

AMERICAN SOCIETY FOR MICROBIOLOGY

KENTUCKY / TENNESSEE BRANCH

2026 SPRING MEETING



April 10-11, 2026



The University of Tennessee, Knoxville
UT Conference Center

KEYNOTE SPEAKERS



Kevin Hockett, Ph.D.
Penn State Univ.



Shaun Brinsmade, Ph.D.
Georgetown Univ.

Showcase & Celebration of Research Accomplishments of

- ❖ Undergraduate Students
- ❖ Graduate Students
- ❖ Medical Students
- ❖ Postdocs
- ❖ Staff
- ❖ Faculty

- ✓ Oral and Poster presentations
- ✓ Awards for outstanding presentations
- ✓ Networking opportunities

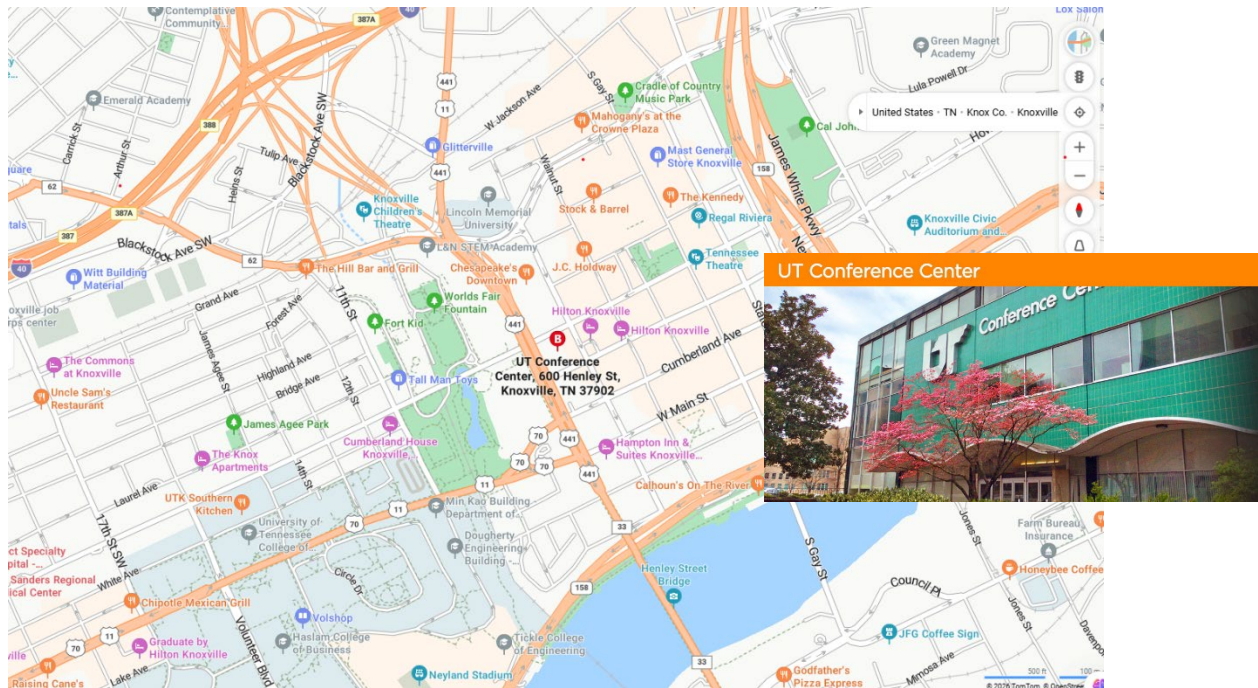
IMPORTANT DEADLINES

Abstract submission: **March 20**
Registration: **March 31**



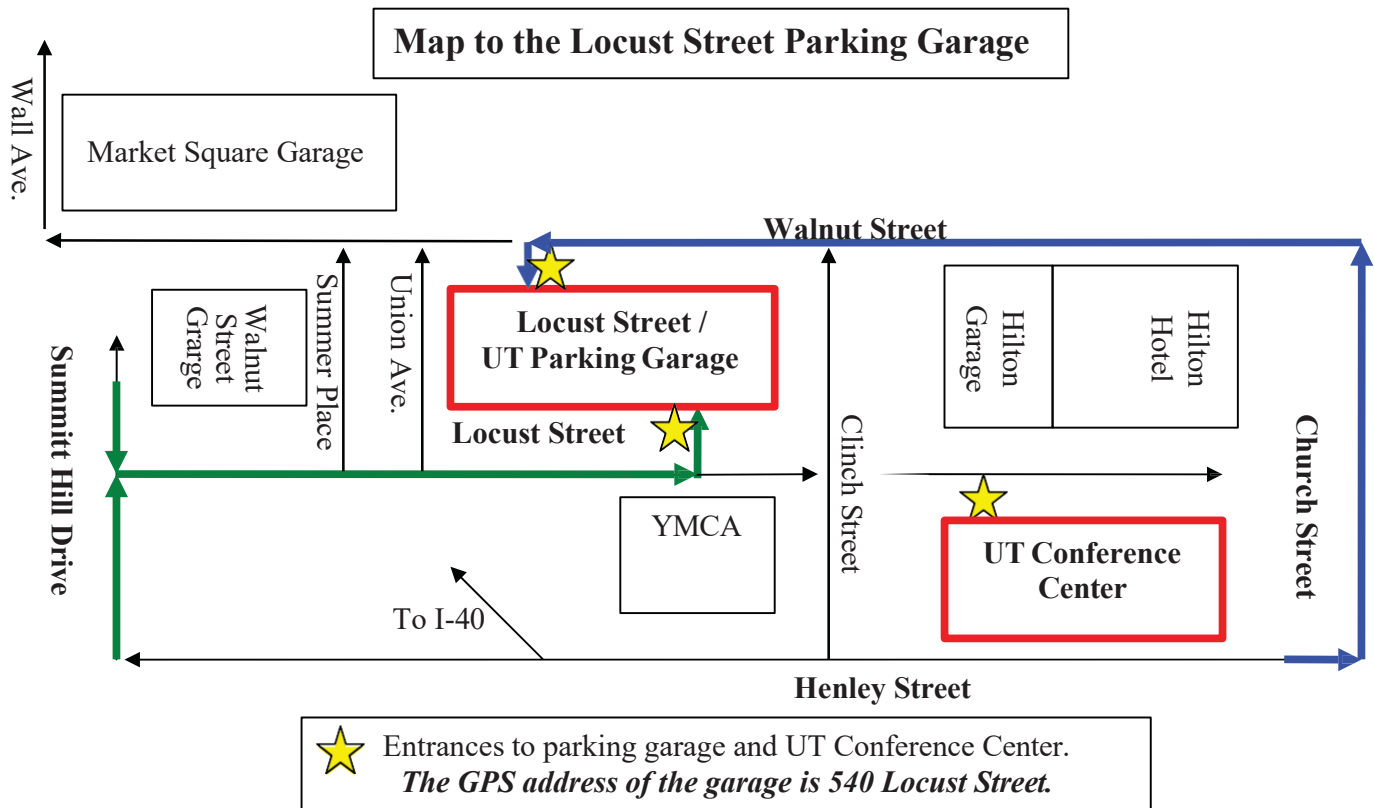
Register & Submit Abstract

The conference will be held at the UT Conference Center (600 Henley St, Knoxville TN 37902) which is located on the 4th floor of the building.



Parking is available at the Locust Street Garage (540 Locust Street, Knoxville, TN 37902). Parking is free on Saturday and after 6 pm on Friday.

A map of the parking and direction to its entrance is on the following page.



If you are traveling North on I-75 or East on I-40:

- Take I-40 East into downtown Knoxville area.
- Take Exit #388 (U.S. 441, Smoky Mountain Exit). (The exit will be to the right.)
- Go through tunnel to Henley Street. Stay to your left on Henley Street to turn left at the second traffic light.
- Turn left onto Church Street.
- Take the second left, which is Walnut Street. Go through the stop sign at Clinch.
- Locust Street Parking Garage is the first entrance on the left.

If you are traveling South on I-75 / I-275:

- Take I-40 East exit. Merge immediately to far right, taking the Henley Street exit ramp.
- Go through tunnel to Henley Street. Stay to your left on Henley Street to turn left at the second traffic light.
- Turn left onto Church Street.
- Take the second left, which is Walnut Street. Go through the stop sign at Clinch.
- Locust Street Parking Garage is the first entrance on the left.

If you are traveling West on I-40:

- Take Exit 389 (Hall of Fame Dr) toward US-441 N. Broadway. Left on Hall of Fame Dr. NE
- Turn right on Summit Hill Dr SE
- Turn left on Locust Street SW
- Locust Street garage entrance is on the left

If you are traveling East on I-40:

- Take Exit 388B onto Henley Street/441 South; bear right immediately toward Route 62 (Western Ave/Summit Hill)
- Turn left on Western Ave. and proceed through the Red Light (Henley Street), past the City of Knoxville fire station and make a right on Locust Street.
- Locust Street Parking Garage is on the left just before the Locust Street and Clinch Street Intersection.

From the Knoxville Airport (McGhee Tyson Airport):

