

Driving Directions to HKRB:

GPS: 760 Press Avenue, Lexington, KY 40536-0679

From Louisville, KY: Take I-65 Southbound, take **exit 131A** to merge onto I-264 Eastbound. Take **exit 19A** to merge onto I-64 Eastbound towards Lexington. Take **exit 115** to merge onto KY-922 Southbound/Newtown Pike towards Bluegrass Parkway/Airport/Lexington. Continue on KY-922 Southbound/Newtown Pike (road will change name to Oliver Lewis Way), turn right onto S Broadway, then left onto Virginia Avenue. Turn right onto Press Avenue. **Parking garage will be on right and HKRB will be on left. Park in HKRB parking lot.**

From Chattanooga, TN: Take I-24 Eastbound toward Knoxville/Atlanta. Take **exit 185B** to merge onto I-75 Northbound towards Knoxville. Take **exit 113** to merge onto US-27/US-68 towards Paris/Lexington. Turn right off the exit onto US27 S/US-68 W/N Broadway. Turn left onto Virginia, then right onto Press Avenue.

From Huntington, WV: Take I-64 Westbound into Kentucky, then take **exit 113** to merge onto US-27/US-68 towards Paris/Lexington. Turn right off the exit onto US27 S/US-68 W/N Broadway. Turn left onto Virginia, then right onto Press Avenue.



**Kentucky - Tennessee Branch
of the American Society for Microbiology
Spring Branch Meeting
April 19 - 20, 2024**

Hosted by the University of Kentucky

**Healthy Kentucky Research Building (HKRB), University
of Kentucky
760 Press Avenue, Lexington, Kentucky**

**Local Organizers – Dr. Yosra A. Helmy
and Dr. Christopher Radka**

Thank you to our sponsors:

University of Kentucky
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American Society of Microbiology – Join ASM

Spring Meeting of the Kentucky - Tennessee American Society for Microbiology Branch Schedule (All Times are in EST)

Friday, April 19, 2024

5:30 PM Registration opens- Healthy Kentucky Research Building (HKRB), University of Kentucky- 760 Press Avenue, Lexington, Kentucky

Opening Ceremony HKRB-150

6:00 pm Dinner served
7:00 pm Welcome, Dr. Korsi Dumenyo, President KY/TN ASM
7:15 pm Amino Acid Sensor Conserved from Bacteria to Humans



Dr. Igor B. Jouline
ASM Distinguished Lecturer
Professor of Microbiology, The Ohio State University

Faculty Talks

8:15 pm Dr. Amelia Pinto, University of Kentucky
Adaptive Immune responses to Powassan virus

8:35 pm Dr. Christopher Lennon, Murray State University
An intein-based biosensor to measure protein stability *in vivo*



Saturday, April 20, 2024

7:30 am Registration opens- Healthy Kentucky Research Building (HKRB), University of Kentucky- 760 Press Avenue, Lexington, Kentucky

Set up posters on the HKRB Lobby

Faculty Talks HKRB-150

8:00 am Dr. Ashlan Kunz Coyne, University of Kentucky
Mechanistic Insights to Combating *Enterobacter cloacae* in Deep Seated Infections

8:20 am Dr. Ernest Osburn, University of Kentucky
Growth potential of soil bacterial communities across global ecosystems

Graduate Student Oral Competition

8:40 am Ashley Dague, Marshall University
Antimicrobial compounds from model mosses *Physcomitrium patens* and *Ceratodon purpureus*

8:55 am Andrew Krusenstjerna, University of Kentucky
Master chromosomal replication initiator DnaA regulates cell morphology and gene expression in the Lyme Disease spirochete.

9:10 am Md Islam, University of Kentucky
Evaluating the in vitro efficacy of next-generation probiotics against *Rhodococcus equi* infection

9:25 am Coffee break

9:40 am Daniel Erickson, University of Louisville
Dietary Sucrose Indirectly Enhances *Clostridioides difficile* Pathogenesis

9:55 am Kent Pham, University of Kentucky
Metagenomic analysis of microbial communities throughout the hemp retting process

10:10 am Ajran Kabir, University of Kentucky
Novel Quorum Sensing Inhibitors as Potential Therapeutics for the control of *Salmonella* infections

Undergraduate Student Oral Competition

- 10:25 am Rachel Turner, Emory and Henry College
Degree of Antibiotic Resistance in Bacterial Isolates from the Holston and Watauga River Watersheds: Variability by Season
- 10:40 am Sumin Karna, University of Kentucky
Exploring Novel Probiotics as Potential Therapeutics Against *Salmonella* Typhimurium

Poster Competition 10:55 am – 12:15 pm

- G01. Insights into the Mechanism *Listeria monocytogenes* Use to Disseminate to the Mesenteric Lymph Nodes Following Oral Infection. **Joshua S. Nowacki**, Grant S. Jones, and Sarah E.F. D’Orazio. University of Kentucky.
- G02. Functional characterization of the nucleic acid binding activity of PlzA, the borrelial cyclic-di-GMP binding protein. **Nerina Jusufovic**, Andrew C. Krusenstjerna, Christina R. Savage, Keira Schinaman, Jessamyn P. Morris, Timothy C. Saylor, and Brian Stevenson. University of Kentucky
- G03. Cyclic-di-GMP Phosphodiesterase STM3615 Regulates *Salmonella* Physiology. **Abigail Pyburn**, Alexandra Pulliam, Erez Mills, and Erik Petersen. East Tennessee State University
- G04. A rapid and cost-effective multiplex PCR method for identification of targeted gene knockouts in *Serratia marcescens*. **Julia Cardot** and Lydia Bogomolnaya. Marshall University
- G05. Isolation of *Salmonella enterica* subsp. *enterica* serotype Mbandaka from foals in Kentucky. **Ajran Kabir** and Yosra A. Helmy. University of Kentucky
- G06. Effects of Cell Free Supernatant of *Pseudomonas fluorescens* on Antibiotic Sensitivity of *Staphylococcus epidermidis*. Sidney Cagle and **Esther Choi**. Union University
- G07. Revealing novel functions of putative cytotoxins in *Chlamydia trachomatis* infection. **Gracie Eicher** and Kenneth A. Fields. University of Kentucky
- G08. Hypoxic growth increases contact-dependent growth inhibition in *Burkholderia dolosa*. **Erica Phillips**, Tanya Myers-Morales, and Erin Garcia. University of Kentucky
- G09. *In vitro* antiviral activity of antimicrobial peptoids against animal herpesviruses. **Denny K. Gao**, Erika L. Figgins, Annelise Barron, and Gill Diamond. University of Louisville
- G10. *In vitro* evaluation of probiotic *Escherichia coli* Nissle 1917 supernatants in inhibiting *Campylobacter jejuni* growth and virulence. **Bibek Lamichhane**. University of Kentucky
- G11. CRISPR-Cas12a is an efficient method to genetically alter *Klebsiella pneumoniae*. **Taylor M. Garrison**, Phoenix Gray, James Collins and Matthew B. Lawrenz. University of Louisville

G13. Advances Towards Saturating Transposon Mutagenesis for *Chlamydia trachomatis*. **Ann Caroline Hawk**, Nur Hamdzah, and Kenneth A. Fields. University of Kentucky

G14. Analysis of epoxide hydrolase enzymatic activity of bacterial proteins elevated in patients with alcohol-associated liver disease. **Urellys Casiano-Esquilin**, Jingzhi Wang, Jeffrey Warner, Josiah Hardesty, Craig McClain, Dennis Warner, Irina Kirpich. University of Louisville

G15. Treatment of Experimental Alcohol-Associated Liver Disease with *Limosilactobacillus reuteri* that Express a Recombinant N3-Fatty Acid Desaturase. **Alice G. Rodgerson**, Josiah E. Hardesty, Jee-Hwan Oh, Jan-Peter van Pijkeren, Craig J. McClain, Dennis R. Warner, Irina A. Kirpich. University of Louisville

G16. Characterization of the universal sugar transport system components PtsI and PtsH in *Enterococcus faecium*. **Michelle Hallenbeck**, Michelle Chua, and James Collins. University of Louisville

G17. Localization and Transmission of Seed Endophytic Bacteria. **Upama Dev** and Luke A. Moe. University of Kentucky

U01. Antimicrobial resistance patterns and virulence traits in *Rhodococcus equi* isolated from foals in Kentucky. **Anaya Ali**, Bibek Lamichhane, Cayla Cayer-McCarthy, Steve Locke, Erdal Erol, and Yosra A. Helmy. University of Kentucky

U02. Flavivirus T-Cell Responses. **Shania Chirinos** and Amelia K Pinto. University of Kentucky

U03. Characterizing Interaction Domains between DdrR and KZA74_19365. **Ethan Newsom**, Deborah Cook, and Janelle Hare. Morehead State University

U04. Evaluation of Novel Small Molecule Therapeutics for Controlling *Rhodococcus equi* Infections in vitro. **Angel Bhusal**, Bibek Lamichhane, Khaled A. Shaaban, Larissa V. Ponomareva, Jon S. Thorson, and Yosra A. Helmy. University of Kentucky

U05. How Sequential Encounters with Closely Related Flaviviruses Change the Immune Landscape. **Julia Andrade**, Mariah Hassert, Jill Plugheber Shania Chirinos, James D. Brien, and Amelia K. Pinto. University of Kentucky

U06. Testing for the Presence of *Naegleria fowleri* in Northeastern Tennessee Recreational Waters. **Mackenzie Nicholas**, Hannah Phillips, and Jennifer Brigati. Maryville College

U07. *In Vitro* and *In Vivo* Antifungal Peptoid Activity Against *Candida auris*. **Isabella M. Griffiths**, Denny Gao, Erika L. Figgins, Lisa K. Ryan, and Gill Diamond. University of Louisville

U08. Antimicrobial profiling of *Serratia marcescens* SM6 using BIOLOG chemical sensitivity assays. **Kate Perkins** and Lydia Bogomolnaya. Marshall University

U09. The Search for the Mystery Source of NtrX Phosphorylation. **Emma Bain** and Benjamin Stein. University of Tennessee at Chattanooga



U10. Dysregulation of Paneth Cell-Specific Genes During SARS-CoV-2 Infection from Patient and Mouse Stool Samples. **Braden Mulcahy**, Erika L. Figgins, Denny Gao, Tanya O. Robinson, Kenneth Wilson, Jon Gabbard, Uyen “Emily” Do, Braden Mulcahy, Lisa K. Ryan, Michelle Galeas Pena, Sarah C. Glover, Gill Diamond. University of Louisville

Lunch

- 12:15 pm Box Lunch Lobby.
Networking and open discussion between the keynote speakers, faculty and trainees
- 12:20 pm Officer Meeting, HKRB Boardroom
All Faculty, Postdocs Welcome

Invited Speaker, HKRB-150

1:15 pm Molecular Mechanisms of Environmental Stress Resistance in Bacteria



Dr. Sean Crosson
ASM Distinguished Lecturer
Professor of Microbiology and Molecular Genetics, Michigan State University

Peggy Cotter Award Recipient, Ryan Doster

2:15 pm Behind the curtains at ASM – what ASM can do for you, how to get involved, and how ASM is evolving.



