms. The C-terminus contains a 225 aa PPP1R42 super family motif of the protein phosphate regulatory subunit and includes leucine-rich repeats. This gene was identified by BLAST in four geographically distant isolates of *R. amblyommatis*; these genes are largely identical except the number of TR repeats varied from 2 to 4. Similar gene was also found in *Rickettsia massilae*. TR-containing fragment of the MCE_8365 gene was amplified from 75 tick DNA which previously tested PCR positive for *R. amblyommatis.* Gel electrophoresis and sequencing detected four different types of amplicons containing 2-5 TR. Amplicons with 4 TR were detected most frequently ($p<0.00001$) and found in all life stages of ticks tested.

Conclusion

This study showed that genetic diversity occurs among *R. amblyommatis* isolates. Whether these variations contribute to regulation of any biological properties of *R. amblyommatis* in ticks and humans is unknown.

Substrate selectivity in the copper and phosphate transporting Mitochondrial Carrier Family Proteins

Laura E. Oldfather, Xinyu Zhu, Katherine M. Buckley, and Paul A. Cobine
Department of Biological Sciences, Auburn University

Mitochondrial carrier family (MCF) is the largest group of proteins localized on mitochondrial inner membrane that translocate various metabolites/molecules into the matrix to maintain essential cellular functions. There are 53 MCF members of this family in humans that appeared to have evolved through duplications and neofunctionalization/specialization. During this evolution MCF substrate promiscuity may have arisen with mutations that facilitate transport of new substrates which may or may not change the transport of the original substrate. An example of an MCF with multiple substrates is SLC25A3 in mammals that transports both copper ions and phosphate. Phylogenetic analysis of this specific MCF reveals that holozoa lost the phosphate-only transporting that can be identified in many other eukaryotic taxa. Using this phylogenetic analysis, we were able to identify multiple taxa that have lost the phosphate-only transporter but maintained multiple copies of the copper transporter and vice versa. We used comparison of these sequences to determine conserved residues that may contribute to the specificity. This allowed us to identify candidate residues that confer the ability to transport copper or phosphate. In particular, organisms with multiple copies of the copper-phosphate transporting MCF have at least on example with histidine 230 converted to glutamine (found in all phosphate only transporting MCF). We investigated the role of this residue by mutating it in the yeast copper-phosphate transporter PIC2. This change elicits increased efficient phosphate transport with little effect on in its copper transporting ability suggesting it plays a role in improving phosphate uptake in organisms with multiple copies of PIC2.

eEF2-sordarin promotes spontaneous forward and reverse translocation.

Zheren Ou, Douglas Wright, Alexey Petrov.
Department of Biological Sciences, Auburn University, Auburn, AL, USA.

The ribosome is a molecular machine that adopts multiple conformations during translation. The body of the small ribosomal subunit rotates relatively to the large ribosomal subunit by about 9 degrees. At the beginning of the elongation cycle, the ribosome awaits A-site decoding and occupies the non-rotated conformation. Upon A-site tRNA binding, the ribosome rapidly catalyzes peptidyl transfer. It is followed by counterclockwise rotations of the small ribosomal subunit that puts the ribosome into the rotated state. The pre-translocation ribosomes are dynamic and exchange between rotated and non-rotated conformations. Eukaryotic elongation factor 2 (eEF2) uses energy of GTP hydrolysis to catalyze translocation, which moves tRNA-mRNA complex and resets the ribosome into the nonrotated state. Despite extensive biochemical and structural studies, the underlying mechanism of translocation remains elusive. We developed a single-molecule fluorescence resonance energy transfer (smFRET) system that allows the observation of intersubunit conformational change of yeast ribosomes in real-time. We observed that pre-translocation ribosomes predominantly occupied a rotated conformation while spontaneously exchanging between non-rotated and rotated states. In the presence of eEF2 and GTP ribosomes underwent irreversible forward translocation. The diterpene antibiotic, sordarin, stabilizes eEF2 on the ribosome in an extended conformation. 80S-eEF2-sordarin complexes underwent multiple rounds of forward and reverse rotations per eEF2 binding event. In the presence of sordarin, neither GTP hydrolysis nor a phosphate release were required for intersubunit movement. These results demonstrate that eEF2 promotes the mid and late stages of translocation by unlocking intersubunit dynamics, with mid and late stages of translocation being thermally driven.

Enhanced method for detection of *Mycobacterium tuberculosis* in ancient skeletal samples

Ariel Owens[1], Daisy McGrath[2], Tsai-Tien Tseng[2]
[1]Department of Geography and Anthropology, Kennesaw State University, Kennesaw, GA
[2]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA

Morphological analysis as a diagnostic method on ancient skeletons for tuberculosis (TB) has been difficult and often inconsistent. With the advent of next-generation sequencing (NGS), this study aimed to enhance available bioinformatic screening methods on ancient DNA (aDNA) to create more suitable workflow and generate insights in relation to TB. NGS data with the accession number of PRJNA422903 were retrieved from the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI). Trim Galore! was first used for quality control and adapter trimming. Kraken2 was then used for taxonomic classification with a custom-built database that was created from genomes of Mycobacteria from NCBI. Quantitation and visualization were carried out with Bracken and Krona, respectively. Our workflow has been applied to 28 Neolithic skeletons representing the Middle Neolithic Brześć Kujawski Group of the Lengyel culture (~4400–4000 BC, 26 individuals), and the Late Neolithic Globular Amphora culture (~3100–2900 BC, 2 individuals). These 28 Neolithic skeletons contained an average of 7.85% of the *Mycobacterium* genus sequencing reads mapping to *Mycobacterium tuberculosis* complex (MTBC). Four of these aforementioned individuals, 12_BK4, 71_BK3, 33_BK4, and 34_BK4, had elevated levels of MTBC within the genus of *Mycobacterium* at 30%, 28%, 38%, and 34%, respectively. Yet, none of these four individuals had skeletal morphological changes due to TB. This work also revealed additional species of MTBC and *Mycobacterium avium* complex (MAC) that were previously unreported by the originator of datasets, including the extensively drug-resistant (XDR) *Mycobacterium tuberculosis* XDR1219 and *Mycobacterium avium hominissuis*. The average percentage of MAC present in the genus *Mycobacterium*, in all 28 individuals, is 6.58%. In conclusion, our bioinformatic workflow has therefore been more robust than previously published approaches and is suitable for future paleopathological studies.

The infection dynamics of lytic phages in physically structured populations of *Escherichia coli*

Peyton Panos[1], Andres Valdez[2], Eduardo Roman[1], Brandon Berryhill[1], Andrew Smith[1], Igor Aronson[3], and Bruce Levin[1]

[1]Department of Biology, Emory University, Atlanta, GA, USA
[2]Department of Biomedical Engineering, The Pennsylvania State University, PA, USA
[3]Department of Biomedical Engineering, Chemistry, and Mathematics, The Pennsylvania State University, PA, USA

Almost all we know about the population and evolutionary dynamics of bacteria and bacteriophage (phage) is based on mass-action differential equation models and their experimental analog, bacteria and phage interacting as individual cells and particles in well agitated liquid culture. In the real world, bacteria do not live in these effectively dimensionless habitats implicit in these models and experiments. Bacteria are likely to exist as colonies growing on surfaces or other physically structured habitats. Under these conditions, phage infect colonies of bacteria, not just individual cells. With mathematical models and experiments with *E. coli* B and its lytic phages T2 and T7, we explore the dynamics of phage infection in colonies of bacteria, and the population dynamics of these bacteria and phage in surface culture.

Our models predicted and experiments observed two forms of infected colonies. One, the phage replicates on the first bacterium it contacts and limits its killing to the immediately surrounding cells, generating a wedge in the colony of otherwise viable cells. Two, the phage spreads though the colony killing almost all the cells. T2 phages generate wedges, “pacman-a-form” colonies, which contain viable cells, while T7 forms “casper-a-form” colonies which are filled with mostly dead cells. These results are manifest at the population level as well. When grown on surface culture with T2, the total number of viable colonies of *E. coli* B is reduced compared to phage-free controls, while *E. coli* B grown with T7 is nearly eradicated. We discuss the implications of this theoretical and experimental study to the ecology and evolution of bacteriophage.

Tracing Mobile Genes Associated with Antibiotic Resistance in the Environment

Isabella Parkhurst[*], Anuradha Goswami[†], J. Jeffrey Morris[†]
*: Presenting Author; †: Corresponding Author
Department of Biology, University of Alabama at Birmingham, AL

As surges in antibiotic resistant bacteria grow more common, the need to better understand antibiotic resistance genes (ARGs) and their ability of dissemination from one bacterium to another in the environment becomes ever more important. While there are studies that can identify the genes in isolated cultures, better understanding how the ARGs disseminate throughout the microbial community is still challenging. When relying solely on metagenomics sequencing for detection errors can arise due to the high complexity of environmental samples and the wide variety of bacteria. Our aim is to help combat this issue through developing a cost-effective laboratory protocol that allows for the identification of ARGs which are present in an environmental sample capable of transferring to a model bacterium (ARG absent *Escherichia coli*). This protocol has been developed using horizontal gene transfer (HGT), with a focus on conjugation. This study developed a method to grow transconjugants of *E. coli* from a leachate sample (contaminated environmental sample) following HGT. To encourage the conjugation necessary for this protocol to work, an environmental sample is grown in close contact with the clinical sample which encourages transfer of plasmids from the environmental sample to the clinical sample of bacteria. This close contact was created by growing the two samples on top of one another, separated by a piece of glass filter paper that because of the pore size still allows for pilus formation which is necessary for the Conjugation process. This protocol has been validated through the trials that have been completed and the transconjugants of these trials have been categorized and studied. The transconjugants exhibited resistance to multiple antibiotics such as Kanamycin and will be utilized to study the mobile ARG carrying conjugative plasmids contributing to ARG dissemination in the environment.