- Take Hwy 129 North to I-40 East.
- From I-40 East, take Exit #388 (U.S. 441, Smoky Mountain Exit). The exit will be to the right.
- Go through tunnel to Henley Street.
- Stay to your left as you exit the Tunnel on Henley Street to turn left at the second traffic light.
- Turn left onto Church Street.
- Take the second left, which is Walnut Street.
- Go through the stop sign at Clinch.
- Locust Street Parking Garage is the first entrance on the left.

Meeting Schedule

**note all times are in EST

Overview:

Friday, April 10th

4:00 – 6:30 PM Registration
 6:00 – 7:30 PM Dinner available
 6:30 – 9:00 PM Keynote session 1; Room 413 ABC
 9:00 PM Adjourn for the day

Saturday, April 11th

7:30 – 11:00 AM Registration
 8:00 – 9:30 AM Breakfast available
 8:30 – 9:30 AM Concurrent Sessions 2 (Room 413ABC) & 3 (Room 406)
 9:30 – 9:45 AM Break
 9:45 – 11:15 AM Concurrent Sessions 4 (Room 413ABC) & 5 (Room 406)
 11:15 – 12:45 PM Poster Presentations (Rooms 413ABC and 400A)
 1:00 – 2:00 PM Lunch Sessions Concurrent for Trainees (Room 413ABC) and Faculty (Room 406)
 2:00 – 3:45 PM Keynote Session 2 (Room 413 ABC)
 3:45 – 4:00 PM Awards Presentation and Close of Meeting (Room 413 ABC)
 Optional: Graduate students mixer

U = Undergraduate or Post-baccalaureate Ga = Graduate student year 1-2

Gb = Graduate student year 3-5 PD = Post Doc

O = Other F = Faculty

P = poster, O = oral

Example: Ga_O1 = Grad student 1-2, Oral, abstract 1, Gb_P3 = Grad student year 3-5, Poster abstract 3

Start time	Abstract number	Speaker/Institution	Title
6:30		Dr. Gladys Alexandre	Opening Remarks
6:35	KN1	Dr. Kevin Hockett Penn State Univ	Utilizing Applied Microbial Ecology in Agriculture
7:15		Break	
7:20	F_01	Dr. Alessandro Occhialini, UTIA	Investigating plant-bacterium horizontal gene transfer (HGT) associated with novel episomal platforms for plastid genetic engineering
7:35	PD_O1	Dr. Elise Kammerdiener, ORNL	Multilayered regulation by RNA thermometers enables precise control of Cas9 expression in <i>E. coli</i>
7:50	PD_O2	Dr. Delaney Beals, ORNL	High resolution metaproteomics reveals protein-level functional redundancy in a defined bacterial community
8:05	PD_O3	Dr. Hannah Hughes, UTK	An RTX adhesin and FhaB homolog modulate surface attachment in <i>Azospirillum brasilense</i>
8:20	PD_O4	Dr. Walter Harrington, St Jude Children's research hospital 2026 Peggy Cotter Award	Duck Duck difference: variation in disease severity and viral shedding in dabbling diving and sea ducks infected with HPAI A(H5N1)□
8:50	PD_O5	Flashtalk: Dr. Alaa Sewid, UTK 2026 Peggy Cotter Award	A New Era of Pathogen Detection: Rapid, Visual, and Field-Ready Diagnostics for Infectious Threats and Antimicrobial Resistance.
9:00		Adjourn	