Dr. Ryan Doster, University of Louisville

2:45 pm coffee break

Postdoctoral Oral Competition

3:00 pm Dr. Tanmoy Mukherjee, University of Kentucky
Transposon Sequencing Reveals a Role of Multiple Pathways in Developing Resistance to
Cathepsin-G in Group A Streptococcus



- 3:15 pm Dr. Lakshay Anand, University of Kentucky
Grapevine Rootstock and Scion Genotypes' Symbiosis with Soil Microbiome: A Machine Learning Revelation for Climate-Resilient Viticulture
- 3:30 pm Dr. Mohammad Rahman, University of Kentucky
IDR-glycosylation of extracytoplasmic proteins contributes to streptococcal pathogenesis
- 3:45 pm Dr. Oscar Vazquez Ciro, University of Kentucky
Discovery of novel mechanisms in the regulation of β -hemolysin/cytolysin in *Streptococcus agalactiae*
- 4:00 pm Dr. Bashir Akhlaq Akhoo, University of Kentucky
Genomic Topography and Evolutionary Trajectories of Cellulose Synthesizing Bacteria: A Deep Dive into *Komagataeibacter*, *Novacetimonas*, and *Gluconacetobacter*

Faculty Talks

- 4:15 pm Dr. Lydia Bogomolnaya, Marshall University
Type 2 Diabetes Changes Systemic Dissemination of *Salmonella*
- 4:35 pm Dr. Irina Kirpich, University of Louisville
Host and Bacterial Epoxide Hydrolases: Relevance to Alcohol-Associated Liver Disease in Humans
- 4:55 pm Dr. Dennis Warner, University of Louisville
Soluble Epoxide Hydrolase Inhibition in a Mouse Model of Alcohol-Associated Liver Disease: Impact on Altered Gut Barrier Function and Gut Microbiota

Closing Ceremony, HKRB-150

- 5:15 pm Dr. Korsi Dumenyo, President KY/TN ASM
Awards Ceremony

Faculty Talks

Faculty Talk #1 Keynote

Amino Acid Sensor Conserved from Bacteria to Humans

Dr. Igor B. Jouline
ASM Distinguished Lecturer
Professor of Microbiology
The Ohio State University

Faculty Talk #2

Adaptive Immune responses to Powassan virus

Dr. Amelia Pinto
University of Kentucky

Powassan virus (POWV) is a tick-borne flavivirus that causes fatal meningoencephalitis in 10-15% of human cases. According to the CDC, a dramatic spike in incidence of POWV infection in the United States has occurred in the last three years. While effective vaccines exist for a related virus, tick-borne encephalitis virus (TBEV), multiple studies have demonstrated that these vaccines do not protect against POWV. Defining the correlates of protection for POWV is a critical step in the development of efficacious POWV vaccines. To this end, we used C57BL/6 mice as a model of POWV infection to determine the protective capacity of POWV specific T cells and antibodies against lethal POWV challenge. Using our model, we have identified two CD8 T cell epitopes and one CD4 T cell epitope within the structural proteins of the virus. Interestingly, POWV-Spooner also appears to contain a unique CD4 T cell epitope. The contribution of POWV-Spooner-specific CD4 T cells, however, remains unknown. We also have preliminary evidence suggesting that POWV-LB induces the production of antibodies that can cross bind WNV. This phenomenon appears to be specific to POWV-LB and was not observed in mice challenged with POWV-Spooner. This data reasserts the importance of establishing correlates of protection for different POWV lineages to inform vaccine design.

Faculty Talk #3

Growth potential of soil bacterial communities across global ecosystems
Dr. Ernest Osburn University of Kentucky
Soil bacteria play key roles in regulating terrestrial ecosystem processes such as nutrient cycling and carbon storage. However, despite the growing catalogue of studies detailing the taxonomic composition of soil bacterial communities, it remains difficult to quantitatively relate bacterial community composition to ecosystem functions. One approach to linking complex bacterial communities to their ecosystem functions is to quantify community-aggregated life history traits. The goal of this study was to explore global patterns and environmental drivers of one fundamental bacterial life history trait: growth potential. We used a dataset of 176 soil metagenomes from 11 biomes on six continents to estimate maximum community-averaged growth rates from codon usage statistics, along with other genomic traits associated with bacterial growth potential (e.g., 16S rRNA gene copy number). Maximum growth rates varied significantly across latitudes, with higher maximum rates found in forested biomes from tropical and temperate/boreal latitudes and lower growth rates in more arid subtropical latitudes. This indicates that bacterial productivity mirrors ecosystem productivity on a global scale. Accordingly, the strongest environmental predictors of growth potential were indicators of productivity (e.g., NPP, distance to equator) and soil properties that vary along productivity gradients, e.g., pH and soil C:N ratios. Our results also demonstrate a tradeoff between growth potential and carbon resource acquisition potential in soil bacteria: maximum growth rates were positively correlated with relative abundances of functional genes involved in energy production/conversion and negatively correlated with relative abundances of genes involved in carbohydrate metabolism. Overall, our results identify macroecological patterns and environmental drivers of bacterial growth potential and link bacterial growth to the decomposition and fate of carbon resources in soil.

Faculty Talk #4

Mechanistic Insights to Combating Enterobacter cloacae in Deep Seated Infections
Dr. Ashlan Kunz Coyne University of Kentucky
<u>Background:</u> Enterobacterales spp. present a pressing challenge, notably with the surge in carbapenem-resistant strains, necessitating alternative treatments. Despite concerns, cefepime shows comparable efficacy to carbapenems in treating Enterobacter spp. bacteremia. Recent data indicate similar mortality rates between high-dose cefepime and carbapenems for AmpC-producing Enterobacterales (AmpC-PE) bacteremia. We aim to optimize cefepime and carbapenem regimens against Enterobacter cloacae using high inoculum ex vivo models. <u>Methods:</u> We evaluated cefepime, meropenem, and ertapenem regimens for bactericidal activity, resistance emergence, and beta-lactamase expression using six E. cloacae isolates in 96-hour ex vivo simulated endocardial vegetation (SEV) models. Isolates were selected to represent characteristics influencing clinician preference between cefepime and carbapenem therapy. SEV models were incubated at 37°C for 96 hours, with clinically relevant antibiotic doses injected. Samples were collected for analysis, and PK target attainment was assessed. Statistical differences were determined using ANOVA with Tukey's post hoc test ($\alpha=0.05$).

Results: Meropenem (2 g every 8 hours via 3-hour infusion) exhibited bactericidal activity ($-\Delta 3.21$ log₁₀ CFU/mL) against *E. cloacae* isolate 0008. Both cefepime and meropenem regimens administered over 3 hours demonstrated significantly greater bacterial killing than their 30-minute counterparts ($-\Delta 1.51$ and $-\Delta 3.39$ log₁₀ CFU/mL, respectively). For *E. cloacae* isolate 0032, both cefepime and meropenem regimens infused over 3 hours showed bactericidal activity ($-\Delta 3.33$ and $-\Delta 3.69$ log₁₀ CFU/mL, respectively). Similar to isolate 0008, the 3-hour infusions of cefepime and meropenem resulted in significantly greater killing compared to the 30-minute infusions ($-\Delta 1.44$ and $-\Delta 2.27$ log₁₀ CFU/mL, respectively). No treatment-emergent resistance was observed in the 96-hour models. Beta-lactamase expression assessments using RT-PCR are pending.

Discussion: Our ongoing work reveals the added benefit of 3-hour extended infusion cefepime and meropenem compared to 30-minute infusions. Further research will assess remaining isolates with beta-lactamase quantification, Emax modeling, and transcriptomic evaluations, as appropriate.

Faculty Talk #5

An intein-based biosensor to measure protein stability in vivo

Dr. Christopher Lennon
Murray State University

BACKGROUND: Biosensors to measure protein stability in vivo are valuable tools for a variety of applications. Previous work has demonstrated that a tripartite design, whereby a protein of interest (POI) is inserted within a reporter, can link POI stability to reporter activity. Inteins are translated within other proteins and excised in self-mediated protein splicing reaction.

METHODS: Here, we developed a novel folding biosensor where a POI is inserted within an intein, which is subsequently translated within an antibiotic resistance marker.

RESULTS: We showed that protein splicing is required for antibiotic resistance, and that housing a stable POI within the intein, compared to an unstable variant, results in a 100,000-fold difference in survival. Further, using a fluorescent protein that matures slowly as the POI, we developed a reporter with two simultaneous readouts for protein folding. Finally, we showed that co-expression of GroEL can significantly increase activity of both reporters, further verifying that protein folding factors can act on the POI in the biosensor.

CONCLUSIONS: As a whole, our work provides a new twist on the traditional tripartite approach to measure protein stability in vivo.

Faculty Talk #6 Keynote

Molecular Mechanisms of Environmental Stress Resistance in Bacteria

Dr. Sean Crosson
ASM Distinguished Lecturer
Professor of Microbiology and Molecular Genetics
Michigan State University

Faculty Talk #7 Peggy Cotter Award Recipient

Behind the curtains at ASM – what ASM can do for you, how to get involved, and how ASM is evolving

Dr. Ryan Doster
University of Louisville

This lecture is aimed to help ASM members at all levels (graduate students through faculty) to better understand the American Society for Microbiology's structure and function. I will highlight different offerings that ASM provides on the local and national level to enhance your science from local meetings to online seminars. We will also discuss different volunteer opportunities at the branch and national level including the position of COMS branch councilor, which I currently hold. Lastly, we will discuss how the ASM organization is changing with a new strategic plan that was recently approved. This strategic plan will reorganize ASM away from the classic ASM communities and provide a new governance structure. We will end with a question/answer session and provide an opportunity for feedback about how ASM can better support our branch on the local level.

Faculty Talk #8

Type 2 Diabetes Changes Systemic Dissemination of Salmonella

Dr. Lydia Bogomolnaya
Marshall University

Background. Type 2 diabetes (T2D) is a risk factor for bacterial infections including those caused by nontyphoidal Salmonella. Individuals with uncontrolled T2D often experience the unusual extraintestinal spread of Salmonella which can lead to life-threatening disorders. The precise underlying mechanism of this predisposition is not clearly understood.

Methods. In this study 8-week-old male TALLYHO (TH) mice were maintained on a standard chow, or on a high fat (HF) diet (45% fat) for the additional 8 weeks to promote diabetes development. As expected, mice on the HF diet gained more weight compared to the animals on a standard chow, and by 16 weeks of age they had diabetic levels of glucose in blood (>300 mg/dL). At that time, TH mice from each diet group were orally infected with a fully virulent bioluminescent Salmonella Typhimurium strain to follow the pathogen spread in individual animals using in vivo imaging.

Results. Mice in both groups developed clinical signs of salmonellosis. However, Salmonella spread in mice with diabetes had an unusual pattern compared to the healthy animals. Because T2D patients have altered gut microbiota with decreased population of butyrate-producing bacteria, we analyzed the intestinal profile of short chain fatty acids, acetate, propionate, and butyrate in TH mice. As expected, concentrations of short chain fatty acids, including butyrate were reduced in the gut of animals on HF diet compared to TH mice maintained on the standard chow. We also found that butyrate supplementation reduced extraintestinal spread of Salmonella in animals maintained on a standard diet. Unexpectedly, oral supplementation of butyrate did not decrease the spread of Salmonella in diabetic mice.

Conclusions. These findings provide novel insights into the pathogenesis of enteric Salmonellosis in the context of T2D.

Faculty Talk #9

Host and Bacterial Epoxide Hydrolases: Relevance to Alcohol-Associated Liver Disease in Humans

Dr. Irina Kirpich
University of Louisville

Background/Aims: Alcohol-associated liver disease (ALD) is a global health concern with limited effective treatments. Our group recently implicated soluble epoxide hydrolase (sEH) as a pathogenic mediator in ALD and showed that sEH inhibition attenuated experimental ALD. sEH is expressed in multiple cells and tissues, and proteins with EH activity are widely expressed by gut microbiota. This study aimed to examine gut microbial EH expression in patients with alcohol-associated hepatitis (AH), a severe form of ALD.