The unusual course of MAC complex osteomyelitis and its progression towards end-stage complications

Sonny Patel[1], Andrew Ensley[1], Muhammad S Khan[1,2], Henna Iqbal[1]
[1]Mercer University School of Medicine, Macon, GA, USA
[2]Atrium Health-Navicent Hospital, Macon, GA, USA

We present the case of a male in his 70s who presented with acute on chronic bilateral lower extremity weakness and back pain, exhibiting no signs of trauma. Physical examination was concerning due to decreased motor strength in bilateral lower extremities. MRI findings revealed a fracture of T5 vertebra, cord compression at T5-T7, and localized epidural hematoma. Chest imaging reported cavitary lung lesions and pleural effusion, raising concerns for possible tuberculosis (TB). However, patient's acid-fast bacilli in sputum, as well as TB serology, were negative. Neurosurgery involving a posterior corpectomy, along with T3-T10 fusion, revealed purulent discharge. Cultures were positive for Mycobacterium Avium Intracellulare from the Mycobacterium Avium Complex (MAC). Cultures from cavitary lung lesions were also positive for MAC. A diagnosis of MAC osteomyelitis was established. The patient was started on IV amikacin, ethambutol, rifamycin, and azithromycin. Despite the treatment, the patient did not have any improvement in his motor strength and was discharged to a rehabilitation facility. However, he returned to the hospital with septic shock and multi-organ failure and was eventually transitioned to hospice care. The manifestation of MAC osteomyelitis, coupled with the development of septic shock and acute kidney injury, represents a unique facet of MAC pathology. We highlight the emergence of novel end-stage symptoms in MAC osteomyelitis, along with protocols aimed to expand the existing literature on MAC complications.

Freshwater growth dynamics of *Salmonella enterica* serovars

Lindsay G. Pauls[1], Nikki W. Shariat[2,3]
[1]Division of Biological Sciences, [2]Department of Microbiology, and [3]Department of Population Health, University of Georgia, Athens, GA

Salmonella is the leading bacterial cause of foodborne illness in the United States, and consumption of fresh produce accounts for 43.3% of illnesses. Contaminated surface water (e.g., creeks and rivers) poses a significant food safety risk for spreading *Salmonella* to crops if it is used for irrigation without treatment. *Salmonella* has over 2,600 distinct serovars, and these differ in their capacity to cause human illness and to survive in different animals or environments. Surveillance studies from across the United States have shown that some serovars are more commonly found in water, suggesting that these serovars are better adapted to survive in water. In a previous study, we determined that serovars Give and Typhimurium were both commonly found in water but that the relative abundance of Give was typically greater. The purpose of this project was to investigate the growth and survival of serovars Give and Typhimurium in a freshwater environment. Two serovar Give (G1 and G2) and two serovar Typhimurium (T1 and T2) isolates were inoculated in triplicate into 150 mL of sterile creek water at a concentration of $6.7x10^5$ CFU/mL. A 100 mL aliquot was removed on days 0, 1, 3, 5, 7 and 14, and the concentration was determined by serial dilution and plating. After 24 hours, the G1 isolate grew by over 1 log CFU/mL and was 3.3 and 2.3 times greater than the T1 and T2 isolates, respectively; additionally, the G2 isolate was 2.3 and 1.6 times greater than the Typhimurium isolates, respectively. By day 14, the amount of G2 was 1.9 times more than the average of both Typhimurium isolates. This higher growth of serovar Give suggests a genetic adaptation that confers better growth and survival in aquatic environments.

Pf phage mediates intraspecies competition in *Pseudomonas aeruginosa*

Federico I Prokopczuk[1], Carlos J Orihuela[1], Eriel Martínez[1]
[1]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL

Introduction: *Pseudomonas aeruginosa* (*Pa*), a significant opportunistic pathogen, often cause infections that exhibit monoclonal characteristics. Nevertheless, the mechanisms through which an infecting strain prevents invasion by novel strains remain elusive. Despite numerous mechanisms of intraspecies competition being studied, the role of filamentous phages remains unexplored.
Objective: We hypothesize that sequence diversity among clinical strains underlies a complex pattern of phage immunity across *Pa* strains, enabling Pf phages to mediate intraspecies competition.
Methods: We performed multiplex PCR with universal and genotype specific (Pf1, Pf4, or Pf5) Pf primers on 70 clinical isolates to categorize the phage they produce. Concurrently, we screened the supernatant of these strains for their ability to form plaques on lawns of those same strains as well as PA01. Creating this matrix, we identified Pf producing strains with wide or narrow host ranges. *In vitro* coculturing using PA01 and candidate clinical strains were performed in broth culture, measuring the change in bacterial populations over time. These experiments were also performed *in vivo* using a mouse open wound model. After seven days, mice were harvested and bacterial populations were quantified.
Results: The matrix of clinical strain supernatants revealed a mosaic of bacterial inhibition that correlates with the presence of different phage genotypes in the supernatants versus the recipient strain. Some strains produced Pf with a wide host range, while others produce Pf that infected no strains. *In vitro* competition studies revealed little difference in bacterial populations during exponential growth, but strains producing infective Pf out competed PA01 more than strains producing non-infective Pf during stationary phase. This is confirmed with our mouse studies, who showed similar results.
Conclusions: These data lay the groundwork for further studies to explore Pf's role in intraspecies competition. Albeit preliminary, this relationship sheds light on the perplexing prevalence of monoclonal *Pseudomonas aeruginosa* infections.

Re-evaluating the gold standard: assessing *Salmonella* prevalence in broiler houses using different sampling methods.

Marco Reina, Emily E. Cason, David Ayala-Velasteguí, Nikki W. Shariat.
Department of Population Health, University of Georgia, Athens, GA, USA.

Broiler chickens are a significant cause of human salmonellosis. New initiatives in the poultry industry have proposed "directed processing" as a new logistical strategy to minimize *Salmonella* cross-contamination in the processing plant. The intention of this approach is to first screen broiler chickens on the farm and then prioritize the processing of the lowest-risk flocks before the highest-risk flocks to reduce the probability of *Salmonella* cross-contamination during processing. Critically, this relies on accurate and reliable on-farm testing. The purpose of this study was to compare different sampling methods to assess *Salmonella* prevalence and load in broiler houses. A total of 24 houses across 10 farms were sampled and six sampling techniques were evaluated: boot-socks, cloacal swabs, electrostatic pad, fecal grab, litter grab, and feather swab. In parallel, *Salmonella* prevalence was assessed by qPCR of a 24-h primary enrichment culture and by culture following selective enrichment and colony isolation from XLT-4 and CASE agars. *Salmonella*-positive samples were quantified by qPCR from a 10-h primary enrichment culture and Ct values were reported. Fisher's Exact Test was performed to establish differences in prevalence among sampling methods. All houses had at least four samples that were positive. When prevalence was assessed by culture there was no difference among sampling methods ($p=0.44$). However, when screened by qPCR differences were observed ($p=0.015$), as boot-socks showed the highest prevalence (41/48; 85%) and litter grab showed the lowest prevalence (25/48; 52%). Within each house, the Ct values were matched between the left and right sides. Boot-socks showed the narrowest distribution for *Salmonella* load, as 65% of the paired samples were within 1 cycle of each other. Overall, boot-socks showed the highest prevalence and the most compact distribution of Ct values, showcasing its potential as an on-farm sampling method to properly determine *Salmonella* prevalence in broiler houses.

Processing interventions reduce *Salmonella* serovar complexity on post-chill chicken carcasses

Amber K. Richards[1], Amy T. Siceloff[1], USDA-FSIS Eastern Laboratory[2], Nikki W. Shariat[1]
[1]Department of Population Health, University of Georgia, Athens, Georgia
[2]United States Department of Agriculture – Food Safety and Inspection Service (USDA-FSIS), Athens, Georgia

Salmonella is a leading bacterial cause of foodborne illness, with about one in five salmonellosis cases attributed to the consumption of contaminated chicken. Traditional *Salmonella* surveillance involves characterizing a single colony from a positive sample. This method favors the most abundant serovar found in a sample, potentially allowing other serovars to remain undetected in the background. CRISPR-SeroSeq is a high-resolution sequencing approach that can detect and quantify the relative frequency of multiple serovars present in a sample. We utilized CRISPR-SeroSeq to analyze *Salmonella* serovar complexity in processing and to determine if processing interventions influence serovar population dynamics. Hot rehang and post-chill young chicken carcasses were collected from processing plants across the United States from August to December 2022. CRISPR-SeroSeq was performed on all *Salmonella*-positive hot rehang (n = 153) and post-chill (n = 38) samples, in both RV and TT enrichment broth. Multiple serovars were detected in 48.4% (74/153) rehang and 7.9% (3/38) post-chill samples. Rehang carcasses contained more serovars per sample (1.6 serovars) than post-chill carcasses (1.1 serovars). There was high serovar concordance between paired rehang and post-chill carcasses (n=31) with 87.1% (27/31) of pairs containing identical serovars. Nineteen different serovars were identified, with serovar Kentucky being the most common in both rehang (72.5%; 111/153) and post-chill (73.7%; 28/38) samples. Serovars Infantis and Enteritidis, two of the most common serovars causing human illness in the United States, were found in both rehang and post-chill samples. Serovar Infantis had a higher prevalence in rehang (39.9%; 61/153) than in post-chill (7.9%; 3/38) samples. These data demonstrate that processing interventions are effective in reducing *Salmonella*, as serovar complexity was reduced in nearly all post-chill samples.