Saturday April 11th

Registration table open: 7:30AM-11:00AM; Breakfast Served 8:00-9:30AM

Session 2:

Moderator: Dr. Carolyn Ibberson Room 413 ABC

Start	Abstract	Speaker/Institution	Title
8:30	Ga_O1	Noah Blevins, ETSU	Isolation and Characterization of Siderophore(s) from <i>Lelliottia amnigena</i>

8:42	Gb_O1	Shannon West, UTK	Staphylococcus aureus physiology and interactions in cystic fibrosis lung infection
8:54	Ga_O2	Braxton Stout, ETSU	Microbial Turf War: Genetic Mapping of Polymicrobial Inhibition in Pseudomonas baetica a390
9:06	Ga_O3	Kayla Reid, UTK	Collection and pathogenicity investigations of Oomycetes on Field Crops
9:18	Gb_O2	Abigail Pyburn, ETSU	From Signals to Survival: How STM3615 Influences Salmonella's Physiology

Session 3:

Moderator: Dr. Hameeda Sultana, Room 406

Start time	Abstract Number	Speaker/Institution	Title
8:30	Gb_O3	Kira Bernabe, UoL	Investigating the role of the ribosomal protein bS21 in vancomycin resistance in Staphylococcus aureus
8:42	Gb_O4	Malabika Bhowmik, LMU	ZFP36L1: An Important Regulator of Innate Antiviral Immunity in Murine Norovirus Infection
8:54	Ga_O4	Victoria Federico, Vanderbilt	Human Milk Oligosaccharides Influence Inflammatory Cytokines Implicated in Preterm Birth: A Magnetic Bead Electrochemical Biosensor-Based Assessment
9:06	Ga_O5	Emma Carpenter, ETSU	Love at First Bind: Decoding the Candida-Enterobacter Attachment Affair
9:18	Gb_O6	Morgan Hetzel, UTK	Acute fasting impairs antimicrobial responses of neutrophils

Session 4:

Moderator: Dr. Lindsey Burcham, Room 413 ABC

Start time	Abstract Number	Speaker/Institution	Title
9:45	PD_O6	Rajasree Chakraborty, Meharry Medical College	Integration competent HIV-1 replication complexes are formed in the cytoplasm of infected cells within
10:00	PD_O7	Jéssica da Conceição Mendonça, UTK	Living in a Lactobacillus World: Group B Streptococcus in the Vaginal Microenvironment
10:15	PD_O8	Maresh Puthiyottu Poyil, UTIA	Active immunization with a recombinant arthropod protein impacts the transmission of rickettsial pathogen from ticks to a vertebrate host
10:30	F_O2	Jason Marion, Eastern Kentucky University	Prevalence of Phenotypic Multidrug-Resistant Enterobacterales in Kentucky (USA) Surface Waters and Evaluation of Sentinel Antibiotics for One Health Surveillance
10:45	F_O3	Brittany Magalis, UoL	Evolutionary indicators of viral cooperative behavior are significant predictors of progression to SAIDS

Session 5:

Moderator: Dr. Brad Binder, Room 406

Start time	Abstract Number	Speaker/Institution	Title
9:45	Gb_O7	Madeline McHugh, UTK	Variability of Spontaneous Prophage Induction is Related to Plasmid Maintenance in Model Marine Bacterium Sulfobacter pontiacus
9:57	Ga_O6	Nada Abdelkader, UKY	Multidrug-Resistant Salmonella enterica from Cattle: Integrated Phenotypic and Genomic Characterization
10:09	Gb_O8	Kennedi Hambrick, UTK	Temperature histories of host and phage influences infection of Microcystis aeruginosa
10:21	Gb_O9	Toyosi Ajide-Bamigboye, UTIA	Microplastic type determines soil microbial restructuring and nutrient cycling dynamics in agroecosystems
10:33	Ga_O7	Katelyn Miller, ETSU	Colorful Competition: The co-culture dynamics and violacein modulation of Raoultella planticola with Chromobacterium violaceum
10:45	Gb_O10	Mahbuba Ferdous, UTK	Cross-kingdom ethylene signaling through bacterial receptors regulates biofilm formation, root colonization, and metabolic wiring

Poster Presentations: 11:15am -12:45pm

1:00-2:00 pm Lunch Sessions:

Trainee Session: (take your lunch)

Room 413 ABC: Moderator: Aanuoluwa E. Adekey

1:00	ASM Young Ambassador Program, ASM Microbe, Future Leaders Mentoring Fellowship and the mBio Early-Career Editorial Board program	Aanuoluwa E. Adekoya (UTK)
1:30	Careers in Industry	Dr. Dora Taggart, President and CEO Microbial insights, Inc.
1:45	Careers in Microbiology: From Constructing Microbial Communities to Building Research Ecosystems: A non-traditional career pathway	Dr. April Armes, UTK

Faculty Session (take your lunch)

Room 406: KY/TN Branch Meeting; Moderator: David Beck

Session 6:

Moderator: Dr. Heidi Goodrich-Blair Room, 413 ABC

Start time	Abstract Number	Speaker/Institution	Title
2:00	KN2	Dr. Shaun Brinsmade, Georgetown University	Lean on me (for lipids): Metabolic synergy between <i>Staphylococcus aureus</i> and <i>Enterococcus faecalis</i> during polymicrobial infection
3:00	F_O4	Ryan Awori, Fullbright visiting scholar UTK	Identification of putative binding sites in tail fibres of tailocins from animal-associated <i>Xenorhabdus</i> bacteria
3:15	F_O5	Dipak Kathay, Lincoln Memorial University	Role of L,D-transpeptidases in the survival of <i>Legionella pneumophila</i>
3:30	F_O6	Andrew Monteith, UTK	Dietary lipids tune staphylococcal Gdh-dependent control of NETosis