Methods: Microbial EH abundance was determined using ShortBRED software. A database of EH-specific gene markers was generated with ShortBRED Identify by inputting a list of proteins with potential EH activity, compiled from the NCBI. Microbial EH genes were quantified via the ShortBRED Quantify function in healthy controls (n=8) and AH patients (n=86) utilizing a publicly available fecal shotgun metagenomic dataset. Differentially expressed EH markers were linked to their associated gut bacteria and correlated with clinical parameters of disease severity.

Results: Nine putative EH genes were significantly elevated in AH patients; there were no differences between males and females. BLAST analysis matched EH gene markers to EH protein families, including the HAD hydrolase family IA, IIA, and IIB, which were potentially encoded by several different bacteria from phyla Bacteroidetes and Firmicutes, including *Enterococcus faecalis*, a pathogenic microbe highly elevated in AH. Multivariate logistic regression analysis revealed that increased IA and IIA hydrolases were associated with decreased blood albumin and elevated AST/ALT ratio, suggesting that products of these hydrolases may negatively impact liver function and contribute to liver injury in these patients.

Conclusions: Individuals with ALD may have increased microbial EH proteins which may contribute to liver injury via the gut-liver axis. Future studies are warranted to further investigate the gut EHs and their role in ALD.

Faculty Talk #10

Soluble Epoxide Hydrolase Inhibition in a Mouse Model of Alcohol-Associated Liver Disease: Impact on Altered Gut Barrier Function and Gut Microbiota

Dr. Dennis Warner
University of Louisville

Graduate Student Talks

Graduate Student #1

Antimicrobial compounds from model mosses <i>Physcomitrium patens</i> and <i>Ceratodon purpureus</i>
Ashley Dague Marshall University
Due to the increasing threat of bacterial antibiotic resistance, it is imperative to supplement the arsenal of tools available to fight this issue. To do this we are working on identifying and characterizing novel antimicrobial natural products from two model mosses, <i>Ceratodon purpureus</i> and <i>Physcomitrium patens</i> . <i>C. purpureus</i> and <i>P. patens</i> strains grown in liquid medium secrete exudates containing secondary metabolites with antimicrobial activities. The female strain (GG1) from <i>C. purpureus</i> did not show any activity against Gram-positive or Gram-negative bacterial species, however, the male strain (R40) from <i>C. purpureus</i> and the Gransden (Gd) strain from <i>P. patens</i> showed potent activity against Gram-positive human pathogens, such as <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> and <i>Enterococcus faecium</i> . Additionally, the secreted metabolites are also effective against the phytopathogen <i>Clavibacter sp.</i> LMG 26808. We next performed partial purification of secreted moss metabolites from <i>C. purpureus</i> R40 using a series of size-exclusion, ion-exchange, and desalting chromatography steps, followed by metabolomics analysis. We identified two candidate compounds, phytosphingosine and DL-erythro/threo sphinganine (d16:0). We next characterized these compounds and showed that DL-erythro/threo sphinganine is bactericidal while phytosphingosine is bacteriostatic for <i>S. aureus</i> ATCC 25923. Overall, our results suggest that the antimicrobial compounds present in <i>C. purpureus</i> R40 and <i>P. patens</i> Gd exudates can potentially add new options for treating infections caused by antibiotic-resistant Gram-positive bacteria. Further analysis of these and other moss antibacterial exudate components will be instrumental in the identification of specific genes and biochemical pathways involved in the bioactive moss metabolite biosynthesis. Supported by USDA grant 58-6060-2-006 and NIGMS grant P20GM103434.

Graduate Student #2

Master chromosomal replication initiator DnaA regulates cell morphology and gene expression in the Lyme Disease spirochete
Andrew Krusenstjerna University of Kentucky
As an infected tick feeds on a mammal, the Lyme disease bacteria <i>Borrelia burgdorferi</i> will experience a burst of replication due to the influx of warm, nutrient-rich blood. All bacteria encode a multifunctional DNA-binding protein, DnaA, which initiates chromosomal replication. Despite having the most complex, segmented bacterial genome, little is known about <i>B. burgdorferi</i> DnaA and its role in maintaining the spirochete's physiology. We utilized inducible CRISPR-interference to deplete cellular DnaA levels to better understand this essential protein. Dysregulation of DnaA significantly slowed growth rates and increased cell lengths. Using fluorescent microscopy, we found these conditional mutants also had increased and irregular spacing of chromosome puncta. The DnaA-deficient spirochetes also exhibited a significant defect in helical structure. RNA-seq of these conditional mutants showed significant changes in the levels of transcripts involved with flagellar

synthesis, elongation, division, and virulence. These findings demonstrate that the DnaA plays a central role in maintaining borrelial growth dynamics and flagellar homeostasis, which are essential at the tick-to-mammal interface.

Graduate Student #3

Evaluating the in vitro efficacy of next-generation probiotics against *Rhodococcus equi* infection

Md Islam
University of Kentucky

OBJECTIVES: *Rhodococcus equi* is a pneumonia-causing pathogen in foals. This zoonotic pathogen can infect immunocompromised people and horses. Clinical signs include fever, labored breathing and cough. Infections, treated with antibiotics, face challenges due to antimicrobial resistance. This study aims to evaluate the effectiveness of new probiotics against *R. equi* in vitro, potentially replacing antibiotics.

METHODS: 38 probiotics were evaluated using an agar well diffusion assay. Selected probiotics and their cell-free supernatant (CFSs) were co-incubated with *R. equi* in co-culture media, and the log reduction was measured between 6 to 120 hours. The biofilm of *R. equi* was assessed using 0.1% crystal violet. Cell culture assays were performed to investigate the impact of probiotics' whole culture, CFSs, and heat-killed cells on the adhesion, invasion, and survival of intra-cellular *R. equi* in murine macrophage. Inhibitory activity against resistant *R. equi* was also observed. Cell viability was determined using the Trypan blue exclusion method. Data was analyzed using two-way ANOVA followed by the Tukey test.

RESULTS: The top six probiotics were selected based on their highest zone of inhibition observed in the agar-well diffusion assay. These selected probiotics exhibited significant inhibition of *R. equi* growth after 12 hours ($p < 0.05$) and completely cleared the bacteria by 120 hours. Moreover, the CFSs of five probiotics showed more than 90% inhibition of biofilm formation and preformed biofilm of *R. equi*. Intracellular survival of *R. equi* was significantly reduced after 24 hours by probiotics' CFSs and heat-killed cells ($p < 0.05$). Furthermore, a mean inhibition zone 1.9 cm was found against resistant *R. equi*. Pre-treatment of the murine macrophages with probiotics and their CFSs resulted in a cell viability rate of 70-90% in *R. equi*-infected cells.

CONCLUSIONS: Selected probiotics provides potential antibacterial action against *R. equi*. Probiotic mediated treatment of *R. equi* infection will bring new insights in managing foal pneumonia.

Graduate Student #4

Dietary Sucrose Indirectly Enhances *C. difficile* Pathogenesis

Daniel Erickson
University of Louisville

Added sugars constitute approximately 13% of the daily caloric intake in an average American diet, with 30% of Americans consuming excessive amounts of sugar. The impact of dietary sugars on the pathogenesis of *C. difficile*, a common enteric pathogen, remains unclear. We used a diet high in sucrose, a sugar commonly consumed but not metabolized by *C. difficile*, to investigate its effect on *C. difficile* infection (CDI). We monitored mice fed this diet for factors such as colonization, bacterial and toxin burden, clinical signs of disease, and tissue damage, following infection with *C. difficile*, with or without antibiotic pretreatment. Our observations revealed that a high-sucrose diet increased

susceptibility to CDI, worsened disease symptoms, and correlated with elevated toxin levels. While mice fed regular chow cleared *C. difficile* within three weeks, mice on a high-sucrose diet maintained a high bacterial burden months after challenge. This led to a higher susceptibility to symptomatic relapse in mice consuming a high-sucrose diet upon retreatment with antibiotics. Interestingly, even without antibiotic pretreatment, which is essential for symptomatic disease, a high-sucrose diet enabled asymptomatic *C. difficile* colonization. This indicates that excessive sugar consumption can augment the susceptibility to CDI conferred by antibiotics. Fiber supplementation, previously shown to alleviate *C. difficile* bacterial burden and CDI symptoms, was insufficient to overcome the disease enhancement caused by a high-sucrose diet. Our findings suggest that dietary sugars can significantly enhance *C. difficile* pathogenesis, even if not directly metabolized by the pathogen. We hypothesize that this could be due to a combination of microbial and host factors, including changes in microbiome structure, metabolome, intestinal inflammation, and the innate immune response.

Graduate Student #5

Metagenomic analysis of microbial communities throughout the hemp retting process

Kent Pham
University of Kentucky

Following harvest, hemp grown for fiber undergoes an in-field, microbe-mediated process called retting, whereby the native microbial community associated with the hemp stalk begins to separate the valuable bast fibers from the woody hurd core. Retting is characterized by degradation of the pectin and hemicellulose layers that connect the bast fibers to the core, which is essential for further industrial decortication. The quality of retting largely dictates the price farmers get for their crop, yet little is known about the key microbial players involved in this process. In this study, hemp stalks were sampled throughout the retting process to track how the microbial community and the functional gene profile changes at each time point. Sampling occurred weekly for 7 weeks starting at harvest (T0) and continuing through optimally retted (T4) and over-retted (T7). DNA extracted from each sample underwent shotgun metagenomic sequencing, generating 2,112,224,662 reads after host removal. Sequencing data was assembled with Megahit 1.2.9 and annotated using the PFams database through IMG/MER's pipeline. After assembly, each time point returned between 300,000 and 1.2 million contigs with GC% content increasing from 50% (T0) to 60% (T4). Fungal reads increased throughout the retting process from 1.6% (T0), to 3.4% (T4), to 6.1% (T7). Trends in pectin and hemicellulose degrading enzyme gene counts varied throughout the retting process. Glucosidase counts specific to fungi increased at T3, peaking at T5. Automated binning with MetaBAT2 generated 10 high quality and 64 medium quality Metagenome Assembled Genomes (High quality > 90% completion, medium quality >50% completion).

Graduate Student #6

Novel Quorum Sensing Inhibitors as Potential Therapeutics for the control of Salmonella infections

Ajran Kabir
University of Kentucky

Background: *Salmonella Typhimurium* is a significant foodborne pathogen. Poultry and poultry products are considered the main source of infection; however, other animals play an important role in infection transmission to humans through contaminated food and water. The development of

multidrug-resistant Salmonella strains has generated an urgency to develop alternative treatment strategies, such as targeting quorum sensing (QS) pathways. QS is a cell-to-cell communication that allows the bacteria to sense its population density and regulate its virulence. This communication is conducted by signaling molecules called autoinducers 2 (AI-2). This study aims to identify QS inhibitors and evaluate their effect on the virulence, and biofilm formation of Salmonella in vitro.

Methodology: We tested 1,900 small molecules (SMs) to assess their impact on QS/AI-2 production of *S. Typhimurium*. Bacterial cultures (100 μ L; OD=0.05) were treated with 1 μ L of each small molecule (SM; 10 μ M - 0.7 μ M) in 96 well plates and incubated for 6 hours at 30 $^{\circ}$ C to assess their effect on bacterial growth. SMs demonstrating no significant impact on bacterial growth were subsequently screened via a bioluminescence assay. Cell-free supernatants of treated bacteria were incubated with *Vibrio harveyi* BB170 to evaluate their effect on AI-2 production. SMs exhibiting the highest inhibitory activity for AI-2 were then selected for their effect on biofilm formation and the expression of virulence, biofilm, and quorum sensing-associated genes using RT-PCR.