Zinc oxide nanoparticles-ciprofloxacin conjugates suppress the expression of efflux pump genes among ciprofloxacin-resistant methicillin-resistant *Staphylococcus aureus* clinical isolates

Mohammad Savari[1,2,3], Effat Abbasi Montazeri[1,2], Zahra Jafarzadeh[1, 2], Daniel Pletzer[4], and Matineh Ghomi[5,6]

[1] Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[3] Department of Biology, School of Arts and Sciences, Emory University, O. Wayne Rollins Research Center, Atlanta, GA 30322, USA (Visiting Scholar)

[4] Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

[5] Chemistry Department, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

[6] Department of Chemical Engineering, Faculty of Engineering, Arak University, Arak, Iran

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global challenge. Ciprofloxacin (CIP) is one of the choice drugs to treat MRSA infections. However, CIP resistance due to Major Facilitator Superfamily (MFS) efflux pumps, such as the Nor family, is responsible for reducing the efficacy of this antibiotic. In this study, we evaluated the antimicrobial susceptibility of methicillin and CIP against 50 clinical *S. aureus* isolates. Phenotypic and genotypic identification of efflux pumps was carried out by the Ethidium Bromide Cartwheel Method (EtBrCw) and PCR, respectively. Zinc oxide nanoparticles (ZnO-NPs) were synthesized and characterized by X-ray diffraction (XRD) pattern and Transmission Electron Microscopy (TEM). The minimum inhibitory concentrations (MICs) of ZnO-NPs and ZnO-NPs-CIP conjugates were estimated against CIP-resistant MRSA isolates. Expression of efflux pump genes from the Nor family among the CIP-resistant MRSA isolates were analyzed after exposure to sub-MICs of ZnO-NPs, ZnO-NPs-CIP, and CIP. Twenty-seven out of 50 *S. aureus* clinical samples were CIP-resistant MRSA, and among these, 19 isolates (70.3%) were EtBrCw-positive. ZnO-NPs-CIP significantly decreased the MIC values eight-fold compared to the ZnO-NPs ($p<0.05$). Furthermore, the expression of the efflux pump genes was suppressed by ZnO-NPs completely, and the expression of genes was significantly reduced by ZnO-NPs-CIP. In summary, ZnO-NPs are able to induce a suppression against the Nor efflux pumps, and when utilized in conjugation with CIP allow the antibiotic to reach its effective concentration, which eventually could be applied as an effective strategy to overcome the efflux-mediated CIP-resistant MRSA challenge.

Mapping Herpesvirus Encephalitis: Insights from Imaging and Initial Clinical Presentation

Marshall Scott[1], Camden Tucker[1], Gretchen Bentz[1], Robert Visalli[1], Emily McMackin[1]
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Macon, GA, USA

Herpesvirus encephalitis is a neurological disorder that occurs when one of the eight human herpesviruses enters the brain causing central nervous system dysfunction. Common symptoms include headache, fever, seizures, altered mental status, and focal neurological deficits. If not treated promptly and accurately, cognitive impairment, intracranial hemorrhage, or even death may occur. Although a definitive diagnosis of herpesvirus encephalitis is made using a polymerase chain reaction (PCR) test, many physicians bolster their diagnosis with magnetic resonance imaging (MRI) due to its 80 to 90% sensitivity in detecting herpesvirus abnormalities. Previous literature shows that herpetic encephalitis typically presents in the temporal and frontal lobes of the brain, however, individual viruses appear to exhibit distinct areas of potential localization. Herpes Simplex Virus 1 (HSV-1) is well documented to involve the medial temporal lobe, but other herpesviruses may mimic this disease. Due to the varying nature of each virus's localization, physicians must consider herpesviruses on their differential as a potential causative agent for encephalitis. This review of 221 PubMed articles looks at the imaging results and initial clinical presentation of immunocompromised and immunocompetent patients with herpesvirus encephalitis highlighting typical intracranial abnormalities.

Targeting Herpesvirus DNA Encapsidation Via Disruption of Portal Protein Interactions

Sandra D. Scott[1], Martin Di Grandi[2], Melissa A. Visalli[1], Robert J. Visalli[1]
[1]Department of Biomedical Sciences, Mercer University School of Medicine, Savannah, GA
[2]Department of Natural Sciences, Fordham College at Lincoln Center, New York, NY

Herpesviruses establish latency after primary infection and can reactivate to cause asymptomatic or symptomatic disease. The development of resistance to current treatments are a concern in immunocompromised, cancer, and transplant patients. Viral DNA encapsidation is a promising novel pan-herpesvirus drug target due to similarities in the portal, scaffold, and capsid protein structures across the *Herpesviridae*. WAY-150138 is a member of a novel class of small-molecule thiourea compounds that can inhibit HSV-1 replication in cell culture, likely functioning as an inhibitor of DNA encapsidation. Previous studies showed that scaffold protein is required for the incorporation of the essential viral portal at the viral capsid vertex. We hypothesized that the mechanism of action of WAY-150138 was via disruption of the scaffold-portal interaction. The purpose of this study was to evaluate the effect of WAY-150138 on portal-scaffold interactions at the molecular level. HSV-1 portal and scaffold proteins were expressed and shown to interact in a Mammalian Two-Hybrid Protein-Protein Assay. The presence of WAY-150138 significantly decreased the interactions between portal and scaffold proteins. When the two-hybrid assay was performed in the presence of increasing concentrations of WAY-150138, a dose-dependent response regarding portal and scaffold interaction was observed. In preliminary experiments, portal proteins with mutations that confer resistance to WAY-150138 still interacted in the presence of compound. This study is the first of its kind to identify an antiviral whose mechanism is disruption of portal-scaffold protein interactions. The conserved portal-scaffold interaction may be exploited as a novel target for the development of new drugs for one of more members of the herpesvirus family.

Nanoparticles against *Xylella fastidiosa*

Deepak Shantharaj[1], Jorge Pereira[2], Swadeshmukul Santra[2], Leonardo De La Fuente[1]

[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA.
[2]NanoScience Technology Center, Department of Chemistry, Department of Materials Science and Engineering, Burnett School of Biomedical Sciences, University of Central Florida, Orlando, Florida, USA.

Xylella fastidiosa is a xylem-limited phytobacterium comprising three major subspecies *fastidiosa, multiplex,* and *pauca*. This pathogen is endemic to the Americas and has spread to some countries in Europe. *X. fastidiosa* can colonize more than 600 species of plants, in some cases surviving as a commensal without causing disease, while in others causing diseases to economically important crops such as grape, citrus, olive, blueberry, pecan, peach, and almond. In the present scenario, disease management is restricted to insect vector control and no antibacterial treatments are commercially available. Antimicrobial compounds have significant limitations for penetration into the xylem. In this study, we evaluated the efficacy of nanoparticles in managing this xylem-limited pathogen. Initially, we evaluated the minimal bactericidal concentration of metal oxide and sulfide nanoparticles against *X. fastidiosa* using in vitro assays. Later we tested some of these compounds in southern highbush blueberries, a crop important in the Southeast USA that is affected by leaf scorch disease caused by *X. fastidiosa*. In greenhouse studies, we tested Zinkicide TMN110 and Na_2S NAC nanomaterials in blueberry by soil drench application. Applications of Zinkicide TMN110 at 2000 ppm and 2500 ppm, reduced *X. fastidiosa* population by ≈ 1-2 $\log_{10}$ units, and disease severity decreased by ≈38%. Na_2S NAC concentration of 1000 ppm and 2500 ppm application mitigated *X. fastidiosa* but caused phytotoxicity. In a broader outlook, nanoparticle's non-phytotoxic concentrations can be useful in mitigating *X. fastidiosa* infections in crop plants.

TESTING OLIGOMERIZATION STATE OF TraT VIRULENCE FACTOR

Kristiana Josifi, Aayushi Sharma, Nabin Ghimire, Tatyana A. Sysoeva
Department of Biological Sciences, The University of Alabama in Huntsville, Huntsville, AL

TraT is a multifunctional protein that serves as a virulence factor in bacteria. It confers numerous benefits onto its cell, such as serum and macrophage resistance, and resistance to bacteriophage, and TraT also prevents the transfer of redundant plasmids through conjugation. Despite many roles and appearances in up to 80% of clinical antibiotic-resistant samples of uropathogenic *Escherichia coli*, prior research has established little about its structure and how it relates to its many functions.

Modeling TraT protein structure suggested that TraT exists as a large ring-shaped undecamer or dodecamer. With this model at hand, we set to establish whether such large oligomers can exist in solution. For that, we proceeded with sequence conservation analysis and developed a method of isolation of recombinant TraT protein.

Multiple sequence alignments of TraT variants from thirty contemporary isolates of antibiotic-resistant *E. coli* were used to identify positions that differ. Nine substitution variants of TraT were found and analyzed for their location in the three-dimensional oligomer model. We also identified a set of amino acids including Glu47 and Ala49 that appear to interact at the interface of the neighboring subunits in the modeled TraT oligomer and their changes are correlated in the alignment.
Recombinant full-length TraT and it is truncated soluble domain were produced in BL21(DE3) cells containing an IPTG-inducible plasmid. Proteins were isolated through nickel-affinity and anion-exchange chromatography and analyzed using size-exclusion chromatography, SDS-PAGE, and Western blot. Results showed that TraT indeed oligomerizes into mixed oligomers and this is largely driven by the soluble domain itself. With the protocol of recombinant TraT isolation, we will use site-directed mutagenesis to measure the impact of the identified amino acids of interest on oligomerization and function of TraT. This will test the oligomer model and bring us closer to establishing the structure and function of this multifunctional virulence factor.

Sequencing the Nucleocapsid of Tomato Spotted Wilt Virus is a Step Towards Understanding the Peanut Breaking Resistance in Alabama.