3:45pm Trainee Awards Presentation and Close of Meeting

U = Undergraduate or Post-baccalaureate Ga = Graduate student year 1-2

Gb = Graduate student year 3-5 PD = Post Doc

O = Other F = Faculty

P = poster, O = oral

Example: Ga_O1 = Grad student 1-2, Oral, abstract 1, Gb_P3 = Grad student year 3-5, Poster abstract 3

Oral Presentations (in order of presentation)

Room 413 ABC	Session 1: Moderator Dr. Gladys Alexandre
KN1	Dr. Kevin Hockett: Utilizing Applied Microbial Ecology in Agriculture
F_O1	<p>Investigating plant-to-bacterium horizontal gene transfer (HGT) associated with novel episomal platforms for plastid genetic engineering</p> <p>Alessandro Occhialini Department of Plant Sciences, University of Tennessee, 301 Agriculture & Natural Resources Building, 2431 Joe Johnson Drive, Knoxville, TN 37996, USA.</p> <p>Novel, cutting-edge plastid genetic engineering tools hold strong potential in agriculture to improve crop traits by transferring valuable transgenes. Engineering of the plastid genome (plastome) can be accomplished using either conventional homologous recombination (HR)-based vectors that mediate precise, site-specific transgene integration, or innovative episomal platforms based on synthetic plastomes (mini-synplastomes) that enable transgene expression from non-integrating plasmid backbones. The risk assessment of potential horizontal gene transfer (HGT) is a critical requirement for regulatory approval prior to the environmental release of these emerging plastid engineering technologies. Although plastids are predominantly maternally inherited, thereby limiting vertical transgene transmission through pollen, their evolutionary origin as endosymbiotic descendants of prokaryotic ancestors may increase the likelihood of HGT to environmental microbial communities. In this study, a naturally competent soil bacterium model <i>Acinetobacter baylyi</i> was utilized to assess the probability of plant-to-bacterium HGT under controlled laboratory experimental conditions. While plant-to-bacterium HGT was detectable in vitro as a rare event, mini-synplastomes did not exhibit a higher HGT frequency compared with conventional chloroplast transformation vectors. Following a comprehensive analysis of potential risks associated with mini-synplastome genetic elements (plastid origin of replications, plasmidic regions containing rRNA genes, and regulatory elements for transgene expression), optimized plasmid variants lacking residual bacterial activity and exhibiting undetectable HGT were developed and characterized. Overall, this work provides a valuable framework for the rational design of plastid transformation platforms with enhanced environmental biosafety for agricultural biotech applications.</p>