Results: Ten SMs with more than 95% inhibition of AI-2 activity without affecting bacterial growth were selected for further evaluation. These compounds possessed inhibition (95-100%) of biofilm formation. Furthermore, all 10 compounds downregulated the expression of genes associated with quorum sensing, virulence, biofilm development, and motility.

Conclusions: Quorum sensing inhibitors offer a promising new strategy to combat Salmonella infections and mitigate the rising threat of antibiotic resistance in this major foodborne pathogen. For further development of these SMs, their toxicity will be assessed on human intestinal and chicken macrophage cell lines.

Undergraduate Student Talks

Undergraduate Student #1

Degree of Antibiotic Resistance in Bacterial Isolates from the Holston and Watauga River Watersheds: Variability by Season

Rachel Turner
Emory and Henry College

Background: Overuse of antibiotics in medical, veterinary, and agricultural industries has led to increased discharge of antibiotics into the surrounding environment which can pose a risk to human health. During the summer, winter, and spring seasons of 2022 into 2023, seasonal levels of bacterial antibiotic resistance were analyzed from three recreational and two wastewater treatment plant locations within the Holston and Watauga River watersheds of northeast Tennessee.

Methods: Microbial sensitivity to 12 antibiotics routinely used in human and veterinary medicine was evaluated following the Kirby-Bauer Disk Diffusion method. We hypothesized fluctuation in antibiotic resistance across sampling sites due to seasonal rainfall and runoff events, and higher resistance in locations downstream of wastewater treatment plants correlated with plant overflows and failures.

Results: Data from all three sampling periods indicates a high prevalence of antibiotic resistance in both watersheds. Thirty-five percent of all isolates (n=224) were resistant to four or more antibiotics, and 21% were resistant to 6 or more antibiotics. In addition, upstream and downstream locations of both wastewater treatment plants exhibited higher occurrences of satellite colonies on Kirby-Bauer Disk Diffusion antibiotic susceptibility testing in the spring 2023 cycle indicating that antibiotic resistance genes are evolving.

Conclusions: Both wastewater treatment plant sites displayed fluctuating patterns of resistance and susceptibility between upstream and downstream samples, indicating the treatment process does not appear to affect the prevalence of antibiotic resistant microorganisms. We interpret that periods of seasonally high rainfall contribute to influxes of fecal bacteria in the Holston watershed through agricultural runoff. Rainfall events also contribute to increases in wastewater facility failures which provide another mode of contamination to the recreational water sites. These data are concerning due to the high volume of human recreation at each sampling site and associated risk of waterborne infectious disease transmission.

Undergraduate Student #2

Exploring Novel Probiotics as Potential Therapeutics Against Salmonella Typhimurium

Sumin Karna
University of Kentucky

Background: Salmonella, a leading cause of foodborne gastroenteritis globally, poses a significant threat due to the alarming rise in antibiotic resistance. With poultry and poultry products acting as major vehicles for human infections, the development of alternative therapeutic strategies is crucial. This study aimed to investigate the efficacy of novel probiotic strains in inhibiting the growth and virulence of Salmonella Typhimurium in vitro.

Materials and Methods: A comprehensive screening of 38 probiotic strains was conducted using an agar-well diffusion assay to evaluate their ability to inhibit the growth of S. Typhimurium. The most promising candidates exhibiting substantial growth inhibition were selected for further investigations. Co-culture assays were employed to assess the inhibitory effects of these probiotics on Salmonella growth in broth media. Additionally, the potential of probiotic supernatants to prevent biofilm formation and disrupt preformed biofilms was examined. The study also explored the impact of probiotics on the adhesion, invasion, and survival of S. Typhimurium in human intestinal cell lines.

Results: Initial screening revealed that all 38 probiotic strains exhibited zones of growth inhibition against S. Typhimurium in the agar-well diffusion assay. Eight strains demonstrating the largest inhibition zones were chosen for further evaluation. In co-culture assays, these selected probiotics significantly suppressed the growth of S. Typhimurium in broth media. Remarkably, four out of the eight probiotics completely inhibited both biofilm formation and preformed biofilms of S. Typhimurium. Furthermore, all the probiotics demonstrated substantial inhibition of S. Typhimurium adhesion, invasion, and survival in human intestinal cell lines.

Conclusion: These findings demonstrate the promising potential of the investigated probiotics in controlling Salmonella infections. Future studies will focus on evaluating the effects of these probiotics on the expression of virulence factors, such as biofilm formation, motility, and invasion, in S. Typhimurium.

Keywords: Probiotics, S. Typhimurium, Antibiotic Resistance, Virulence factors

Early Career/Postdoc Talks

Postdoc #1

Transposon Sequencing Reveals a Role of Multiple Pathways in Developing Resistance to Cathepsin-G in Group A Streptococcus
Dr. Tanmoy Mukherjee University of Kentucky
<p>Background: Group A Streptococcus (GAS) is a human pathogen associated with numerous diseases including life-threatening diseases. GAS can evade the host immune defense mechanisms including the most potent cationic antimicrobial peptide, Cathepsin-G (Cat-G), a highly cationic serine protease found in the azurophilic granules of neutrophils and a key component of innate immunity. Recent studies have shown that bacteria can disarm Cat-G using various mechanisms.</p> <p>Methods: To identify the genetic factors conferring resistance/susceptibility to Cat-G against GAS, we used genome wide screen called Transposon Sequencing (Tn-Seq).</p> <p>Results & Conclusions: Tn-Seq analysis unveils the intricate strategy employed by GAS to develop resistance or susceptibility against Cat-G, involving signal transduction, PTS system, Zinc uptake, peptidoglycan modification, and cell separation machinery. Seven genes (<i>adcR</i>, <i>mapZ</i>, <i>murA</i>, <i>vfr</i>, <i>dacA</i>, <i>ciaH</i>, <i>gacH</i>) were chosen for further investigation. $\Delta dacA$, a low molecular weight penicillin binding protein (PBP) with putative DD carboxypeptidase activity, demonstrated heightened sensitivity to Cat-G, whereas $\Delta gacH$, involved in adding glycerol phosphate modification to the cell wall, imparted increased resistance. This investigation elucidates the mechanisms underlying both resistance and sensitivity to Cat-G in GAS. Furthermore, among the examined genes, Δvfr was found to be sensitive to histone, LL37, and human phospholipase A2 (hglIA), while $\Delta dacA$ was sensitive to LL37, and $\Delta mapZ$ was sensitive to hglIA. These findings highlight novel insights into GAS's responses to innate immunity antimicrobials, previously unexplored in studies related to resistance against such agents.</p>

Postdoc #2

Grapevine Rootstock and Scion Genotypes' Symbiosis with Soil Microbiome: A Machine Learning Revelation for Climate-Resilient Viticulture
Dr. Lakshay Anand University of Kentucky
<p>Background: In light of climate change's impact on agriculture, developing resilient crop varieties is crucial. A plant's microbiota plays a vital role in its productivity, affecting nutrient absorption, disease resistance, and overall health. However, the genetic factors in plants that influence microbial community formation are still unknown. This study investigates the potential of Machine Learning to predict grapevine rootstock and scion genotypes based on soil microbiota despite environmental variations.</p> <p>Methods: The research analyzed soil microbial bacteriome data from 281 vineyards across 13 countries and five continents, encompassing 34 different <i>Vitis vinifera</i> cultivars grafted onto various rootstocks. Several machine learning (ML) algorithms, including Random Forests, Adaptive Boost, Gradient Boost, Support Vector Machines, Gaussian and Bernoulli Naïve Bayes, k-Nearest Neighbor,</p>

and Neural Networks, were employed. Two filtering criteria were applied: the first retained sparse classes to ensure class diversity, and the second excluded sparse classes to assess model robustness against overfitting.

Results: Both filtering criteria yielded high F1weighted scores (>0.8) for most classes across most ML algorithms. Notably, the successful prediction of rootstock and scion genotypes from soil microbiomes indicates that both plant parts significantly influence the microbiome composition.

Conclusion: These findings lay the groundwork for identifying plant genes that can enhance breeding programs, improve plant productivity, and sustain sustainability by improving the plant-microbiota relationship.

Postdoc #3

IDR-glycosylation of extracytoplasmic proteins contributes to streptococcal pathogenesis

Dr. Mohammad Rahman
University of Kentucky

Despite lacking a tertiary structure, intrinsically disordered regions (IDRs) of proteins play a range of functional roles including cell signaling and protein folding in eukaryotes. However, the functions of bacterial IDRs are poorly understood. In this study, deep learning algorithms were used to predict extracytoplasmic IDRs in the proteome of three human pathogens- *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus mutans*. We identify that streptococci possess a subset of proteins harboring long-extracytoplasmic IDRs enriched with serine/threonine residues that are O-glycosylated with N-acetylgalactosamine (GalNAc) by pgf operon in *S. mutans*, and glucose by GtrB-glycosyltransferase in *S. pyogenes* and *S. pneumoniae*. Peptidyl-prolyl isomerase PrsA and penicillin-binding protein Pbp1A are identified as the major glycoproteins. We found that GtrB catalyzes the first step of O-glycosylation in the cytosol transferring UDP-glycan to the lipid carrier, undecaprenyl phosphate. Furthermore, loss of IDR glycosylation in PrsA resulted in a defect in biofilm formation in *S. mutans*. Biochemical and functional characterization demonstrates that IDR does not affect PrsA stability and is protected with GalNAc from proteolysis by an unknown protease in *S. mutans*. Also, PrsA expression and the degree of glycosylation in *S. mutans* strongly depend on the length of IDR. These data suggest that O-glycosylation of serine/threonine-rich IDRs in streptococcal membrane-proteins contributes to pathogenesis.

Postdoc #4

Discovery of novel mechanisms in the regulation of β -hemolysin/cytolysin in *Streptococcus agalactiae*

Dr. Oscar Vazquez Ciros
University of Kentucky

Streptococcus agalactiae [Group B *Streptococcus* (GBS)] is a commensal bacterium colonizing the gastrointestinal and genital tracts of human healthy adults. However, GBS is an important pathogen in patients with some previous comorbidities and it is also the leading cause of neonatal infections. The transition of GBS from a commensal bacterium to an invasive pathogen is associated with the expression of the virulence factor β -hemolysin/cytolysin (β -H/C). Nevertheless, the factors triggering this switch remain unknown. To identify the regulatory factors involved in the control of the expression of β -H/C, we used transposon mutagenesis of the bacterium grown on a chemically defined media. This approach revealed that *phoU*, a gene involved in the regulation of the phosphate

uptake by inhibiting the PHO regulon under high concentration of phosphate, acts as an activator of β -H/C production, suggesting that phosphate pool is a signal triggering the expression of β -H/C. Another interesting regulator is a CaaX-like membrane protease, acting as a repressor of expression of β -H/C and some other important secreted virulence factors. CaaX-like protease was found to have a possible interaction with some other protein or proteins, additionally it has a predicted catalytic site that might be involved in its regulatory role, suggesting the role of the enzyme in cleavage of quorum sensing peptides involved in the regulation of virulence factors expression.

Postdoc #5

Genomic Topography and Evolutionary Trajectories of Cellulose Synthesizing Bacteria: A Deep Dive into Komagataeibacter, Novacetimonas, and Gluconacetobacter

Dr. Bashir Akhlaq Akhooon
University of Kentucky

Background: Bacterial cellulose possesses unique structural and chemical properties suitable for applications in electronics, medicine, and pharmaceuticals. However, the widespread adoption of bacterial cellulose has been hindered by limitations in bacterial performance capabilities. A deeper understanding of the molecular mechanisms underlying cellulose production could unlock its commercial potential for various industries.