Abdelaal H.A. Shehata[1], Michael A. Mayfield[1], Wilson Clark[1], Alexandra C. Rios[1], Alana Jacobson[1], Amanda Strayer-Scherer[1], Kathleen M. Martin[1]
[1]Department of Entomology and Plant Pathology, Auburn University, Auburn AL

Two-thirds of the peanuts in the United States are grown in Georgia, Florida, and Alabama. The Orthotospovirus, *Tomato spotted wilt virus* (TSWV), is an ambisense RNA virus composed of three segments encoding six proteins. TSWV infects over 900 plant species in 70 botanical monocot and dicot plant families. TSWV was first recorded in Alabama, Georgia, and Florida in the 1980s and has become a major obstacle to peanut production over the past three decades. There are at least nine different species of thrips that transmit TSWV, however, the western flower thrips (*Frankliniella occidentalis*) and tobacco thrips (*Frankliniella fusca*) are of the most concern in Alabama. Peanut cultivars bred for resistance were used to manage TSWV in the field, however, an increase in the severity and incidence of symptoms was observed in 2021 and again in 2022. Our hypothesis is that mutations in the genome of TSWV could be the reason for this change. The nucleocapsid gene was utilized to determine the sequence variability as a first step toward understanding these changes. In 2021, 11 peanut samples were collected from the Wiregrass Research and Extension Center, and in 2022, the study was expanded to 115 peanut samples collected across the state from three different site locations, Wiregrass Research and Extension Center, Brewton Agricultural Research Unit, and Gulf Coast Research & Extension Center. Samples were selected based on symptoms of wilt and leaf ring spots. The sequencing results and the protein alignments indicate that six amino acid changes were found in the samples collected in 2021 and two more amino acid changes were found in the samples collected in 2022. These changes could be involved in the breaking resistance of the peanut to TSWV in Alabama.

Predicting the Likelihood of Detecting *Salmonella* in Creeks using Weather and Land-Use Factors

Sherwin Shirazi[1,2], Nikki W. Shariat[1]
[1]Department of Population Health, University of Georgia, Athens, GA, USA
[2]Department of Statistics, University of Georgia, Athens, GA, USA

Salmonella contamination in freshwater sources, such as creeks, can pose a significant threat to public health and agricultural produce safety. This study aimed to develop a predictive model for detecting *Salmonella* in creeks by analyzing temporal, land-use, and weather data gathered every month for two years from four distinct creek systems in Georgia. Weather data was sourced from the nearest weather station, while land-use data utilized the USGS National Land Cover Database 2021 Land Cover dataset, considering watershed topography. Three generalized linear mixed models were constructed, treating the creek system as a random effect and the season as a fixed effect. Iterative variable selection was performed within each model to identify the best predictors of *Salmonella* detection based on comparison of Bayesian Information Criterion (BIC). A 5-fold cross validation was used to evaluate the performance of each model. Among the three models evaluated, Model C, incorporating both weather and land-use variables, outperformed the others with a model accuracy of 84.27%. Model A, containing only weather variables, had an accuracy of 78.65%, and Model B, containing only land-use variables, had an accuracy of 73.03%. Model C identified minimum temperature ($p = 0.0054$), humidity ($p = 0.0075$), wind speed ($p = 0.0006$), and radiation ($p = 0.0082$) as significant weather predictors. The likelihood of detecting *Salmonella* in creeks increased with higher minimum temperatures, greater humidity, greater wind speed, and greater radiation. Additionally, the model revealed that an increased log ratio between wetlands and herbaceous land ($p = 0.0005$) was a significant land-use predictor for *Salmonella* detection. However, these two categories made up small proportions of the total surrounding land-use. Data from this work allows for stakeholders to make informed decisions and to implement targeted intervention strategies in areas and during periods where *Salmonella* presence is more probable.

High-resolution *Salmonella* surveillance in post-harvest meat and poultry products

Amy T. Siceloff[1], Renee Smith[1], Dayna Harhay[2], and Nikki W. Shariat[1]
[1]Department of Population Health, University of Georgia, Athens, GA
[2]United States Department of Agriculture – Agricultural Research Services, Clay Center, NE

Despite significant reduction of *Salmonella* incidence during processing, meat and poultry products remain a considerable source of foodborne salmonellosis. Conventional *Salmonella* monitoring relies on the identification of a single serovar from a sample, which causes some serovars to remain undetected. This limitation can have important public health implications since different *Salmonella* serovars have varying virulence levels. This study was designed to determine the incidence of multiserovar *Salmonella* populations in meat and poultry following antimicrobial processing interventions. Deep serotyping with CRISPR-SeroSeq is a next-generation sequencing approach that quantifies the relative abundance of serovars within mixed populations. We applied CRISPR-SeroSeq to analyze 409 surveillance samples collected at slaughter facilities across the United States (pork: n=141; chicken: n=200; beef: n=38; turkey: n=31). Concurrently, up to two colonies were isolated and serotyped using molecular methods. CRISPR-SeroSeq identified 54 serovars, including 7/10 most frequently found by the CDC. There was an average of 1.5 serovars per sample, with a maximum of seven serovars detected within a single sample, and 34% (139/409) of samples contained multiple serovars. Alternatively, conventional isolation and serotyping methods found 49 serovars (average of 1.1 serovars per sample) and 8% (33/409) of samples contained multiple serovars. For 99% (404/409) of samples, serovars matched between serotyping and CRISPR-SeroSeq. Serovar profiles differed between commodity types, although Typhimurium was the top serovar in pork (26%; 37/140) and turkey (32%; 10/31) samples, and Infantis was most often found in chicken (41%; 82/200) and beef (21%; 8/38) samples. Within 20% (81/409) of samples, CRISPR-SeroSeq identified additional *Salmonella* serovars of concern that were undetected by traditional culture methods. Population analyses by CRISPR-SeroSeq revealed a third of post-harvest, post-intervention samples contain multiple serovars, demonstrating the complexity of *Salmonella* populations and the limitations of conventional methods. This study highlights the importance of comprehensive pathogen surveillance monitoring in food production systems.

Here I Doff, Turn the Phage: Validating a Hospital's Equipment Protocol with Bacteriophages

Andrew P. Smith[1], Brandon A. Berryhill[1,2], Kylie B. Burke[1,3], Jill S. Morgan[4], Jessica Tarabay[4], Josia Mamora[4], Jay B. Varkey[3,4], Joel M. Mumma[3], Colleen S. Kraft[3,4,5]

[1] Department of Biology, Emory University, Atlanta, GA, USA

[2] Program in Microbiology and Molecular Genetics (MMG), Graduate Division of Biological and Biomedical Sciences (GDBBS), Laney Graduate School, Emory University, Atlanta, GA, USA

[3] Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

[4] Emory Healthcare, Atlanta, GA, USA

[5] Department of Pathology and Laboratory Medicine, Emory University School of Medicine; Atlanta, GA, USA

Infection prevention (IP) is a central aim of hospitals. Administrators and healthcare workers bolster IP using a variety of personal protective equipment (PPE) and healthcare protocols, all of which theoretically eliminate contamination events. However, these protocols are not necessarily empirically tested. The individual pieces of PPE ensembles are often tested, but the ensembles as a whole and their doffing protocols are not experimentally validated. Here we examined a protocol for doffing a high-containment ensemble of PPE built to work with high-consequence infectious diseases. We began by watching a film depicting the doffing protocol and hypothesized what we describe as contamination pathways, that is, ways that infectious agents could travel from the outside of the PPE to the healthcare worker through the doffing protocol. We confirmed the existence of the contamination pathways through fluorescence testing, as well as a previously validated method for testing contamination via bacteriophages (phages). We employed three genetically marked phages placed on different areas of the PPE, allowing us to track three contamination pathways as a healthcare worker doffs. All healthcare workers had high phage contamination from at least one of the three phages. We incorporated simple changes and performed another set of phage-based tests. With the amended doffing protocol, we recovered no viable phages, indicating our changes eliminated the contamination pathways. Our results not only emphasize the need to empirically validate hospital protocols, but also reveal the solvency that can emerge as a result of this testing.

Salmonella prevalence in wild bird feces on fresh produce is correlated to fecal moisture

Jared C. Smith[1,2], Sofia Varriano[3], Laurel L. Dunn[4], William E. Snyder[3], Nikki W. Shariat[1,2]
[1]Department of Microbiology, University of Georgia, Athens, GA, USA
[2]Department of Population Health, University of Georgia, Athens, GA, USA
[3]Department of Entomology, University of Georgia, Athens, GA, USA
[4]Department of Food Science &Technology, University of Georgia, Athens, GA, USA

Over 40% of salmonellosis cases are linked to fresh fruits and vegetables. To develop appropriate and effective mitigations that can prevent outbreaks, it is critical to identify sources of foodborne pathogen transmission to fresh produce. Wild birds have been linked to transmission of foodborne pathogens such as *Salmonella* on to produce via their feces, but basal prevalence and vector species have yet to be defined in the southeastern United States. To fill this knowledge gap, a total of 773 fecal samples were collected from above-ground produce plants (peppers, tomatoes, squash, cucumbers, eggplants, okra) on 108 farm visits across the Southeast from May to October in 2021 and 2022 ranging from small to large operations (<0.5 acres and >100 acres, respectively). Incidence of viable Salmonella was determined by culture (2.1%) and also using a PCR targeting the *invA* gene (9.75%). Fecal moisture affected the likelihood of *Salmonella* recovery, as *Salmonella* was identified more often from moist feces than dry ($\chi^2(1, n = 773) = 6.55, p < 0.05$). When *Salmonella* culture-positive feces were moist, organisms in the Enterobacteriaceae family were significantly enriched in the microbiome compared to samples without *Salmonella*. Physical bird counts were performed on site to compare observed species to those identified molecularly from fecal samples. A total of 1123 individuals were counted, including 51 species. Molecular identification was completed on 161 bird feces which identified 24 total species contributing to fecal deposits on produce and linked five species to viable *Salmonella*. This work indicates that while wild birds can transmit pathogens to produce via their feces, the incidence appears to be low and dependent on moisture. These results can be used by growers to make informed decisions about where to use limited resources for bird control.