PD_01	<p>Multilayered regulation by RNA thermometers enables precise control of Cas9 expression in E. coli</p> <p>Elise K. Kammerdiener 1,2, Sarah K. Garcia 1,3, Margaret K. Bales 1,2,3, Dawn M. Klingeman 1,2, Adam M. Guss 1,2, Richard J. Giannone 1,2, Robert L. Hettich 1,2, Carrie A. Eckert 1,2, William G. Alexander 1,2 1Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, United States 2Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN 37830, United States 3Bredesen Center, University of Tennessee, Knoxville, TN 37996, United States</p> <p>Cas9-mediated genome editing enables the generation of large, diverse genetic libraries. However, in many bacteria, poorly regulated Cas9 activity leads to unintentional cell death, which overshadows its potential utility. This "leaky" Cas9 expression can inadvertently alter the abundances of gRNA library members, a phenomenon referred to as library skew. To improve control of Cas9 expression, we employed a multilayered regulatory approach where inducible promoters regulating transcription were fused with RNA thermometers (RNATs) regulating translation. When we combined the heat inducible promoter with an RNAT to drive Cas9, we observed significant temperature-dependent increases in cell survival along with the near elimination of library skew. This strategy of layering inducible promoters with RNATs should be generalizable to all bacterial Cas9-based technologies including CRISPRi, CRISPRa and highly parallel CRISPR editing (e.g. CREATE).</p>
PD_02	<p>High-resolution metaproteomics reveals protein-level functional redundancy in a defined bacterial community</p> <p>Delaney G. Beals (1), Jonghyun Kim (1), Robert L. Hettich (1), Dale A. Pelletier (1), Mitchel J. Doktycz (1) (1) Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA</p> <p>Functional redundancy is frequently invoked to explain the stability of microbial communities, yet it is often inferred from shared genomic potential rather than directly measured from realized activity. Consequently, it remains unclear whether closely related strains that encode similar functions are functionally interchangeable within multispecies communities. Here, we use strain-resolved metaproteomics to assess functional redundancy among closely related <i>Pseudomonas</i> strains embedded within a defined bacterial community derived from the <i>Populus</i> root microbiome. We constructed a ten-member synthetic community in which a single <i>Pseudomonas</i> representative was systematically replaced with one of nine alternative isolates while holding all other community members and growth conditions constant. By quantifying protein expression across the entire community before and after stabilization, we evaluated functional overlap ranging from individual proteins to emergent physiological states. Despite substantial conservation of genomic content among <i>Pseudomonas</i> isolates, metaproteomic profiles resolved these strains into two statistically distinct clusters. These clusters were characterized by differential allocation toward biosynthetic, growth-associated functions versus stress-associated maintenance functions. These differences were driven primarily by strain-specific regulation of conserved core pathways rather than by accessory gene content. Additionally, variation in <i>Pseudomonas</i> functional state propagated to the broader community, affecting the relative abundance and proteomic responses of non-<i>Pseudomonas</i> members. Together, these results demonstrate that functional redundancy among closely related bacteria is context- and scale-dependent, emerging from shared functional capacity while remaining constrained by regulatory and physiological divergence. This work highlights the importance of measuring realized activity to understand how diversity within a genus shapes community function and stability.</p>
PD_03	<p>An RTX adhesin and FhaB homolog modulate surface attachment in <i>Azospirillum brasilense</i></p> <p>Hannah Hughes & Gladys Alexandre University of Tennessee Knoxville</p> <p>Background: Surface attachment is an important step to many bacterial behaviors, including biofilm formation and host colonization. For the soil bacterium <i>Azospirillum brasilense</i>, attachment to plant roots promotes beneficial colonization of the root surface that can be leveraged to improve plant growth and crop yield. Although attachment is a crucial step in this interaction, the key factors driving <i>A. brasilense</i> attachment are not well understood. Methods: To investigate this process, we examined the impact of a highly expressed, yet uncharacterized beta roll RTX surface protein, designated 28875. Extraction of the protein from a native context revealed an association with a larger protein similar to <i>Bordetella pertussis</i> FhaB, which is required for <i>Bordetella</i> to infect host cells. We generated <i>A. brasilense</i> mutants deficient in these proteins and carried out phenotypic analysis to determine any role in attachment. Results: The Δ28875 mutant demonstrates increased biofilm formation while the ΔfhaB mutant is unable to form a biofilm. However, upon incubation with plant roots, Δ28875 cells show a deficiency in attachment. Tests for cell-cell attachment (i.e., flocculation) have not shown significant differences from the parent strain. Conclusions: We propose that 28875 interacts with the FhaB-like protein to form a surface structure that interacts with plant roots and mediates attachment. When 28875 is absent, plant root attachment decreases, but general attachment (via biofilm) increases. This working model is similar to what has been shown for <i>Bordetella</i> FhaB, which also demonstrates increased biofilm upon deletion of its partner RTX protein. Our results suggest RTX-FhaB homologs have a more expanded role than previously thought, modulating surface attachment even in non-pathogenic bacteria.</p>
PD_04	<p>Duck Duck Difference: Variation in disease severity and viral shedding in dabbling, diving, and sea ducks infected with HPAI A(H5N1) D1.1</p> <p>Walter Harrington¹, Johanna Harvey², Jeffery Sullivan³, Jonathan Dain⁴, Karlie Woodard¹, Trushar Jeevan¹, Ahmed Kandeil¹, Jeri Carol Crumpton¹, Patrick Seiler¹, Lisa Kercher¹, Nichola Hill⁴, Diann Prosser³, Richard Webby¹</p> <p>1Dept. of Host-Microbe Interactions, St. Jude Children's Research Hospital, Memphis, TN 2Dept. of Natural Resources Science, University of Rhode Island, Kingston, RI 3Eastern Ecological Science Center, U.S. Geological Survey, Laurel, MD 4Dept. of Biology, UMass Boston, Boston, MA</p> <p>Highly pathogenic avian influenza (HPAI) A(H5N1) clade 2.3.4.4b viruses have caused a global panzoonotic event in wild birds, with more than 17,000 wild bird mortalities reported in the United States since 2022, a substantial underestimate of the true impact. Continued spillover into a broad range of mammalian species has heightened concern regarding viral adaptation and pandemic potential. Although surveillance data suggest that mallard ducks play a major role in the geographic dissemination of these viruses, experimental studies evaluating infection dynamics across duck species beyond mallards remain limited. Here, we report experimental challenge studies in four duck species representing dabbling, diving, and sea ducks, including mallards, lesser scaup, common eiders, and surf scoters, using the dominant circulating HPAI A(H5N1) genotype D1.1 from the 2024 to 2025 migratory season. Mallards and lesser scaup exhibited high survival, whereas all common eiders and surf scoters succumbed to infection within seven days post-challenge. Across species, oropharyngeal viral titers were generally higher than cloacal titers, with surf scoters exhibiting significantly higher oropharyngeal shedding compared to other species, while common eiders displayed the lowest cloacal titers. Surviving mallards mounted robust hemagglutination inhibition antibody responses to the challenge virus. A subset of mallards primed with H3N8 before HPAI A(H5N1) challenge showed complete survival and reduced oropharyngeal and cloacal viral titers compared to mallards that were challenged with HPAI alone. Despite clear seroconversion to A(H5N1), no detectable H3N8-specific hemagglutination inhibition antibodies were observed. Measurements of nucleoprotein-specific antibodies showed that LPAI exposure generated weak and variable NP responses, while HPAI infection produced strong and consistent NP antibody responses. Taken together, these results demonstrate pronounced species-specific differences in susceptibility, viral shedding, and immune responses following HPAI infection, and highlight the importance of incorporating non-mallard duck species into ecological, transmission, and spillover risk assessment models.</p>