Methods: In this study, we conducted a comparative pangenomic analysis of 79 microbial genomes sourced from three cellulose-producing genera: Komagataeibacter, Novacetimonas, and Gluconacetobacter. High-quality genomes were selected to delineate core and accessory genomes, elucidate phenotypic variations, and discern evolutionary relationships among lineages.

Results: Our phylogenetic and comparative genomic analyses unveiled strains with distinct genome compositions and evolutionary lineages, correcting past misclassifications. A detailed comparative genomic assessment exposed structural disparities in carbohydrate uptake genes and the bacterial cellulose biosynthesis (bcs) operon structure, both within and between genera. These structural differences elucidate variations in cellulose production efficiency and yield. Moreover, prediction of biosynthetic gene clusters using genome mining techniques revealed a vast reservoir of biosynthetic gene clusters within these genera, capable of producing diverse secondary metabolites such as polyketide synthases, Non-ribosomal peptides, Terpenes, Ribosomally synthesized and post-translationally modified peptides and others.

Conclusions: This comprehensive multi-genome study significantly advances our understanding of genetic diversity among cellulose-producing genera. The insights gleaned serve as a valuable roadmap for laboratory-based experiments aimed at harnessing bacterial cellulose production for industrial applications.

Graduate Student Posters

Graduate Poster #1

Insights into the Mechanism *Listeria monocytogenes* Use to Disseminate to the Mesenteric Lymph Nodes Following Oral Infection

Joshua S. Nowacki, Grant S. Jones, and Sarah E.F. D’Orazio
University of Kentucky.

Background. *Listeria monocytogenes* (Lm) is a Gram-positive facultative intracellular bacterium which can be orally acquired through contaminated food. Lm can translocate across the intestinal epithelium, enter the lamina propria and spread to the mesenteric lymph nodes (MLN) through an unknown mechanism. Previous work has shown that Lm can invade conventional dendritic cells (cDC) and adhere to the surface of monocytes, but does not replicate well in either of these host cells. Both of these cell types are known to be migratory, therefore we hypothesize that Lm may be capable of using cDC and monocytes, in addition to free floating, to traverse the lymphatics to the MLN.

Methods and Results. To determine if either cDC or monocytes are involved in transporting Lm to the MLN, various methods were used to either decrease or increase cellular trafficking to the MLN in vivo. We used animals lacking C-C chemokine receptor type 2 (CCR2^{-/-}), a receptor essential for monocyte egress from the bone marrow, and observed no change in MLN bacterial burdens. Alternatively, animals were pre-treated with anti-VEGFR3 (Vascular Endothelial Growth Factor Receptor 3) clone AFL4, an antibody used to block C-C chemokine receptor type 7 (CCR7) expressing cells from infiltrating the MLN, however no impact on the amount of CCR7 expressing cells in the MLN was found. Finally, animals were treated with FMS-like tyrosine kinase ligand (Flt3L) to increase differentiation of progenitor cells into cDC. Pretreatment of animals with Flt3L prior to Lm infection resulted in a subsequent increase in cDC and were found to result in increased bacterial burdens in some tissues.

Conclusions. This research suggests that there is some redundancy in pathways Lm may use to enter the MLN, with modulation of any one pathway having limited effect on subsequent bacterial burden.

Graduate Poster #2

Functional characterization of the nucleic acid binding activity of PlzA, the borrelial cyclic-di-GMP binding protein

Nerina Jusufovic, Andrew C. Krusenstjerna, Christina R. Savage, Keira Schinaman, Jessamyn P. Morris, Timothy C. Saylor, and Brian Stevenson
University of Kentucky

Background: The Lyme disease spirochete, *Borrelia burgdorferi*, must integrate environmental cues to properly regulate gene expression and maintain survival during the enzootic life cycle. *B. burgdorferi* has a two-component signaling system which produces the signaling molecule c-di-GMP. Upon binding of this molecule by PlzA, the only universally encoded c-di-GMP binding protein in *B. burgdorferi*, expression of c-di-GMP responsive genes is modulated. PlzA and c-di-GMP are required for *B. burgdorferi* survival in the tick vector and maintaining the enzootic life cycle. The mechanism of this regulator was previously unknown. The presented work further characterizes PlzA nucleic acid binding properties ultimately to better define the PlzA regulon.

Methods: Recombinant proteins of wild type, truncated, and mutant PlzA proteins were generated. Fluorescently labeled probes derived from nucleic acid sequences of the B31 strain of *B. burgdorferi* were made and used in electrophoretic mobility shift assays (EMSA) to assess the nucleic acid binding function of the recombinant proteins. Competition EMSAs with mutagenized and truncated DNA competitors were performed to determine potential sequence motifs. Computational docking of PlzA and nucleic acids was used to identify amino acid residues involved in nucleic acid binding. Residues of interest were then switched to alanine via site-directed mutagenesis.

Results and Conclusions: Through electrophoretic mobility shift assays, we show that PlzA is a c-di-GMP dependent nucleic acid binding protein. PlzA predominantly interacts with the major groove of DNA and prefers longer and AT-rich sequences. Biochemical characterization coupled with computational analyses identified regions in the N-terminal domain as important for PlzA binding. Mutagenesis of several residues in these regions impacted PlzA-DNA binding affinity. *B. burgdorferi* plzA-mutant strains are currently being produced to determine the consequences of aberrant PlzA-nucleic acid binding function on borrelial physiology. Our studies will further inform mechanisms by which the Lyme disease pathogen regulates gene expression for infection.

Graduate Poster #3

Cyclic-di-GMP Phosphodiesterase STM3615 Regulates Salmonella Physiology

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East Tennessee State University

Foodborne-related diseases pose a persistent and widespread global health threat. A highly clinically relevant etiological enteric pathogen in both humans and animals is Salmonella, which demands a comprehensive understanding of the molecular mechanisms governing Salmonella survival and adaptation. The second messenger cyclic-di-GMP relates to bacterial infections by influencing processes such as biofilm formation, flagellar motility, and virulence. Previous work has shown that the Salmonella Typhimurium cyclic-di-GMP-metabolizing enzyme STM3615 is required for proper survival within both macrophages and mice. Here, we examined the role of STM3615 in Salmonella physiology. Using an agar plate containing dyes designed to identify cell death, we determined that survival of an STM3615 deletion mutant decreased in the stationary phase. We turned to microscopy and found that this mutant also displayed a shortened bacterial morphology. Considering that both of these phenotypes are associated with the regulation of bacterial division, we exposed the STM3615 mutant to A22, an antimicrobial that targets the bacterial replication machinery and found that it displayed dramatically reduced survival. Observing the following phenotypes prompted focused testing of STM3615's specific domains: two transmembrane domains, a periplasmic domain, a HAMP domain, and a cyclic-di-GMP phosphodiesterase domain. The periplasmic domain, rather than the PDE domain, emerged as the primary mediator of bacterial morphology and division regulation. A protein fold prediction algorithm suggested STM3615 is potentially interacting with a periplasmic partner to mediate this response. Using random transposon mutagenesis, we identified mutants associated with the Rcs outer membrane and periplasmic damage response pathway that reverted to wildtype phenotypes. Future research aims to address the role of STM3615 by testing the hypothesis that it interacts with a periplasmic protein partner, thereby regulating cell morphology. By investigating this mechanism, we can uncover a novel mechanism involved in the regulation of bacterial division. This investigation is motivated by the practical need to enhance our understanding of bacterial infections, with potential implications for targeted interventions, therapeutic strategies, and preventive measures.

Graduate Poster #4

A rapid and cost-effective multiplex PCR method for identification of targeted gene knockouts in *Serratia marcescens*

Julia Cardot and Lydia Bogomolnaya
Marshall University

Background. The lambda Red-based system of gene replacement has been widely used in bacterial genetics for over 20 years. However, in some bacterial species this approach results in the formation of many false positive clones with off-target antibiotic resistance cassette integration. Though colony PCR is a quick way to screen for true mutants, the technique suffers from an inability to standardize the amount of DNA present in a sample while also being labor and material demanding when screening large numbers of unique colonies. Multiplex colony PCR allows for multiple colonies to be screened for a gene of interest in one reaction vessel and reduces the amount of materials and time needed to obtain a result.

Methods. In this study, multiplexing technique was combined with colony PCR towards identifying a *nucA* gene target in a mixture of WT and mutant *Serratia marcescens* containing a kanamycin resistant cassette. Ten unique colonies were suspended in water and the optical density (OD600) of the multi-colony mixture was adjusted to 0.12 - 0.15. A set of primers for the *nucA* target gene, and a single forward primer which read from the cassette, were added to a reaction tube containing an aliquot of the multi-colony mixture. After addition of commercially available PCR master mix, tubes underwent colony PCR. Upon completion of the reaction, visualization on agarose gel showed bands correlating with both the WT and a variety of additional mutant sizes. The complexes containing the expected kanamycin-resistant *nucA* deletion sized band were selected and the individuals in the complex were genotyped by colony PCR. A true mutant colony was confirmed.

Results. 200 colonies were screened in 25 multiplex PCR reactions and four potential candidate mutants were identified.

Conclusions. This technique minimized the time and materials necessary to screen large amounts of potential candidate mutants.

Graduate Poster #5

Isolation of *Salmonella enterica* subsp. *enterica* serotype Mbandaka from foals in Kentucky

Ajran Kabir and Yosra A. Helmy
University of Kentucky

Background: *Salmonella* is a zoonotic bacterial pathogen causing foodborne illnesses worldwide. Poultry and poultry products are considered the main source and reservoir of infection; however, other animals play an important role in infection transmission to humans through contaminated food and water. The inappropriate or excessive use of antimicrobials in both agriculture and human medicine has contributed to the development and spread of antimicrobial-resistant (AMR) strains of *Salmonella*. This study aimed to determine the prevalence, genomic characteristics, virulence factors, and antimicrobial resistance profiles of *Salmonella* isolates from foals in Kentucky.

Methodology: A total of 118 horse fecal samples were collected from various horse farms in Kentucky. Samples were enriched in tetrathionate broth and subsequently cultured on XLT4 plates. *Salmonella* serotypes were confirmed using polymerase chain reaction (PCR). Based on its PCR-determined virulence profile, a single isolate from six months old foal was selected for whole-genome sequencing (WGS) on the Illumina MiSeq System.

Results: Analysis of 118 samples revealed a 11.9% prevalence of *Salmonella* spp. Whole genome sequencing revealed that our sequenced isolate belongs to pathogenic sequence type ST413 responsible for several outbreaks in Africa, Europe, and potentially other continents. This isolate was identified as *Salmonella enterica* subsp. *enterica* serotype Mbandaka on serotyping. Virulence

profiling revealed 127 genes linked to host cell invasion, immune evasion, biofilm formation, and systemic infection, indicating significant pathogenic potential. Alarming, our isolate harbors genes conferring resistance to five antibiotic classes, including colistin which is considered a last-resort antibiotic for human infections.

Conclusion: The spread of such bacteria from animals or contaminated food to humans poses a significant public health risk. By studying the complete genomes of other isolates and analyzing transmission patterns, valuable insights can be gained that will ultimately help in the development of effective prevention and control strategies.