Identification of the genetic factors that are critically required for the survival of *Salmonella* Typhimurium in milk

Greeshma Bharathan[1], Emma Holden[2], Mark Webber[2], and Shabarinath Srikumar[1,3]
[1]College of Agriculture and Veterinary Medicine, UAE University, Al Ain, UAE
[2] Quadram Institute Bioscience, Norwich Research Park, Norwich, Norfolk NR4 7UQ, UK.
[3]Department of Poultry Science, College of Agriculture, Auburn University, Alabama, AL36830

Milk is known to be highly nutritious, and contains various antimicrobial factors such as lactoferrin, lactoperoxidase and so on. Yet, pathogenic bacteria such as *Salmonella* survives in the milk matrix and cause outbreaks. *Salmonella* Typhimurium, a foodborne pathogen causing gastroenteritis, can quickly alter their transcriptional profile and adapt to new environments. The adaptation to iron-scarce environments, such as lactoferrin containing milk, involves the production of siderophores that will scavenge environmental iron for *Salmonella.* Even though the adaptation to an iron scarce host environment is well characterized for the pathogen, similar studies on food matrices is rare. The siderophore based adaptation to iron scarce environment is regulated by the DNA binding ferric uptake regulator (Fur). The growth kinetics of a *S.* Typhimurium 4/74Δ*fur* in two milk matrices, bovine and milk, was compared to that of the isogenic wild type. The absence of the *fur* gene hindered the growth of *Salmonella* in both milk matrices. Fur repressed the siderophore production. Using ferric chloride and streptonigrin, we demonstrated that pathogenic inhibition happened due to a cytoplasmic iron accumulation and consequent iron toxicity in *S.* Typhimurium 4/74Δ*fur.* To obtain a more global picture of gene involvement in the *Salmonella* milk survival, we resorted to transposon mutagenesis based TraDIS-Xpress – a massively parallel transposon mutagenesis approach using transposon-located promoters to assay the impact of disruption or altered expression of all genes in the *Salmonella* genome. Genes such as *nirD* (NADH nitrite reductase), STM14_1440 (possible bacteriophage protein), *celAB* (chitobiose phosphotransferase system, chitobiose import) were critically necessary for the survival of the *Salmonella* in milk matrices. We identified the transcriptional factors and genes that are critical for the survival of *Salmonella* Typhimurium in milk, a theme that can be expanded upon to mitigate the pathogen's ability to survive in milk.

Discovering Natural Product Producers from the Microbiome of Arctic Marine Sediments

Alex Swystun[1], Emma Smith[3], Kevin Kiser[2], Patrick Erwin[2], R. Thomas Williamson[3], Blake Ushijima[2], Wendy K. Strangman[3].
Department of Marine Science[1],
Department of Biology and Marine Biology[2],
Department of Chemistry and Biochemistry[3],
University of North Carolina Wilmington, Wilmington, NC 28403.

Natural products are a prolific source of potential drug leads.[1] Bacteria make a diverse array of potent natural products and exploration of new environments often translates to the discovery of bacteria that have the capacity to produce novel compounds. In the current research, fifty-seven bacterial strains from a series of deep Arctic marine sediment cores, an underexplored area in natural product research, were isolated in UNCW's Drug Discovery Lab and identified based on 16S rRNA gene sequencing. Chemical extracts of ten of these strains have been tested at Wayne State University Oncology Center for anti-tumor activity, and at George Mason University Center for Biodefense and Infectious Disease Research against a panel of multi-drug resistant pathogens. Additionally, thirty-three bacterial extracts have been screened for their ability to produce compounds called metallophores that bind metal ions and display distinctive binding of different metals.[2] Metallophores in their apo- and metal-bound states can be critical for ecological bioactivity and future pharmaceutical applications.[3] To increase assay efficiency, metal dyes were adapted to liquid spray HP TLC plates for high throughput testing of the microbial library. The isolate's extracts were spotted on TLC plates with the goal of discovering new compounds specific to a variety of transition metals including iron, copper, zinc, cobalt, and manganese. Metal-specific chelators will next be screened by collaborators to assess bioactivity and determine potential pharmaceutical applications.

Metagenome-wide characterization of shared antimicrobial resistance genes in sympatric people and lemurs in rural Madagascar

Brooke M. Talbot[1], Julie A. Clennon[2], Fara Rakotoarison[3], Lydia Rautman[2], Sarah Durry[4], Leo J. Ragazzo[2], Patricia C. Wright[3,5], Thomas R. Gillespie[1,2,3,4*], Timothy D. Read[1,6]

[1]Program in Population Biology, Ecology, and Evolutionary Biology, Emory University
[2]Department of Environmental Sciences, Emory University
[3]Centre ValBio, Ranomafana, Madagascar
[4]Department of Environmental Health, Rollins School of Public Health, Emory University
[5]Institute for the Conservation of Tropical Ecosystems, Stony Brook University
[6]Division of Infectious Diseases, School of Medicine, Emory University

Tracking the spread of antibiotic resistant bacteria is a critical step in reducing the global morbidity and mortality of human and animal infections. Since antibiotic resistance genes (ARGs) can be conferred through mobile genetic elements to susceptible bacteria, it is critical to examine bacterial microbiome structures to quantify the burden and potential of antibiotic resistance spread. Further, there is a need to understand the role that wild animals may play in transfer. In this study, we used metagenomics to identify and compare the abundance of bacterial species and ARGs detected in the gut microbiomes from sympatric humans and wild mouse lemurs in a forest-dominated, roadless region of Madagascar at the interface of Ranomafana National Park. We examined whether demographic factors among human residents contributed to differences in ARG abundance. Finally, we compared the genomic similarity of common ARGs between human and lemur microbiomes. The diversity of bacterial species and ARGs between human and lemur microbiomes were distinct in both alpha and beta diversity measure, with human microbiomes having a higher burden of ARG abundance but a similar number of detectable alleles to lemur microbiomes. Home village was the only demographic factor correlated with ARG abundance. Human and lemur microbiomes shared 14 distinct ARGs which were highly conserved in nucleotide identity between sample source pairwise comparisons. Synteny of ARG-associated assemblies revealed that there is a distinct multidrug-resistant gene cassette carrying *dfrA1* and *aadA1* present in human and lemur microbiomes without evidence of geographic overlap, suggesting that these resistance genes could be widespread in this ecosystem. This study highlights the need to investigate intermediary processes of ARG spread that could maintain populations of drug-resistant bacteria.

Thason

Interaction Between *Staphylococcus aureus* and *Staphylococcus epidermidis* During Biofilm Formation

Staphylococcus aureus, a residential bacterium of the human skin and mucous membranes, is a serious opportunistic pathogen. One factor that contributes to the virulence of *S. aureus* is the ability to form biofilms. A biofilm is a complex community of microbes encased in a protective matrix that can exist on living and non-living surfaces. Biofilms are ubiquitous in nature and negatively impact many industries related to human health, such as the healthcare and food industry. Previous studies have shown that *Staphylococcus epidermidis*, a more prevalent and less pathogenic residential bacterium of the human skin, can inhibit biofilm formation by *S. aureus*. In this study, the results showed that *S. aureus* biofilm formation is inhibited when *S. epidermidis* is the initial colonizer. Growth curve data indicated that *S. epidermidis* grows slower than *S. aureus*; therefore, if both species are inoculated simultaneously, *S. aureus* can grow quicker and establish a biofilm before *S. epidermidis* can have an impact. Studying competitive bacterial interactions, such as that between *S. aureus* and *S. epidermidis*, could provide more information about the mechanisms of biofilm formation and ways to disrupt these harmful communities.

Pro-Inflammatory Caspase-1 Regulates the Innate Immune Function of Amyloid-Beta

Amanda N. Tuckey[1,2], Jonathon P. Audia[1,2]

[1]Department of Microbiology and Immunology, University of South Alabama Whiddon College of Medicine, Mobile, AL, USA
[2]Center for Lung Biology, University of South Alabama Whiddon College of Medicine, Mobile, AL, USA

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. The Centers for Disease Control and Prevention estimates 1.7 million cases per year in the United States. Compounding the acute problems associated with the infection, approximately half of survivors develop post-sepsis syndrome and attendant chronic, inflammation-driven complications. Amyloid-β (Aβ) has recently emerged as a pleiotropic innate immune effector that may function both as an initiator of the host response to infection and as a downstream activator of deleterious inflammation. First, Aβ is an antibacterial, antiviral, and antifungal peptide. Second, Aβ is a known activator of inflammation via pattern recognition receptor signaling, including the NLRP3-caspase-1 inflammasome. Intriguing, *in silico* analysis identified a putative caspase-1 cleavage site within Aβ, highlighting a possible novel regulatory relationship between these two innate immune effectors. *In vitro*, we have shown that caspase-1 prevented spontaneous Aβ fibril formation; a hallmark feature associated with Aβ pathophysiology. To add onto this finding, we also synthesized the two peptide fragments corresponding to the predicted caspase-1 cleavage site (termed small and large fragments) and tested for effects on Aβ fibril formation. *In vitro*, an equimolar mixture of the small and large Aβ fragments demonstrated very modest fibril formation. However, when added to full length Aβ, the small fragment inhibited and the large fragment stimulated spontaneous fibril formation. Future studies will examine the effects of caspase-1 cleavage on Aβ antimicrobial activity including potential regulatory roles for the small and large Aβ fragments.