<p>PD_O5</p>	<p>A New Era of Pathogen Detection: Rapid, Visual, and Field-Ready Diagnostics for Infectious Threats and Antimicrobial Resistance.</p> <p>Alaa Sewid University of Tennessee, Knoxville</p> <p>Rapid and accessible pathogen detection is critical for controlling infectious diseases and antimicrobial resistance. We developed a DNzyme-based loop-mediated isothermal amplification (DNzyme-LAMP) platform that enables rapid, sensitive, visual, and low-cost detection of pathogens without the need for complex instrumentation. This approach provides highly sensitive and specific colorimetric readouts, supports smartphone-based analysis, and opens new avenues for scalable, deployable solutions in real-time outbreak monitoring and public health applications.</p>
<p>Room 413ABC</p>	<p>Session 2 – Moderator Dr. Carolyn Ibberson</p>
<p>Ga_O1</p>	<p>Isolation and Characterization of Siderophore(s) from <i>Lelliottia amnigena</i></p> <p>Noah Blevins East Tennessee State University</p> <p>Background Every year antibiotic resistant bacteria contribute to millions of deaths, with the number only set to grow each year. As such, it is vital to develop new, effective treatments for antibiotic resistant bacteria. Because of the difficulty of developing new antibiotics, it is more efficient to find ways to bypass resistance altogether. To obtain iron and other vital metals in harsh environments, many bacteria produce compounds called siderophores. These compounds use specialized pathways to traverse the cell, which can be exploited by attaching antibiotics to slip into the bacteria like a Trojan horse. However, not all siderophores can enter all bacteria, and not all bacteria produce enough siderophores to be economically viable as the basis of a treatment. Therefore, it is important to test a wide variety of novel bacteria to determine if their siderophores could be used in new medications, such as the subject of this project, <i>Lelliottia amnigena</i>.</p> <p>Methods To maximize siderophores production by <i>L. amnigena</i>, the source and concentration of carbon, nitrogen, iron, and various salts in the minimal media used to grow the bacteria was optimized. Additionally, the growth time, aeration ratio, and temperature were tested to find the ideal growth conditions. Following growth, optical density of the media and of an Arnov's Assay of the supernatant of the media were used to determine the quantity of bacteria and siderophores, respectively. After optimization, by using column chromatography followed by RP-HPLC of supernatant from centrifuged media, siderophores can be purified from media for additional testing.</p> <p>Results and Conclusions Spectral analysis and amino acid hydrolysis via thin layer chromatography are expected to determine the chemical composition and metal affinity of <i>Lelliottia amnigena</i>'s siderophores. After optimization, a formulation that produces over ten times the original production of siderophores has been created.</p>
<p>Gb_O1</p>	<p>Staphylococcus aureus physiology and interactions in cystic fibrosis lung infection</p> <p>Shannon R. West¹ and Carolyn B. Ibberson¹ ¹Department of Microbiology, University of Tennessee, Knoxville</p> <p>Cystic fibrosis (CF) is a life-threatening genetic disease that affects nearly 40,000 people in the United States and an estimated 100,000 people worldwide. CF disease is caused by the mutation of a gene for a cellular ion channel protein, the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR mutation impairs mucus clearance from epithelial cell surfaces throughout the body causing a variety of intestinal and pulmonary symptoms. In the lungs, this mucus accumulation leads to bacterial infections, inflammation, and tissue damage, making pulmonary complications the primary cause of morbidity and mortality in people with CF (pwCF). These lung infections are often polymicrobial, difficult to treat, and persist over the lifetime of pwCF. Despite the polymicrobial nature of CF lung infections, clinical reporting and metagenomic data show <i>Staphylococcus aureus</i>, including methicillin-resistant <i>S. aureus</i> (MRSA), as the most prominent pathogen. Metatranscriptome analysis of CF sputum also confirms high transcriptional activity and co-occurrence of <i>S. aureus</i> with other CF pathogens such as <i>Pseudomonas</i> and <i>Haemophilus</i> as well as commensal bacterial genera (<i>Veillonella</i>, <i>Prevotella</i>, <i>Rothia</i>, and <i>Streptococcus</i>). However, the physiology of <i>S. aureus</i> in these infections and how it is shaped by the polymicrobial community is understudied. Additionally, the specific interactions that occur between <i>S. aureus</i> and these co-infecting microbes in CF lung infection are not well characterized. Therefore, we employed an in vitro CF system (SCFM2), cell-culturing, transcriptomics, and metabolomics to study how these co-infecting microbes impact the growth and global gene expression of <i>S. aureus</i>. The results of these experiments show that <i>S. aureus</i> has distinct responses to the different microbes, highlighting how community member composition can alter the pathogenicity of a major CF pathogen. Current investigations are utilizing a quantitative framework to calculate how accurately these in vitro conditions capture the in situ physiology of <i>S. aureus</i>.</p>
<p>Ga_O2</p>	<p>Microbial Turf War: Genetic Mapping of Polymicrobial Inhibition in <i>Pseudomonas baetica</i> a390</p> <p>Braxton E Stout, BS; Sean J Fox, PhD (PI) East Tennessee State University</p> <p>The genus <i>Pseudomonas</i>, often recognized for its role in human disease, also possesses a remarkable capacity to inhibit competing microbial species through polymicrobial interactions. This project investigates the antimicrobial potential of environmental <i>Pseudomonas</i> isolates and their relevance in targeting clinically significant pathogens. Soil samples collected from the Appalachian region were screened for antimicrobial activity against pathogens commonly associated with hospital-acquired infections. One isolate, identified as <i>Pseudomonas baetica</i> a390, demonstrated broad-spectrum inhibitory activity, producing zones of inhibition against thirteen pathogens, including <i>Salmonella enterica</i> and <i>Salmonella arizonae</i>. To elucidate the underlying mechanism of inhibition, transposon mutagenesis was performed using the EZ-Tn5 system to generate loss-of-function mutants. Four mutants (C1, C2, H8, H9) were identified based on reduced inhibitory phenotypes. Subsequent genomic mapping through restriction digest, plasmid rescue, and RATE-PCR enabled identification of transposon insertion sites. Notably, sequencing of Mut4 (H9) revealed disruption of a gene encoding a putative coniferyl aldehyde dehydrogenase (CALDH), implicating this pathway in antimicrobial activity. Functional assays comparing wild-type and mutant strains demonstrated altered effects on both planktonic growth and biofilm formation of <i>S. arizonae</i>. Additionally, a <i>Caenorhabditis elegans</i> infection model was employed to assess host survival and microbial burden in co-culture conditions. Together, these findings provide insight into the genetic basis of polymicrobial inhibition by <i>P. baetica</i> a390 and highlight the role of metabolic pathways, including CALDH-associated functions, in mediating antimicrobial interactions. This work contributes to a broader understanding of microbial competition and identifies potential targets for novel antimicrobial strategies.</p>