Graduate Poster #6

Effects of Cell Free Supernatant of *Pseudomonas fluorescens* on Antibiotic Sensitivity of *Staphylococcus epidermidis*

Sidney Cagle and **Esther Choi**
Union University

Staphylococcus epidermidis is a human skin commensal, but also one of the leading causes of hospital infections due to its ability to form biofilms. Biofilms, microbial aggregates, present significant challenges due to their accumulation on implants and medical devices. Given the resistance of biofilms to antibiotics, alternative therapies are crucial. This study investigated the impact of combining *Pseudomonas fluorescens* culture supernatant (CS) with antibiotics on *S. epidermidis* biofilms. Antibiotic sensitivity testing revealed increased zone of inhibition and lower minimum inhibitory concentration values in samples with CS compared to untreated. These findings suggest the potential of *P. fluorescens* CS with antibiotics as a promising treatment for biofilm-related infections. Future research could explore CS efficacy on other clinical *S. epidermidis* strains, analyze its gene expression, and evaluate its clinical efficacy.

Graduate Poster #7

Revealing novel functions of putative cytotoxins in *Chlamydia trachomatis* infection

Gracie Eicher and Kenneth A. Fields
University of Kentucky

Chlamydia trachomatis is the leading cause of bacterial STIs in the US, with treatment being expensive due to prevalent reinfection. *Chlamydia* species display a considerable degree of genome conservation. However, a plasticity zone (PZ) harbors considerable genetic variations among serovars and strains. Within the PZ of *C. muridarum*, is a series of genes encoding three highly similar proteins, TC0437-0439, with homology to a single protein (CT166) found in the urogenital strains of *C. trachomatis*. These proteins are putative cytotoxins that are expected to inhibit host-cell actin polymerization. Initial studies have shown that when CT166 is ectopically expressed, the host-cell actin is disrupted and cell rounding occurs; this supports the hypothesis that this protein is contributing to cytotoxicity of *Chlamydia*. Our research shows that the CT166 and TC0438 have the catalytic motif necessary for glycosyltransferase activity. We used FRAEM mutagenesis to delete the tc0437-0439 genes in *C. muridarum* effectively creating a toxin deletion mutant. When the mutant was used in infections, our results showed that the toxin does not contribute to immediate toxicity, mediated by collapse of the actin cytoskeleton during infection. Instead, deletion did cause a defect in invasion. Immunofluorescence and trypsin degradation assays provided evidence to support surface

localization of CT166 in *C. trachomatis*. Together, this data suggests that CT166 might not have a role in immediate toxicity but is localized to the surface of Chlamydia and aids in invasion and/or attachment. Further investigation will need to be done to elucidate the relevant function of these proteins and to identify the specific targets.

Graduate Poster #8

Hypoxic growth increases contact-dependent growth inhibition in *Burkholderia dolosa*

Erica Phillips, Tanya Myers-Morales, and Erin Garcia
University of Kentucky

Background: Contact dependent growth inhibition (CDI) systems are found in many proteobacterial species. These systems are known to produce an antibacterial polypeptide when in direct contact with “kin” bacteria, inhibiting recipient cell growth unless a cognate immunity protein is produced. Little is known about factors regulating CDI system gene expression and their potential role in promoting survival under environmental or host mediated stress. *Burkholderia dolosa* contains four distinct CDI systems, (encoded by *bcpAIOB* genes), making this a model organism to study CDI.

Methods: To understand when CDI gene expression occurs, we utilize *lacZ* reporter strains to quantify promoter activity of CDI genes. CDI mediated antagonism is measured by calculating competitive index of inhibitor vs target strains co-cultured together in a known ratio and condition.

Results and Conclusions: We have found that CDI antagonism, as well as the activity of multiple *bcpA* promoters, increased during hypoxia. The FixLJ two-component regulatory system and FixLJ-regulated DNA-binding protein FixK are known to respond to low-oxygen stress in *B. dolosa*. Surprisingly, we found that the absence of *fixK* increased *bcpA* promoter activity and antagonism, suggesting that FixK may negatively regulate *bcpAIOB* expression. Preliminary data suggests that increases in CDI mediated antagonism during hypoxic growth may be due to the *fixLJ* two component system. Future studies will define the role of FixLJ/K on CDI activity and identify additional regulator(s) of CDI system gene expression.

Graduate Poster #9

In vitro antiviral activity of antimicrobial peptoids against animal herpesviruses

Denny K. Gao, Erika L. Figgins, Annelise Barron, and Gill Diamond
University of Louisville

Bovine alphaherpesvirus 1 (BoHV-1), equid alphaherpesvirus 1 (EHV-1), and suid herpesvirus 1 (SuHV-1) are several species of herpesvirus that lead to outbreaks in cattle, horse, and swine populations, respectively. These herpesviruses cause respiratory and genital illnesses, and may lead to pneumonia, abortion, and death. While vaccines do exist and are widely used, treatments for these viruses are not effective at clearing the virus from infected animals, leading to lifelong infections necessitating quarantine or culling of infected animals. Therefore, development of antiviral drugs to clear these viruses from infected animals is a pressing need. Antimicrobial peptides (AMPs) are naturally occurring broad-spectrum antimicrobial agents which represent promising candidates as antiviral drugs due to the lack of acquired resistance. These AMPs, such as cathelicidin, bind to and disrupt the viral envelope, destroying the virus via osmotic pressure. However, AMPs are often subject to in vitro digestion by proteolytic enzymes, reducing their effectiveness. We have been examining the antimicrobial potential of a class of drugs called peptoids, which are a novel class of N-substituted

glycine oligomers which mimic the structure of the cathelicidin LL-37. These peptoids are predicted to mimic the activity of LL-37 to enable viral disruption, while also resisting protease degradation due to their slightly altered structure, increasing bioavailability. We therefore hypothesize that these compounds will exhibit potent in vitro antiviral activity against animal herpesviruses. Preliminary screening of several peptoids reveals both MXB5 and MXB9 are highly effective at clearing both BoHV-1, EHV-1, and SuHV-1. Plaque assay and qPCR revealed a significant reduction in plaques and viral DNAs, respectively, for these viruses. Subsequent experiments reveal that these peptoids continue to remain effective at concentrations as low as 5 µg/mL. These results support the development of peptoids, including MXB5 and MXB9, as novel antiviral agents for treating animal herpesvirus infections.

Graduate Poster #10

In vitro evaluation of probiotic *E. coli* Nissle 1917 supernatants in inhibiting *Campylobacter jejuni* growth and virulence

Bibek Lamichhane

University of Kentucky

Background: Probiotic *E. coli* Nissle 1917 (EcN) is a widely recognized probiotic known for its ability to inhibit the growth of foodborne pathogens. Among these pathogens, *Campylobacter jejuni* is a prevalent enteric pathogen transmitted to humans primarily through the consumption of contaminated poultry and poultry products. With the emergence of antibiotic-resistant strains of *C. jejuni*, there is a pressing need for alternative therapeutic options. Our study aims to evaluate the effectiveness of EcN supernatants in inhibiting the growth and biofilm formation of *C. jejuni* in vitro.

Materials and Methods: Initially we evaluated the effect of EcN supernatants for their impact on *C. jejuni* growth using an agar well diffusion assay. Subsequently, we assessed their effects on inhibiting biofilm formation and pre-formed biofilms, as well as on adhesion, invasion, and survival of *C. jejuni* in human intestinal cells. Furthermore, we evaluated their effect on the expression of genes related to virulence factors, biofilm, and quorum sensing of *C. jejuni* using RT-PCR analysis.

Results: EcN supernatants (collected at 3, 12 and 24h of incubation) exhibited high inhibition zone against *Campylobacter* growth in agar well diffusion assay and significantly inhibited *C. jejuni* growth when cocultured in liquid media. They effectively reduced pre-formed biofilms by up to 82% and biofilm formation by 75%. Pre-treatment of HT-29 MTX human intestinal cells with EcN supernatants significantly ($p < 0.05$) inhibited *C. jejuni* adhesion, invasion, and intracellular survival. Also, EcN supernatants downregulated the expression of genes associated with *C. jejuni* virulence, biofilm formation, and quorum sensing.

Conclusion: In the future, we aim to characterize EcN-derived bioactive molecules and evaluate their effectiveness against *C. jejuni*. Our study will help the advancement and development of EcN-derived bioactive compounds as viable alternatives to antibiotics for treating *C. jejuni* infections.

Keywords: *C. jejuni*, foodborne pathogens, *E. coli* Nissle 1917, antibiotic alternatives.

Graduate Poster #11

CRISPR-Cas12a is an efficient method to genetically alter *Klebsiella pneumoniae*

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Klebsiella pneumoniae is a serious public health threat and major cause of hospital acquired infections. The rising incidence of multidrug resistance among *K. pneumoniae* strains has resulted in difficulty treating infections. Therefore, there is an urgent need to better understand *K. pneumoniae*'s pathogenesis to help develop new therapeutic approaches. However, traditional methods to generate site-specific mutations in *K. pneumoniae* have proven to be difficult and time consuming. Thus, we sought to develop a more efficient and rapid method to generate deletions in *K. pneumoniae* utilizing a CRISPR-Cas12a system. To achieve this, we generated a plasmid-based system containing the Cas12a gene from *Acidaminococcus* sp. controlled by a tetracycline inducible promoter. We also integrated a cloning region downstream of a small RNA promoter to allow for quick engineering of the plasmid to contain a protospacer to target specific genes and gene-specific flanking regions for repair/resolution. To test the efficacy of this single plasmid-based system, we engineered plasmids to target genes related to metal-acquisition in *K. pneumoniae* (e.g., the ZnuABC ABC transporter and the yersiniabactin siderophore). We transformed the plasmid into *K. pneumoniae* KPPR1 and induced the Cas12a system using anhydrous tetracycline. Cultures were diluted into fresh media containing inducer every 24 h. At each passage, the bacterial culture was plated, and clones were screened for loss of the CRISPR-Cas12a plasmid and deletion of the targeted genes. Via this system, we were able to rapidly recover in frame deletions within 72 hrs, with 90% recovery by passage four. Whole genome sequencing confirmed the deletion and no off-target mutations were recovered. Together these data indicate that this CRISPR-Cas12-mediated mutagenesis system can quickly and reproducibly generate in frame deletions in *K. pneumoniae*.

Graduate Poster #13

Advances Towards Saturating Transposon Mutagenesis for *Chlamydia trachomatis*

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Chlamydia is an obligate intracellular pathogen with a biphasic development cycle, both of which pose challenges for genetic manipulation. Reverse genetics is possible with targeted gene inactivation, but advances in forward genetics is lagging. In response to this limitation, efforts have been dedicated to developing a system for transposon mutagenesis in the chlamydial species. Previous attempts in *Chlamydia muridarum* were inefficient. Efforts to apply this system in *Chlamydia trachomatis* utilized an expression plasmid to overcome apparent toxicity of the transposase. This resulted in inefficient transposition that did not support purification of isolates from the complex mutant pool. Additionally, runaway transposition, due to a stably maintained shuttle vector, was an issue. Our study describes a novel approach to enhance transposon mutagenesis efficiency in *C. trachomatis* by reengineering the system. We employ the pKW expression plasmid, which leverages inducible control and fluorescence reporting. Unlike previous attempts, our method allows for curing of the plasmid without the need for antibiotic selection. Additionally, we can implement a high-throughput screening approach to efficiently purify and confirm individual mutants. Ongoing efforts to optimize the system aim to enhance mutagenesis efficiency and streamline the isolation process. The refinement of our transposon mutagenesis system is essential to overcome the existing limitations in chlamydial genetics.