Unraveling the Oxidative Stress Response Mystery: Sulfur Starvation Induces Fe dyshomeostasis in *Pseudomonas aeruginosa*

Chidozie G. Ugochukwu[1], Tonia S. Schwartz[2], Tonya N. Zeczycki[3], Holly R. Ellis[3] & Douglas C. Goodwin[1]

1. Department of Chemistry and Biochemistry, Auburn University, AL
2. Department of Biological Sciences, Auburn University, AL
3. Department of Biochemistry and Molecular Biology, Brody School of Medicine, East Carolina State University, NC

Bacteria typically thrive on sulfate as their preferred sulfur source for growth and metabolism. However, when sulfate becomes scarce, they undergo an adaptive transformation that enables the utilization of non-preferred organosulfur compounds. This shift involves intricate metabolic reprogramming, including the activation of sulfur-scavenging and antioxidant genes. Despite this, the mechanistic details behind the expression of antioxidant genes during sulfur starvation have remained elusive. To unveil this mystery, our study investigates the effects of sulfur starvation on *Pseudomonas aeruginosa* (PA), a human opportunistic pathogen known for causing life-threatening infections.

In our experiment, we examined six samples of PA: three replicates were grown in sulfur-free media (treatment group), and three were supplemented with 500 μM sulfate (control group). We employed RNA sequencing (RNAseq) and label-free proteomics to quantify the transcriptome and proteome. Subsequently, we conducted enrichment and network analyses of the detected differentially expressed features to gain insight into overrepresented biological pathways.

RNAseq analysis identified 5658 genes, with 1053 exhibiting differential expression in the treatment group compared to the control. Similarly, our proteomics analysis revealed 659 differentially expressed proteins out of 2321 reliably quantified proteins. Remarkably, sulfur starvation led to a significant downregulation of Fe-uptake genes and pathways, including the pyoverdine (*pvd*), pyochelin (*pch*), and heme-uptake (*phu* and *has*) operons in the treatment group. Additionally, we observed a marked decrease in genes associated with virulence pathways, such as quorum sensing, multidrug-resistant (MDR) efflux pumps, phenazine biosynthetic genes, and hydrolytic enzymes regulated by the ferric uptake regulator (Fur). Conversely, there was a substantial upregulation of genes related to Fe-storage (*bfrB*), reactive oxygen species (ROS) metabolism (*sodB*, *lsfA*, *ohR,* and *ahpC*), and sulfur acquisition and assimilation (*msu*, *ssu*, and *cys* operons).

Our study reveals the intricate connection between sulfur metabolism and Fe homeostasis in PA. The findings indicate that sulfur starvation prompts an Fe-replete response in PA, activating antioxidant genes to combat the oxidative effects of free intracellular Fe. Moreover, we uncovered how sulfur starvation suppresses virulence pathways in PA, offering intriguing possibilities for future clinical investigations.

The Role of Cell Free DNA in Diagnosing Periprosthetic Joint Infection

Melanie Valencia and Reagan Williams

Mercer University School of Medicine, Savannah, GA

Periprosthetic joint infections (PJI) are incredibly difficult to eradicate, therefore prior to devising a treatment plan, the presence of PJI and the identity of the pathogen must be confirmed. Conventional methods have included bacterial culture, which lack sensitivity and inflammatory markers, which lack specificity. Nevertheless, throughout the bloodstream and the synovium there's free floating DNA from cell excretions and apoptosis that can provide insight into the infection. The use of cell free DNA (cfDNA) in diagnosing PJI is especially of interest as biofilm, commonly formed in PJI, is known to excrete extracellular DNA. Yet, it is not well understood how cfDNA analysis should be interpreted and integrated into clinical decision-making. Therefore, the National Library of Medicine was searched for clinical trials using cfDNA for detection of PJI in the past 5 years (2018-2023). The mean concentration of cfDNA in participants with PJI was 20 times greater than the control with a sensitivity of 96.2 and specificity of 100% of detection of infection [1]. In comparison to standard bacterial cultures, implementation of cfDNA increased pathogen detection by 8%, detected pathogens otherwise not found, and decreased the time to retrieve the results by 3 days [2]. Furthermore, cfDNA analysis is improving in specificity through next-generation sequencing, making it possible to identify antibiotic-treated and antibiotic-resistant bacteria [3]. However, next-generation sequencing has some drawbacks, as its increased sensitivity can lead to false positives due to the body's residential bacteria [3]. Nonetheless, one of the greatest limitations of cfDNA is its limited availability within the body, yet the sensitivity of cfDNA remains greater than current methods of detection. In conclusion, the concentration of cfDNA provides evidence of a periprosthetic joint infection and it may also contain fractions of cfDNA from the pathogen, facilitating the identification of the pathogen and antibiotic-resistant strands.

Modeling transmission chain heterogeneity from experimental data

Vega

Heterogeneity is inherent in biological processes. Many distributions of biological data are characterized by asymmetry/skew and long, sometimes bumpy tails, and it is difficult to generate models that fit the relevant features of these distributions. Here we show, using experimental data from small host models, how distributions of both transmission and sensitivity to colonization affect the shape of transmission chains, with distributions of secondary infections as the primary measure of interest. These results provide novel insights into the effects of individual heterogeneity on the outcomes of the stochastic process of transmission.

Evaluation of ACC deaminase activity of rhizobia isolated from soybean nodules

Kasun T. Wanninayaka, Alvaro Sanz-Saez, and Yucheng Feng.
Department of Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL, USA

1-Aminocyclopropane-1-carboxylate (ACC) is the immediate precursor of the plant stress hormone, ethylene, which regulates plant growth and stress response. Soil bacteria, including rhizobia, can break down ACC using the enzyme ACC deaminase and reduce the inhibitory effects of ethylene in plants during stress periods. The objectives of this study were to screen rhizobia isolated from soybean nodules for ACC deaminase activities and determine the capability of selected isolates to reinfect soybean. We found that the ACC deaminase activities of 49 isolates ranged from 0.34 to 3.53 μmol α-ketobutyrate/mg protein/h. Six of these rhizobial isolates were selected to evaluate their nodulation capability and nitrogen fixation potential in soybean using Leonard jars in a growth chamber with uninoculated plants serving as the controls. At the end of the one-month growth period, all inoculated plants produced nodules, whereas the uninoculated plants did not. Analysis of variance performed on plant physiological parameters showed significant differences among inoculation treatments ($p < 0.05$). However, inoculation with isolates of higher ACC deaminase activity did not always lead to higher chlorophyll content, nodule number, and dry weight. These isolates will be used in future research to determine the role of ACC deaminase activity in rhizobia on soybean drought tolerance.

Characterization of a new *Pseudomonas aeruginosa* Queuovirinae bacteriophage

Lauren Whiteley[1], Marvin Whiteley[1]
[1] School of Biological Sciences, Georgia Institute of Technology

The ESKAPE pathogen *Pseudomonas aeruginosa* is a common cause of chronic wound and cystic fibrosis lung infections, as well as acute burn and nosocomial infections. Many of these infections are recalcitrant to conventional antibiotic therapies due to both traditional antibiotic resistance mechanisms and antimicrobial tolerance. Recent successes with bacteriophage (phage) therapy to treat chronic human *P. aeruginosa* infections has led to a renewed interest in isolating and characterizing new *P. aeruginosa* phages. Here, we isolated and characterized a new lytic phage (termed PIP, Pili Infecting Phage) capable of infecting *P. aeruginosa* PA14. PIP is a tailed phage with an icosahedral head and flexible tail containing a circular genome that is 57,462 bp in length. Phylogenetic analysis reveals that PIP belongs to the subfamily Queuovirinae and genus Nipunavirus, but is highly divergent in gene content from known Nipunaviruses. By isolating and characterizing a *P. aeruginosa* strain that spontaneously evolved resistance to PIP, we show that the receptor for PIP is Type IV pili. In sum, we isolated a new *P. aeruginosa* phage species with a unique genome, thus increasing the diversity of phages known to infect this important human pathogen.

The Role of Quorum Sensing Response Regulator LuxR on *Flavobacterium covae* Biofilm Formation and Virulence in Channel Catfish (*Ictalurus punctatus*)

Rachel E. White[1], Emily M. Churchman[1], Tingbi Xu[1], Miles D. Lange[2], Stacey LaFrentz[1], Benjamin R. LaFrentz[2], and Mark R. Liles[1]

[1]Department of Biological Sciences, Auburn University, Auburn, Alabama, USA
[2]United States Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, Auburn, Alabama, USA

Columnaris disease leads to massive losses in catfish aquaculture due to the bacterial pathogen *Flavobacterium covae*. It has been speculated that biofilm formation on skin and gill surfaces may contribute to pathogenicity. Previous research has shown that catfish mucus significantly upregulates the quorum sensing response regulator LuxR in *F. covae* biofilms. Since quorum sensing is known to regulate biofilm production and virulence in other aquatic pathogens, we hypothesized that LuxR has a similar role in *F. covae.* A *luxR* deletion mutant (Δ*luxR*) was constructed in *F. covae* C#2 using an allelic exchange method. Microtiter biofilm formation was quantified for the wild-type and Δ*luxR* mutant. A significant increase of biofilm production was observed in the Δ*luxR* mutant compared to the wild-type strain. Interestingly, when supplemented with 10 mM mannose, the wild-type strain exhibited an increase in biofilm formation while the Δ*luxR* mutant remained unaffected. These results suggest a phenotypic difference in biofilm formation due to the *luxR* deletion. Recently, the Δ*luxR* mutant has been complemented with a plasmid containing the *luxR* gene and the biofilm experiments will be repeated with the wild-type, Δ*luxR* mutant and complemented Δ*luxR* mutant. Future research includes evaluating the transcriptome of these strains to identify differentially expressed genes and determining the role of LuxR in virulence by *in vivo* infection of channel catfish. Collectively, these data will yield greater insights into the role of LuxR in *F. covae* biofilm formation and pathogenesis.