Ga_O3	<p>Collection and pathogenicity investigations of Oomycetes on Field Crops</p> <p>Kayla Reid, Gayan Kariyawasam, Heather Kelly University of Tennessee</p> <p>Several soil-borne oomycete pathogens, including <i>Globisporangium ultimum</i>, cause <i>Pythium</i> seed rots. These pathogens thrive in moist soils and wide temperature ranges, leading to stand loss by rotting the seed or killing the seedlings of commonly rotated row crops in the Southeastern U.S. Given that <i>G. ultimum</i> is a soil-borne pathogen, isolation techniques as well as selective media are a necessity in obtaining pure cultures while suppressing the growth of true fungi. In this study, we compared the growth of <i>G. ultimum</i> to three common contaminating true fungi, by measuring colony growth over time on: potato dextrose agar (PDA) amended with Streptomycin; pimaricin (5mg/L), ampicillin, rifampicin, and pentachloronitrobenzene (P5ARP); and P5ARP with amended with two different rates of tebuconazole. The P5ARP with 150 µL/L tebuconazole media (P5ARP+T2) provided optimal oomycete growth, and the least true fungi growth. P5ARP+T2 was then used to isolate oomycetes from cotton roots collected across the Cotton Belt in 2025. Using 16 newly collected isolates and a known pathogenic isolate of <i>G. ultimum</i> a seed pathogenicity assay was conducted on cotton, corn, and soybean seed. Results indicated that isolates were most virulent on cotton, followed by soybean, and least virulent on corn. In both the cotton and soybean assay, all isolates were pathogenic however, isolates TN 21A and MS 3-28 displayed lower virulence. Of the 16 isolates evaluated, 7 isolates had a higher virulence than the non-treated check on corn but had a lower severity rating compared to cotton and soybean. These results better inform crop rotation decisions to better manage <i>Pythium</i> seedling diseases in field crops. Future directions include species identification through sequencing and additional collections of oomycete species from soil across the Cotton Belt.</p>
Gb_O2	<p>From Signals to Survival: How STM3615 Influences Salmonella's Physiology</p> <p>Abigail E. Pyburn, Alexandra Pulliam, and Dr. Erik Petersen Department of Biomedical Health Sciences, College of Public Health, East Tennessee State University</p> <p>Foodborne-related diseases pose a widespread global health threat. A clinically relevant etiological agent in both humans and animals is <i>Salmonella</i>. To survive in varying environments, <i>Salmonella</i> must constantly sense and regulate a wide variety of internal mechanisms. The secondary signaling molecule cyclic-di-GMP (c-di-GMP) is one mechanism used to regulate such adaptations by influencing processes such as biofilm formation, flagellar motility, and virulence. Previous work has shown that the <i>Salmonella Typhimurium</i> c-di-GMP-specific phosphodiesterase (PDE) STM3615 is one of three PDEs required for proper survival within mouse macrophages and virulence during systemic infection. Deletion of STM3615 also resulted in bacteria with decreased survival in stationary phase, an altered rod shape, and sensitivity to peptidoglycan stress. Here, we examine the role of STM3615 in <i>Salmonella</i> physiology. Focused testing of STM3615's specific domains revealed that the periplasmic domain, rather than the enzymatically active PDE domain, is the primary mediator of the observed physiological phenotypes. A transposon reversion screen and protein-protein interaction assays suggest that STM3615 interacts with members of the Rcs bacterial phosphorelay stress response system. Future research aims to address the link between these two systems by testing the hypothesis that STM3615 participates within the Rcs stress pathway, and that the Rcs pathway regulates STM3615 PDE activity. By investigating this regulatory interaction, we seek to define a novel mechanism linking c-di-GMP signaling with the Rcs stress response, providing new insight into how bacterial pathogens coordinate stress adaptation and its physiological state.</p>
Room 406	<p>Session 3: Moderator Dr. Hameeda Sultana</p>
Gb_O3	<p>Investigating the role of the ribosomal protein bS21 in vancomycin resistance in <i>Staphylococcus aureus</i></p> <p>Kira Bernabe, University of Louisville; Kathryn Ramsey University of Louisville</p> <p>As the macromolecule in the cell responsible for protein synthesis, the bacterial ribosome is a major target for antibiotics. The ribosomal protein bS21 is located in the 30S subunit close to the anti-Shine Dalgarno sequence and mRNA exit channel. Although its precise role in protein synthesis is not well established, it has been implicated in regulation of translation initiation in several bacterial species. <i>Staphylococcus aureus</i> (<i>S. aureus</i>) is a Gram-positive coccus that is part of human normal flora but can cause serious disease. Typically, antibiotic resistant <i>S. aureus</i> infections are treated with vancomycin, a glycopeptide antibiotic that targets the cell wall. Vancomycin-intermediate <i>S. aureus</i> (VISA) and vancomycin-resistant <i>S. aureus</i> (VRSA) are of elevated concern because vancomycin is considered a drug of "last resort". Mutations in the gene that encodes bS21 have been detected in clinical isolates of VISA and at least some of these mutations are predicted to lead to loss of functional bS21. The link between loss of a ribosomal protein and resistance to a cell-wall targeting antibiotic in <i>S. aureus</i> remains unknown. Our goal is to elucidate the molecular mechanism that leads cells without bS21 to have increased vancomycin resistance. We confirmed that <i>S. aureus</i> cells with a clean deletion of the gene encoding bS21 have increased resistance to vancomycin compared to wild type cells, consistent with a VISA phenotype. Similarly to clinically relevant VISA strains, cells lacking bS21 also have thicker cell walls. Current experiments focus on understanding how the change in ribosome composition impacts gene expression and cell wall thickness. Together, our studies reveal a connection between ribosome heterogeneity and drug resistance in <i>S. aureus</i>.</p>
Gb_O4	<p>ZFP36L1: An Important Regulator of Innate Antiviral Immunity in Murine Norovirus Infection</p> <p>Malabika Bhowmik, Tooba Momin, Mrigendra Rajput Lincoln Memorial University</p> <p>Background: Norovirus is a leading cause of acute gastroenteritis worldwide and a major health concern due to its high transmissibility rate and lack of licensed vaccines or antivirals. Innate immune responses are critical for antiviral defence, and autophagy contributes to this process by viral degrading and regulating antiviral signalling. However, several viruses, including norovirus, exploit autophagy to enhance its replication. The role of ZFP36L1 in regulating norovirus replication remains poorly defined. This study investigated the role of ZFP36L1 on murine norovirus (MNV-1) replication and autophagy.</p> <p>Methods: RAW_{264.7} and HEK293T cells were stably transduced for ZFP36L1 overexpression or knockdown using lentiviral vectors. Cells were infected with MNV-1 (MOI 0.01), with wild-type and scrambled shRNA infected cells were used as controls. Viral titers were measured over 36 hours post-infection using TCID₅₀. Autophagy was modulated using rapamycin (induction) and 3-methyladenine (3MA: inhibition) and its effects on viral replication and ZFP36L1 expression was measured using qPCR and western blot (WB). Relationship between autophagy machinery and ZFP36L1 was further examined by inducing autophagy through LC3 overexpression (via gene transfection) or rapamycin treatment, and inhibiting autophagy using 3-MA and measured for ZFP36L1 expression. While the effect of ZFP36L1 expression on autophagy-related genes, including Atg13, Beclin1, Bcl-2, and p62, was analysed using qPCR and WB.</p> <p>Results: ZFP36L1 overexpression significantly reduced whereas ZFP36L1 knockdown increased MNV-1 titers ($p < 0.05$). MNV-1 infection increased autophagy measured by Atg13 and LC3II. Autophagy induction enhanced viral replication, suggesting viral utilization of this pathway for its efficient replication. ZFP36L1 overexpression suppressed basal and induced autophagy, (decreased Atg13 and LC3-II levels and increased p62 expression). These findings indicate that ZFP36L1 modulates autophagy and influences MNV-1 replication.</p> <p>Conclusions: ZFP36L1 contributes to restricting murine norovirus replication by limiting autophagy machinery. These findings expand the role of ZFP36L1 as a broad-spectrum antiviral regulator.</p>