Graduate Poster #14

Analysis of epoxide hydrolase enzymatic activity of bacterial proteins elevated in patients with alcohol-associated liver disease

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Background/Aim: Alcohol-associated liver disease (ALD) is often associated with gut bacteria dysbiosis, therefore an understanding of how the gut microbiota contributes to ALD development/progression may reveal new mechanisms and help discover novel therapies. Our group focuses on epoxide hydrolases (EHs), enzymes involved in lipid/xenobiotic metabolism, as potential pathogenic players in ALD. EHs are expressed by various host cells/tissues and are widely expressed by the gut microbiota. Previous fecal metagenomic analysis performed by us identified several bacterial proteins with predicted EH activity which were significantly elevated in patients with ALD vs non-ALD controls. The aim of the current study was to test for EH enzymatic activity of 4 identified proteins.

Methods: Synthetic genes (in a eukaryotic expression vector) for each were created and expressed in *E. coli*. Proteins were purified on nickel columns and their ability to hydrolyze the epoxy-fatty acids [9(10)- and 12(13)-EpOME] were determined by measuring a reduction of these substrates by LC-MS/MS.

Results: As compared to a negative control (no enzymatic activity), two proteins of interest revealed EH activity with substrate specificity to both 9(10)- and 12(13)-EpOMEs; one protein demonstrated EH activity with specificity to only 12(13)-EpOME; and the last protein revealed no EH activity. Comparison to positive control (purified soluble EH) confirmed these results.

Conclusions: In this study we confirmed EH enzymatic activity for three bacterial proteins. The next step is to identify bacterial species expressing these proteins and examine their abundance in our patient population, as well as validate the results in the large patient cohort.

Graduate Poster #15

TREATMENT OF EXPERIMENTAL ALCOHOL-ASSOCIATED LIVER DISEASE WITH LIMOSILACTOBACILLUS REUTERI THAT EXPRESS A RECOMBINANT N3-FATTY ACID DESATURASE

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Alcohol-associated liver disease (ALD) is a significant health care issue and results in deleterious effects on numerous organs, specifically the liver and gut. We have shown that n6-polyunsaturated fatty acids (PUFAs) exacerbate, while n3-PUFAs attenuate ALD in mouse models. This is important because the Western diet is rich in n6-PUFAs. Our goal was to test a novel approach to increase endogenous n3-PUFAs via engineered *Limosilactobacillus reuteri* (LR) that releases a recombinant n3-fatty acid desaturase (Fat-1). Wild type C57BL6/J mice were given an all-liquid diet containing ethanol (EtOH) for 10 days followed by a single EtOH binge. Mice were gavaged daily with LR-Fat-1 or LR-Con (109 cfu/day) or PBS. n3 and n6 PUFAs were measured in multiple tissues. Various end-points associated with liver and intestinal health, and gut microbiota were assessed. Compared to controls, LR-Fat1-treated mice had increased levels of n3-PUFAs, DHA and EPA, in liver and cecal contents. Fecal metagenomic analysis in these mice revealed significant increases in phylum Bacteroidota and

decreases in phyla Pseudomonadota, Verrucomicrobiota, and Actinomycetota. LR-Fat-1 treatment also resulted in several changes at the species level, including an increase in butyrate-producing *Senimuribacter intestinalis*. Elevated level of liver n3-PUFAs in LR-Fat-1 treated mice was associated with a modest reduction in plasma ALT levels (marker of liver injury). Lastly, there was a reduction in hepatic, plasma, and ileal levels of pro-inflammatory cytokines in LR-Fat-1 treated mice. These data suggest that increased levels of n3 PUFAs has beneficial effects on EtOH-mediated alterations in the gut microbiota and liver injury.

Graduate Poster #16

Characterization of the universal sugar transport system components PtsI and PtsH in *Enterococcus faecium*

Michelle Hallenbeck, Michelle Chua, and James Collins
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Background: Vancomycin-resistant Enterococci (VRE) pose a serious public health risk. Clinical *Enterococcus faecium* isolates are enriched in phosphotransferase system (PTS) sugar transporters. PtsI and PtsH are the universal components essential for sugar-specific transporter function and have been implicated in other physiological processes in multiple species. Here we examine their roles in biofilm formation, stress resistance, and competitive fitness.

Methods: We used a novel CRISPR-Cas12 system to generate clean the deletion mutants Δ ptsIH and Δ ptsH in *E. faecium* NCTC7171. Growth on simple sugars and the roles of PtsH and PtsI in biofilm formation, competitive fitness, and resistance to stressors including, autolysis, cold shock response, acid resistance, and copper toxicity were examined.

Results: The Δ ptsIH and Δ ptsH mutants demonstrated growth defects in rich media, likely because of their inability to utilize PTS-transported sugars. When grown in co-culture, the wild-type significantly outcompeted the Δ ptsIH. The ptsIH mutant was also outcompeted by the wild-type in a mouse GIT model of infection. The Δ ptsIH mutant displayed significantly reduced biofilm formation compared to the wild-type, but the Δ ptsH mutant displayed typical wild-type biofilm levels. Interestingly, complementation of the Δ ptsIH mutant restored wild-type growth, but not biofilm formation. PtsIH appears to have no role in stress resistance.

Conclusions: Our results indicate that the universal PTS genes ptsI and ptsH play a significant role not only in sugar metabolism but also in biofilm formation, and that the absence of both PtsI and PtsH results in a significant fitness defect both in vitro and in vivo. To date, we have been unable to generate a ptsI mutant, but we developed a novel anhydrous tetracycline (ahTC)-inducible CRISPR interference (CRISPRi) system to repress gene expression and used it to target ptsI and ptsH. The ptsI knockdown strain displayed significantly reduced biofilm formation, supporting a possible role for PtsI.

Graduate Poster #17

Localization and Transmission of Seed Endophytic Bacteria

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Seed endophytic bacteria can transmit vertically to the next generation, reflecting a potential beneficial role from germination through survival to crop production. Despite potential plant growth

and promotion properties, there is little known about the selection and localization of seed endophytes or their impact on plant germination. We aim to isolate and characterize seed endophytic bacteria from maize. Endophytic seed bacteria are present in all parts of the seed and all stages of the plant life cycle, and to explore the specificity of bacterial transmission during seed germination, we will examine field-grown untreated seeds to isolate seed endophytic bacteria and characterize the endophytic microbiome using both culture-dependent and metagenomic analysis. With knowledge of the endophytic microbial content, we will explore the localization and transmission of individual microbes during germination. We will use confocal laser scanning microscopy coupled with fluorescence in situ hybridization to characterize the localization of bacteria within the seed. The confocal microscopy will enable us to investigate the colonization pattern of the endophytic bacteria within seeds and seedlings during seedling establishment. This work will enable future work on manipulating the maize seed microbiome to enhance plant health and yield.

Undergraduate Poster Competition

Undergraduate Poster #1

Antimicrobial resistance patterns and virulence traits in *Rhodococcus equi* isolated from foals in Kentucky

Anaya Ali, Bibek Lamichhane, Cayla Cayer-McCarthy, Steve Locke, Erdal Erol, and Yosra A. Helmy.
University of Kentucky

Background: *Rhodococcus equi*, an intracellular pathogen causing pneumonia in foal. The infection is primarily treated with antibiotics, typically a macrolide and rifampin combination. However, rising antibiotic resistance has necessitated the need to investigate its resistance pattern and virulence for effective disease management. This study aims to evaluate the prevalence of antibiotic resistance and virulence traits in *R. equi* isolated from horses.

Methods: *Rhodococcus equi* isolates were collected from necropsied at the Veterinary Diagnostic lab at the University of Kentucky horses. *R. equi* was isolated on blood agar, and the resistance profile of the isolates was determined by broth microdilution assay using the Sensititre™ EQUIN2F Vet plates. Antibiotic susceptibility was confirmed by comparing Minimum Inhibitory Concentration (MIC) values with established standard ranges for antibiotics. Furthermore, the prevalence of different virulence genes was determined within the isolates using PCR.

Results: All of 27 *R. equi* isolates were susceptible to amikacin and resistant to cefazolin. However, intermediate sensitivity was observed to ampicillin, ceftazidime, ceftiofur, enrofloxacin, oxacillin, and penicillin. Similarly, 29.6% of the isolates were resistant to rifampin and erythromycin whereas, 33% were resistant to clarithromycin. Moreover, four isolates were identified as multi-drug resistant, demonstrating resistance to three or more tested classes of antibiotics. Similarly, out of 12 isolates tested for the presence of virulence genes, 83% were positive for VapA, 100% for VapB, 66% for VapC, 50% for VirR, 75% for VirS, 83% for iupS, and 50% for IupT.

Conclusion: In the future, we will focus on evaluating the ability of biofilm formation in *R. equi* isolates. The isolates will also be tested for genes associated with antimicrobial resistance. This study

intends to offer insights into the status of antibiotic resistance development and provide a comprehensive understanding of both antibiotic resistance and virulence patterns in *R. equi*.
Keywords: *R. equi*, pneumonia, antibiotic resistance, virulence

Undergraduate Poster #2

Flavivirus T-Cell Responses

Shania Chirinos and Amelia K Pinto
 University of Kentucky

In Dr. Pinto's lab, we have been working on a project with Flaviviruses, specifically Zika and Dengue. Using previous work from a previous graduate student, Mariah Hassert, she discovered the differences in T-cell responses from T-cell cross reactivity during flavivirus infections. My project revolves around having T-Cell response after having a previous flavivirus. More specifically, having Zika then a Dengue infection. After growing virus, we will infect mice. After infection we will wait different time frames to then infect a second flavivirus (dengue). Another aspect of this project is same timeframe but different amounts of virus. After harvesting the mice, we will look at the different immune responses. The results we hope for are to see how detrimental a previous flavivirus can be to an immune system. Long term effects we are looking for is having a previous flavivirus before a second flavivirus infection can lead to drastic reduction in immune response. We hope to create a vaccine to help the immune response be effective when it comes to multiple flavivirus. This is a major issue in third world countries as well as areas prone to mosquitoes infections.

Undergraduate Poster #3

Characterizing Interaction Domains between DdrR and KZA74_19365

Ethan Newsom, Deborah Cook, and Janelle Hare
 Morehead State University

Background: *Acinetobacter baumannii*'s response to DNA damage provides this drug-resistant, opportunistic pathogen with increased mutagenesis opportunities. Instead of a LexA repressor, coregulators UmuDAb and DdrR repress its error prone polymerases. UmuDAb and LexA each bind DNA to repress these polymerases, while DdrR, a protein unique to *Acinetobacter*, binds UmuDAb to provide additional repression. Identifying other proteins that DdrR interacts with could provide new drug targets as the CDC has declared the pathogen "an urgent public threat". We previously screened a two-hybrid library created using the BACTH (bacterial adenylate cyclase) system and found that our DdrR bait interacted with a library insert encoding just 17 residues of a putative DNA-binding protein, KZA74_19365.

Methods: We will verify this finding by constructing a new plasmid encoding KZA74_19365 for two-hybrid assay analyses. We also aim to identify specifically where in the 184 residue KZA74_19365 protein DdrR interacts by creating additional plasmids encoding different forms of truncated KZA74_19365 proteins.

Results: An I-TASSER model of the KZA74_19365 protein predicted that the positive library insert corresponds to a long helix region of the protein. Protein sequence alignments between KZA74_19365, UmuDAb, and LexA show that it lacks an AG self-cleavage site and the serine and lysine active site needed for self-cleavage. We designed primers to produce the full-length protein for insertion into the pUT18c plasmid.

Conclusions: From the I-TASSER results we predict DdrR will interact with the long helical-region of the entire KZA74_19365 because it previously interacted with a fragment from that region. KZA74_19365's lack of an AG self-cleavage site indicates a different mechanism of action that we hope to explore in the future. If interaction between KZA74_19365 and DdrR is confirmed further testing will be conducted to determine its role in the pathogen.