Interactions between *Pseudomonas aeruginosa* aggregates and polymorphonuclear leukocytes in the cystic fibrosis lung

Oriana M. Williams[1], Sophie E. Darch[1]
[1]Department of Molecular Medicine, University of South Florida, Tampa, FL, USA

Pseudomonas a*eruginosa* (*Pa)* is a pathogen known to colonize the airways of the lungs contributing to chronic infection in patients with cystic fibrosis (CF). In CF *Pa* forms small dense clusters of 10-1,000 cells called aggregates. Growth as an aggregate allows *Pa* to resist antibiotic intervention and become tolerant to host immune responses. The interaction of *Pa* and polymorphonuclear leukocytes (PMNs) during chronic CF infections is characterized by a lack of microbial clearance by PMN mediated mechanisms. We hypothesize that PMNs and its byproduct, neutrophil elastase, directly impact spatial organization and growth rate of *Pa* aggregates. Changes in spatial structure likely influence aggregates ability to regulate virulence pathways that allow tolerance to host immune responses. However, the mechanisms that underpin this are not fully understood. We hypothesize that PMNs influence the genetic and physiological response of *Pa* aggregates. Quantifying regulation of genes in the presence of PMNs may indicate what mechanisms *Pa* employs to resist immune response during aggregate growth. A challenge to studying bacterial infection in the CF lung environment is creating physiological conditions like the heavily mucus filled airways. Here, aggregates are grown in a synthetic sputum media (SCFM2) to mimic the CF lung environment. To determine how PMNs in a CF lung environment affect spatial organization and growth rates of *Pa* aggregates, confocal microscopy was used to track aggregate size and growth rate after the addition of human elastase. We found a significant reduction in the size of *Pa* aggregates after the addition of elastase, however there was not complete clearance of *Pa* aggregates in SCFM2. Visualizing aggregates and PMNS using SCFM2 provides valuable insights into the role of aggregate spatial organization during host-pathogen interactions in CF. Additionally, determining genes important for aggregate survival will allow better understanding of how immune tolerance mechanisms in *Pa* function.

Intranasal Povidone Iodine Provides Prophylaxis Against *S. Aureus* Periprosthetic Joint Infections

Reagan B. Williams and Melanie Valencia
Mercer University School of Medicine, Savannah, GA

Staphylococcus aureus (SA) is a gram-positive bacterium and a known cause of surgical site infections (SSI). With known antibiotic-resistant strains, the treatment of SA has become more challenging. One of the greatest concerns with SA is its ability to form a biofilm on prosthetics, such as total joint arthroplasties (TJA). While TJA has become a highly successful procedure, surgical site infection (SSI) has remained the most common reason for failure. SA is currently the most frequent cause of SSI, and surgeons have been using nasal decolonization with Mupirocin as an optimal strategy for prevention in patients receiving orthopedic implants. However recently, Mupirocin has shown a decline in decolonization and reduced efficacy in SSI prevention from supposed bacterial resistance. Studies show that povidone-iodine (PI) solutions offer promising results for SA decolonization. Previously a disadvantage to PI was its inactivation by nasal secretions, although recent improvements to PI formulations have helped overcome this. Therefore, prior to implementing PI as a standard protocol, the cost analysis and current antibacterial efficacy of different PI formulations, including off-the-shelf PI products and a PI-based skin and nasal antiseptic (SNA), for nasal decolonization. The National Library of Medicine was searched for all published articles evaluating intranasal administration of povidone iodine as prophylaxis for periprosthetic joint infection within the past 10 years (2013-2023). Approximately 10% of SA strands showed resistance to mupirocin. Thus, SNA was significantly more effective at decolonizing *S. aureus* over the 4-hour time interval ($P = .003$). However, there was no significant difference found over a 24-hour period among the off-the-shelf PI group, SNA group, and saline swab (control) group. Furthermore, MRSA displays even less susceptibility to mupirocin compared to MSSA ($p<0.001$). PI is the preferred prophylaxis for patients with MRSA colonization because it displays uniform sensitivity to MRSA, PI is unaffected by antibiotic resistance, and only requires one application, leading to greater patient adherence to protocols. Prevention of SSI proves to beneficial to patient outcomes as it can greatly decrease the length of hospital stay by 50%. The cost analysis predicts a possible $74.74 savings per patient with the use of povidone-iodine prior to total joint arthroplasty. Overall, the use of PI solutions prior to TJA is beneficial in controlling nasal SA over short periods of time. PI-based SNA proved to have the greatest antibacterial efficacy compared to other solutions and thus should be a considered regime for intranasal decolonization in patients with considerable risk for infection. The use of intranasal PI solutions also has shown a cost savings of $74.72 per patient, furthering the benefit of this potential treatment. Therefore, intranasal PI could provide an optimal alternative to mupirocin for patient decolonization. Future research should be directed toward the potential risks of developing bacterial resistance against PI. As bacterial strains continue to develop resistance to intranasal prophylaxis, it's important for microbiologists, and surgeons alike, to remain vigilant in their attempt to further develop effective prophylactic solutions.

Evaluation of oral adjuvanted killed *Aeromonas hydrophila* vaccines in channel catfish (*Ictalurus punctatus*)

Allison Wise[1,2], Craig Shoemaker[1], Troy Bader[1], Benjamin Beck[1]
[1]USDA-ARS Aquatic Animal Health Research Unit, Auburn, AL 36832
[2]College of Veterinary Medicine, Auburn University, Auburn, AL 36832

Virulent *Aeromonas hydrophila* (vAh) attributes nearly $35 million dollars in economic losses within the southeastern catfish aquaculture industry. This bacterial pathogen infects channel and hybrid catfish inducing skin necrosis, internal and external hemorrhaging, and exophthalmia. Farmers can lose over 50% of a harvest yield in less than a week when infected, increasing the urgency for more effective preventative measures. Oral bacterin vaccination with the inclusion of an adjuvant is a promising solution providing ease of administration for farmers, while decreasing stress put onto the catfish. Bacterin vaccines typically mediate a high antibody response and even more so when boosted. Research has been conducted demonstrating adjuvant's ability to also increase protection and duration of protection, thus this study's first objective is to determine whether the addition of an adjuvant increases vaccine efficacy. Over the years, multiple strains of vAh have caused catastrophic mortalities in both Mississippi (S14-452) and Alabama (ALG-15-097). Due to the variation within vAh strains, objective two is to determine whether bacterin vaccines with adjuvants demonstrate cross-protection amongst S14-452 and ALG-15-097. Bacterin vaccines were made for S14-452 (1.10×10^7) and ALG-15-097 (1.0×10^7). To test objectives one and two, channel catfish (13 g) were separated into treatments (ALG-15-097, ALG-15-097 + adjuvant, S14-452, S14-452 + adjuvant, adjuvant only, sterile TSB). Feed was top-coated with each treatment and fed for 7 days at 2% body weight and boosted at 9 weeks. At 3 and 12 weeks post-vaccination and boost, treatment groups were challenged with ALG-15-097 (3.4×10^7 CFU mL^{-1}), and cumulative percent mortality was evaluated to determine efficacy and cross-protection. At 3 weeks, ALG-15-097 (49.3% ± 9.23) experienced significantly less mortality ($P < 0.05$) compared to adjuvant only (92% ± 6.93) and placebo groups (97.3% ± 2.31). Cross-protection and an increase in efficacy appears promising at 12 weeks after treatments have been vaccinated. Results from this study allows for the development of more efficacious vaccine components and delivery on catfish farms, ultimately, preventing mass mortality due to vAh.

Characterization of SAP in Group B *Streptococcus* as a Novel Virulence Factor Contributing to Disease

Jacob Wood[1], Aidan Flanagan[1], Cody Cutts[1], Alexander Westermann[2], Till Sauerwein[3], Konrad U. Foerstner[3], Melanie Higgins[1], Brandon J. Kim[1]
[1]University of Alabama; [2]Helmholtz Institute for RNA-Based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI); [3]TH Koeln University of Applied Sciences

The blood-brain barrier (BBB) is a highly specialized collection of brain endothelial cells (BECs) that surround the vessels in the brain and allow for the selective passage of substances. This regulation is vital to protect the brain from pathogens such as Group B *Streptococcus* (GBS), which can cause diseases like bacterial meningitis. RNA sequencing of GBS during infection revealed one up-regulated gene encoding for an LPXTG motif, which indicates a surface anchoring region on the protein. Due to their extracellular nature, these proteins have a likelihood to contribute to virulence. The identified protein, *Streptococcus agalactiae* pullulanase (SAP), is of interest because of its known ability to bind extracellular polysaccharides. Previous literature has shown SAP's ability to bind other types of cells, but interaction with BECs is a novel approach. Using *E. coli* expression vectors, we will selectively target and express 2 separate fragments, along with the full-length protein for purification. The fragments and full-length targets will be used to characterize novel interactions of SAP with BECs by conducting assays using an induced pluripotent stem cell model of BECs to accurately represent the BBB. This work will support the identification of SAP as a novel virulence factor that plays a role in GBS interaction with the BBB. Considering the absence of a GBS vaccine and the challenges involved in treating GBS infections, this work has far-reaching applications in the development of vaccines, antibiotics, and specialized therapies to diminish GBS virulence, ultimately reducing disease prevalence

Impact of choline binding protein-F in pneumococcal pathogenicity and host tissue damage

Vipin C, Katherine L. Kruckow, Eriel Martínez, Jennifer N. Luck and Carlos J. Orihuela*
Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL, United States

Streptococcus pneumoniae (Spn) is one of the most important opportunistic pathogen causing invasive infections. Choline binding proteins are a family of surface bound proteins found in *S. pneumoniae* involved in a wide range of physiological functions, including virulence. Here, we describe the importance of *CbpF*, a member of CBP group in organ specific pneumococcal infection. We bioinformatically compared the genomic content of the *S. pneumoniae* hypervirulent genetic lineages GPSC10 and GPSC27 (TIGR4) to that of the less virulent GPSC12 genetic lineage to identify genes that may play a crucial role in virulence/pathogenicity. To understand the role of *CbpF* in pathogenesis and host organ damage, we infected 10-week-old female C57BL6J mice intratracheally with Wild-Type TIGR4 or its isogenic *CbpF* deficient mutant (*ΔCbpF* TIGR4). The health index, weight, bacterial titers in the blood, were recorded at 24h interval post-infection as was overall survival. At time of death, lungs and hearts were excised and histopathological analyses of the organs was performed by examination of H&E-stained tissue sections and immunofluorescence-based detection of bacteria in the organs. Bioinformatic analysis revealed that C*bpF* was conserved only in the hypervirulent lineages GPSC10 and GPSC27. Compared to the WT-TIGR4 infected group, *ΔCbpF* TIGR4 infected group of mice had better health index scores. Over a 24-hour period, both groups lost weight and had a rise in bacterial titer in their blood however the WT-TIGR4 group had a moderately higher bacterial blood titer. Microscopic analysis showed greater number of foci of *S. pneumoniae* infection in both WT-TIGR4 infected lung and heart tissues compared to *ΔCbpF*-TIGR4. Our results suggest *CbpF* plays an important role in disease severity caused by *S. pneumoniae*, particularly with regard to the formation of foci of infection.

Genome evolution of the microsporidian pathogen genus *Nosema*

Xiao Xiong[1], Chris J Geden[2], Dapeng Zhang[3], Daniel Bergstralh[4], John H Werren[4], Xu Wang[1,5,6]

[1]Auburn University, [2]USDA Agricultural Research Service, [4]Saint Louis University, [5]University of Rochester, [5]HudsonAlpha Institute for Biotechnology, [6]Alabama Agricultural Experiment Station

Nosema is a diverse genus of microsporidian parasites, which are fungal symbionts and pathogens of insects and other arthropods. In this study, we report high-quality assemblies (14,397,169 bp) of *Nosema muscidifuracis* genomes and comparisons to other *Nosema* genomes. *N. muscidifuracis* infects parasitoid wasp species of *Muscidifurax zaraptor* and *M. raptor* (Hymenoptera: Pteromalidae). Using PacBio long-read sequencing technology, a novel composite 4-bp (TAGG)n and 5-bp (TTAGG)n telomeric repeat motif was discovered at the ends of chromosomes, which represent the first identified telomeres for *Nosema*. In total, 2,782 protein-coding genes were annotated, of which 449 shared orthologous genes with six other genome-sequenced *Nosema* species. Comparative phylogenomic analyses revealed incongruency in the *Nosema* and host species trees, indicating a host switch event between parasitoid wasps and bees. In *N. muscidifuracis*, a highly significant ACCC motif was found within 20 bp upstream of the translation start codon ATG. This motif is present in 90% of highly expressed genes, in sharp contrast to ~20% in lowly expressed genes, and therefore serves as a candidate *cis*-element for positive regulation of gene expression. Strikingly, similar (C)3 and (C)4 motifs were also discovered in other distant related *Nosema* species, suggesting a conserved *cis*-regulatory mechanism. The unusually low GC-content (22.6%) drives a substantial A-T bias in the third codon position in protein-coding genes in *N. muscidifuracis*. Cytogenetic analyses revealed a substantial *Nosema* load within the ovaries of *M. raptor* and *M. zaraptor*, consistent with a heritable component of infection and per ovum vertical transmission. The parasitoid-*Nosema* system is laboratory tractable, and therefore can serve as a model to inform future genome manipulations of *Nosema*-disease microsporidian pathogen as potential cures for this disease. Our study also provides novel insights into the genetic architecture, gene regulation, and genome evolution of *Nosema* species and will enhance the understanding of host-parasite interactions.

The global distribution of hypervirulent *Aeromonas hydrophila* and its recombinant vaccine control

Xu, et al.

Aeromonas hydrophila is an important opportunistic pathogen to humans, and fish. Over the past decade, vast mortalities of farmed fish due to motile *Aeromonas* septicemia (MAS) have occurred in channel catfish (*Ictalurus punctatus*) farmed in the United States and in grass carp (*Ctenopharyngodon idella*) farmed in China. While *A. hydrophila* is typically regarded as a secondary pathogen, the emerging hypervirulent *A. hydrophila* (vAh) corresponds to a unique sequence type (ST251) that acts as a primary pathogen causing high fish mortality. In the US, vAh isolates have been isolated in Alabama, Mississippi, and Arkansas, resulting in the cumulative loss of over 35 million pounds of channel catfish in AL alone.

Due to the significant threat of vAh, a global survey was conducted to investigate its distribution, and phylogeny. Isolates from Southeast Asia, South Asia, Europe, Northern America, and Africa were tested for *myo*-inositol utilization, *gyrB* phylogeny, core genome phylogeny, and virulence factors. Five strains isolated from Cambodian pangas catfish (*Pangasius pangasius*) and Vietnamese striped catfish (*Pangasianodon hypophthalmus*) were identified as vAh, based on the results of phylogenetic analysis, pairwise comparison of average nucleotide identity and predicted virulence factors.

Multiple vAh gene pathways/clusters were observed to be upregulated within channel catfish by *in vivo* transcriptome analysis. The *in vivo* expressed proteins were analyzed for cellular location, solubility, and antigenicity. Based on these results, 12 proteins were selected to produce recombinant protein and evaluation as vaccine candidates. Those proteins, like MatB, FlpD, CpaA/B, and Flp/Fap, were PCR amplified and cloned into TOPO vector, and expressed by induction with IPTG in *E. coli*. Ongoing experiments are evaluating the use of purified recombinant proteins like MatB and CpaA/B to evaluate their immunogenicity by testing with vAh-challenged channel catfish serum. The best candidates will be tested as recombinant vaccines to protect channel catfish against vAh infection.

PspA-mediated aggregation protects *Streptococcus pneumoniae* against desiccation on fomites

Jessica R. Lane, Muralidhar Tata, Rahena Yasmin, Hansol Im, David E. Briles, and Carlos J. Orihuela
Department of Microbiology, University of Alabama at Birmingham, AL, USA

Streptococcus pneumoniae (*Spn*) is an opportunistic pathogen that resides in the nasopharynx. Pneumococcal surface protein A (PspA) is an established *Spn* virulence factor that protects the bacterium by inhibiting killing activity of antimicrobial peptide lactoferricin (LF). PspA is known to interact with mammalian GAPDH (m-GAPDH), however the details of this interaction are not clear. Mice were colonized with wildtype *Spn* and an isogenic ΔPspA mutant. Nasal lavage fluid was examined microscopically. Recombinant PspA, GAPDH (m-GAPDH and *Spn*-GAPDH (*Streptococcus pneumoniae* GAPDH)) were expressed in *E. coli* and purified using cobalt-affinity resin. Flow cytometry was used to determine binding of FITC-labeled GAPDH to wildtype and ΔPspA *Spn*. Formation of a multimeric complex consisting of PspA-GAPDH-LF was tested for using Surface Plasmon Resonance (SPR). To test the impact of PspA-mediated GAPDH-dependent aggregation, *Spn* was suspended in saline containing GAPDH and LF at distinct concentrations and the size of the aggregates were calculated from images acquired microscopically. We validated the ability of wildtype *Spn* to bind to mGAPDH and LF in PspA dependent manner from microscopic examination of nasal lavage elutes from wildtype and ΔPspA *Spn* colonized mice. In *in vitro* GAPDH was found to mediate bacterial aggregation in concentration and PspA dependent manner, with LF disrupting the aggregation. SPR experiments confirmed that GAPDH and PspA formed a multimeric complex on *Spn* surface that was dissociated by LF. Bacteria in aggregates survived desiccation, indicating PspA plays a role in transmission on fomites. PspA mediates bacterial aggregation during colonization that promotes survival on fomites when nasal lavage is desiccated. Importantly, its effects can be countered by host LF. Our data presented here describes a new role for PspA

Preliminary Analysis of Tennessee River Bloom at Water Treatment Plant

Natalie Yates[1], Brett Harris[1], and Eric Becraft[1]
[1]Department of Biology, University of North Alabama, Florence, AL, USA

Understanding the bacterial and algal populations of our local rivers is important to the health of the local environment. Occasionally, freshwater microbial blooms will occur due to the high input of organic nutrients, potentially complicating industrial processes and releasing dangerous toxins. In the early summer of 2023, the Tennessee River that separates Florence and Muscle Shoals, Alabama experienced a bloom complicating the water filtration process at the Muscle Shoals water treatment plant. Uniquely, the organism causing the bloom was also observed in high abundance post-filtration. The following are the preliminary efforts to identify the cyanobacterial or algal contributors to the freshwater bloom which will help direct future management efforts. Samples were collected from the Tennessee River and filtered water at the treatment plant and immediately processed through a 0.2 um filter and stored at -20°C. DNA was extracted and amplified at the 16s ribosomal RNA V3-V4 region and sequences were classified. Multiple cyanobacteria and algal species were identified at greater than 1% of the population in both the river and post-filtration samples. No cyanobacterial or algal species were in high abundance as the bloom had subsided by August; however, some of the species identified are known to contribute to freshwater blooms. Future experiments will consist of targeted qPCR analyses, seasonal sampling, and direct bloom sampling if the organism increases in abundance again in early summer 2024.