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Undergraduate Poster #4

Evaluation of Novel Small Molecule Therapeutics for Controlling *Rhodococcus equi* Infections in vitro.

Angel Bhusal, Bibek Lamichhane, Khaled A. Shaaban, Larissa V. Ponomareva, Jon S. Thorson, and Yosra A. Helmy
University of Kentucky

Background: *Rhodococcus equi* is a significant equine pathogen responsible for chronic bronchopneumonia in foals under 6 months old. As a zoonotic agent, it can also cause life-threatening necrotizing pneumonia in immunocompromised individuals. While antibiotics are the standard treatment, the emergence of antibiotic resistance necessitates the development of alternative therapeutic approaches. This study aims to investigate the potential of small molecules as novel strategies for controlling *R. equi* infections in vitro.

Materials and Methods: An initial screen evaluated approximately 1900 compounds at 10 μM concentration for their ability to inhibit *R. equi* growth. Highly potent compounds demonstrating 100% inhibition were selected for further evaluation. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these promising candidates were determined. Additionally, their efficacy in disrupting preformed *R. equi* biofilms and their activity against antibiotic-resistant strains were assessed.

Results: Ten small molecules exhibited remarkable growth inhibition (100%) of *R. equi* in the primary screen. Strikingly, their MIC and MBC values ranged from as low as 0.078 μM to 0.156 μM , respectively. Five of these compounds demonstrated complete (100%) inhibition of preformed biofilms at 10 μM , while the remaining compounds exhibited over 90% inhibition at 1.25 μM . Notably, these small molecules retained their potency against antibiotic-resistant *R. equi* strains.

Conclusion: These findings underscore the potential of novel small molecules as promising alternative therapeutics for controlling *R. equi* growth, biofilm formation, and antibiotic resistance in vitro. Future studies will investigate the effects of these compounds on the intracellular survival of *R. equi* within phagocytic cells and their impact on the expression of virulence factors, including those involved in biofilm formation and invasion.

Keywords: *Rhodococcus equi*, small molecules, antibiotics, alternatives, foals, bronchopneumonia, biofilms

Undergraduate Poster #5

How Sequential Encounters with Closely Related Flaviviruses Change the Immune Landscape

Julia Andrade, Mariah Hassert, Jill Plugheber, Shania Chirinos, James D. Brien, and Amelia K. Pinto.
University of Kentucky

Flaviviruses, including Zika virus (ZIKV), dengue virus (DENV), West Nile virus (WNV), yellow fever (YFV), and Japanese encephalitis virus (JEV), endanger over half of the world's population. Some are present in many of the same regions, increasing the likelihood that people get exposed to multiple Flaviviruses over their lives. Additionally, Flaviviruses share large amounts of genetic information and, consequently, antigenic overlap (2-5). With the novel detection of Zika in the Americas in 2015, it is essential to understand how exposure to other Flaviviruses impacts ZIKV immunity and how it can impact disease outcomes.

Our goal is to understand how these overlaps with similar Flaviviruses change the landscape of flavivirus T cell immunity and the host-pathogen interactions that occur during infection.

Undergraduate Poster #6

Testing for the Presence of *Naegleria fowleri* in Northeastern Tennessee Recreational Waters

Mackenzie Nicholas, Hannah Phillips, and Jennifer Brigati
Maryville College

This study aimed to optimize and use a PCR assay that detects general free-living amoebas (FLAs), *Naegleria* sp., and *Naegleria fowleri* in recreational waters in Northeastern Tennessee. Knowledge about the presence of *N. fowleri* in recreational waters is important to the public because of the extreme and fatal illness that it causes: primary amebic meningoencephalitis (PAM). Twenty locations were sampled from Blount, Anderson, Knox, Loudoun, Jefferson, and Sullivan Counties in the months of July, August, and September 2023. Twelve of those samples were positive for FLAs as seen by visual feeding trails on non-nutrient agar plates with a lawn of *E. coli*. Nine were confirmed as positive via PCR for FLAs, 4 were positive for *Naegleria* sp., and none were positive for *N. fowleri*. Despite the negative results for *N. fowleri*, this study suggests that users of recreational waters should still take precautions to avoid exposure to *N. fowleri*, especially in the summer months.

Undergraduate poster #7

In Vitro and In Vivo Antifungal Peptoid Activity Against *Candida auris*

Isabella M. Griffiths, Denny Gao, Erika L. Figgins, Lisa K. Ryan, and Gill Diamond
University of Louisville

Background: Invasive candidiasis is a serious fungal infection caused by *Candida* species which can enter the bloodstream and affect major organs, particularly in immunocompromised patients. Recently discovered *Candida auris* rapidly develops resistance to antifungal treatment and kills roughly one in three infected patients, posing a significant public health threat. Resistance to the few existing antifungals and their associated toxicity necessitates new antifungal therapies. Host defense peptides (HDPs) demonstrate intrinsic, broad-spectrum antifungal properties with little resistance, but manufacturing cost, protease vulnerability and limited efficacy of artificial HDPs render them impractical. One novel alternative exists in host defense peptide mimetics known as peptoids. We studied in vitro activity of eight peptoid compounds, oligomers of N-substituted glycines which mimic the antimicrobial peptide, LL-37, a cathelicidin. Our aim was to establish these peptoid compounds as a potential treatment of invasive candidiasis.

Method: Peptoids were tested against ten *C. auris* strains and five other *Candida* species using MIC, MFC, synergy, resistance, and time-kill kinetics assays. In vivo efficacy was examined in an

immunocompetent C57Bl/6 female mouse model of systemic infection. Mechanistic activity was visualized using TEM and fluorescent confocal microscopy of peptoid against *C. auris* clinical isolates 381 and 382.

Results: MIC values measured 2-64 $\mu\text{g/ml}$ against all *Candida* species and MFC values measured as low as 8 $\mu\text{g/ml}$. Compounds MXB-4 and MXB-5 showed strong synergistic effect with fluconazole and caspofungin, and serial daily passage of MXB-5 at sub-MIC concentrations showed no resistance up to three weeks. Imaging indicated increased membrane porousness and time-kill kinetics revealed complete killing of fungal colonies around one hour. A systemic candidiasis mouse model treated with MXB-5 subcutaneously 3 hr post-infection resulted in clearing of infection at 24 hr with the 5 mg/kg dose.

Conclusions: Our results propose these peptoids as a potential antifungal treatment, particularly as an alternative therapy for resistant strains.

Undergraduate Poster #8

Antimicrobial profiling of <i>Serratia marcescens</i> SM6 using BIOLOG chemical sensitivity assays
Kate Perkins and Lydia Bogomolnaya Marshall University
The emergence of bacterial drug resistance is a global public health concern. <i>Serratia marcescens</i> , the Gram-negative bacterium from the order Enterobacteriales, is an emerging pathogen with increasing clinical importance due to its intrinsic resistance to several classes of antibiotics. <i>S. marcescens</i> causes diseases of the central nervous system such as meningitis, urinary tract infections, pneumonia, bloodstream infections, various respiratory diseases, and many different types of wound infections in people with weakened immune systems. To identify additional compounds that could be used for treatment or prevention of <i>S. marcescens</i> infections, we utilized the Biolog Phenotype MicroArray technology. Phenotype MicroArrays (PM) consists of a panel of ten 96-well plates containing different classes of chemical compounds. Each PM plate contains twenty-four chemicals of varying structures and function in four different concentrations. The entire panel allows to test for sensitivity to 240 antimicrobials. In this ongoing project, we currently screened 96 compounds and found that 25 of them can inhibit bacterial growth. Of those, many compounds were not previously reported to have antimicrobial activity against <i>S. marcescens</i> . After initial screening, the identified anti- <i>S. marcescens</i> candidate compounds will be tested individually to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Our project will fill a critical knowledge gap in understanding <i>S. marcescens</i> sensitivity to antimicrobials. Obtained information would provide the additional results required to develop a strategy to control <i>S. marcescens</i> infection.

Undergraduate Poster #9

The Search for the Mystery Source of NtrX Phosphorylation
Emma Bain and Benjamin Stein University of Tennessee at Chattanooga
Bacteria are abundant in the environment and use two-component signaling systems (TCSs) to sense their surroundings. These systems consist of a histidine kinase (HK) and a response regulator (RR). The

HK senses a signal and autophosphorylates on histidine residue, followed by phosphotransfer to the response regulator (RR). The phosphorylated RR then substantially causes a cellular response such as transcriptional changes. Although many TCSs work in this manner, some diverge and can have alternative proteins that initiate or modulate phosphorylation. In *Caulobacter crescentus*, the NtrYX system is one such complex TCS. Based on primary sequence, the NtrY protein is a histidine kinase, whereas NtrX is the response regulator. However, we have observed that NtrY is not needed to phosphorylate NtrX. Therefore, an unidentified component is responsible for phosphorylation of NtrX. With the use of Phos-tag electrophoresis, we have eliminated known kinase candidates that are close in sequence to NtrY. These candidates include: FixL, NtrB, and ChvG. In the future, we plan to utilize pulldown methods to investigate potential small molecule or protein interactors that may phosphorylate NtrX.

Undergraduate Poster #10

Dysregulation of Paneth Cell-Specific Genes During SARS-CoV-2 Infection from Patient and Mouse Stool Samples

Braden Mulcahy, Erika L. Figgins, Denny Gao, Tanya O. Robinson, Kenneth Wilson, Jon Gabbard, Uyen “Emily” Do, Braden Mulcahy, Lisa K. Ryan, Michelle Galeas Pena, Sarah C. Glover, Gill Diamond.
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Paneth cells, which are specialized epithelial cells found exclusively in the small intestine, play a crucial role in maintaining gut homeostasis by releasing antimicrobial peptides. Additionally, they secrete lectins that can impede viral entry by binding to pathogens. Recently identified glycan-binding lectins, known as Intelectins 1 and 2, have diverse functions in the human body, including cell interactions, modulation of signaling pathways, and contributions to innate immune responses. Despite the challenges posed by invasive procedures, our research has shown the feasibility of analyzing changes in intestinal gene expression by examining exfoliated cells in stool samples. Given the association between SARS-CoV-2 and various gastrointestinal symptoms, it is plausible that the virus disrupts the gut immune response, including the expression of genes specific to Paneth cells. We hypothesized that Paneth cell-specific genes in the gut would be dysregulated during infectious conditions such as a SARS-CoV-2 infection. To investigate this hypothesis, we obtained stool specimens from hospitalized SARS-CoV-2 patients and healthy human controls. We then performed total RNA extraction and quantitative RT-PCR (qRT-PCR) to evaluate mRNA levels specific to Paneth cells and non-specific genes. Our findings demonstrate a novel approach to detecting and quantifying Paneth cell-specific mRNA levels from stool samples. Specifically, SARS-CoV-2 positive patient samples exhibited decreased mRNA levels of Paneth cell-specific genes, including Intelectin-2, HD-5, HD-6, sPLA-2, alpha-1-anti-trypsin, and trypsin 2. In contrast, non-Paneth cell-specific genes like Intelectin-1 did not show altered mRNA levels, suggesting that SARS-CoV-2 predominantly affects Paneth cells specifically. These results were corroborated in a mouse model of SARS-CoV-2 infection, where stool samples from infected mice exhibited significantly reduced expression of Paneth cell-specific genes such as PLA2G2A and Lysozyme. These findings validate our hypotheses and pave the way for further investigation into the impact of viral infections on intestinal gene expression.