Ga_O4	<p>Human Milk Oligosaccharides Influence Inflammatory Cytokines Implicated in Preterm Birth: A Magnetic Bead Electrochemical Biosensor-Based Assessment</p> <p>Victoria Federico (1), Dr. Hannah Richards (1), Dr. Julie Talbert (1), Jocelyn Leal (2), Dr. Jennifer Gaddy (1), Dr. Steven Townsend (1), Dr. David Cliffe(1) (1): Vanderbilt University (2): Haverford College</p> <p>Background: Preterm birth is the leading cause of death in children under five years old. Elevated levels of inflammatory cytokines in amniotic fluid have been linked to preterm birth and perinatal infections, including those caused by Group B Streptococcus (GBS) and Escherichia coli (E. coli). Human milk oligosaccharides (HMOs) have antimicrobial properties against pathogens such as GBS and E. coli. Recent studies indicate that HMOs may also modulate immune responses by inhibiting certain inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) which are known to be implicated in preterm birth.</p> <p>Methods: To investigate how HMOs may modulate PTB-relevant inflammation in THP-1 macrophages, we optimized magnetic bead electrochemical sandwich assay (MBESA) biosensors to quantify interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) following lipopolysaccharide (LPS) infection and HMO treatment. The MBESA harnessed the reduction-oxidation reaction of 3,3',5,5'-tetramethylbenzidine to generate amperometric signal, offering a faster, novel alternative to the traditional enzyme-linked immunosorbent assay (ELISA) methods for measuring cytokine levels.</p> <p>Results: The MBESA quantified inflammatory levels following LPS infection HMO treatment with great precision. This study demonstrated that HMOs exert cytokine specific and dose dependent effects on inflammatory signaling in THP-1 macrophages, shaping both baseline and LPS-induced cytokine secretion. HMOs selectively suppressed IL-1β output at low doses while enhancing TNF-α signaling.</p> <p>Conclusions: These findings position HMOs as dual immunomodulators of macrophage cytokine secretion, in addition to solidifying the MBESA as a rapid and sensitive alternative for analytical quantification. This work serves as a foundational step toward exploring HMOs as therapeutic agents against inflammation-induced PTB. Future efforts will focus on integrating MBESA biosensors for IL-1β, TNF-α, and IL-6 with a fetal membrane-on-a-chip system, facilitating a multiplexed approach to investigating inflammatory signaling and immunomodulation by HMOs in the PTB context.</p>
Ga_O5	<p>Love at First Bind: Decoding the Candida-Enterobacter Attachment Affair</p> <p>Dr. Sean Fox, Emma Carpenter Department of Biomedical Health Sciences, East Tennessee State University</p> <p>Polymicrobial interactions play a crucial role in combatting infections in today's healthcare system. The fungal pathogen, Candida albicans, and bacteria, Enterobacter, both exist normally in the human microbiota, having the chance to become opportunistic causes of co-infections. C. albicans have a unique characteristic of being a dimorphic fungus that can switch from a yeast to a hyphal form. These hyphal filaments increase the likelihood of allowing them to become invasive into host tissues, play a role in drug resistance, along with being imperative for biofilm formation and attachment. Enterobacter cloacae is a gram-negative bacterium that is part of a group of pathogens that are predominantly multidrug resistant and a leading cause of hospital-acquired infections. A study examining VA patient populations found that Enterobacter was the third most common co-infection with C. albicans. Our lab has previously discovered that E. cloacae adhere to C. albicans hyphae and inhibits Candida growth. This project aims to investigate these polymicrobial interactions and identify the genetic elements involved in Candida-Enterobacter attachment. To examine this attachment, we established the optimal binding time for C. albicans and E. cloacae to be at four hours of co-incubation. Prior work from our laboratory examined attachment between the wild type strains of Candida and Enterobacter compared to both the Candida agglutination like sequences (ALS) mutants and our own transposon library Enterobacter mutants. With these results, we were able to narrow down which mutants appeared to have contributing factors. Further attachment assays will be performed to quantify results, as well as sequencing the disrupted genes. Investigating this interaction is important due to the increase in antibiotic and antifungal resistance and their ability to coexist in biofilms, which leads to reduced treatment efficacy. Understanding their contribution to common healthcare infections could lead to a complex interpretation of diagnostic approaches and therapeutic strategies.</p>
Gb_O6	<p>Acute fasting impairs antimicrobial responses of neutrophils</p> <p>Morgan L. Hetzel, Helene Hoover, Calleigh P. Reber, Jadan Zavestoski, Dyanna Rameriz, Ariful Islam, Aliyah Collins, Bailey E. Holder, Ashley Wise-Mitchell, Tania Wong Fuk Lung, Tim E. Sparer and Andrew J. Monteith Microbiology Department, The University of Tennessee, Knoxville</p> <p>Acute fasting is common in perioperative care and may create a transient window of vulnerability to hospital-acquired infection. Because Staphylococcus aureus is a major cause of bacteremia in this setting, we asked how a short-term 12-hour fast alters neutrophil metabolism and antibacterial function. Human neutrophils isolated after acute fasting displayed reduced glycolytic and mitochondrial activity and exhibited impaired oxidative burst and neutrophil extracellular trap release in response to S. aureus. In a murine model, a single 12-hour fast markedly increased susceptibility to staphylococcal bacteremia without increasing tissue bacterial burdens. Instead, acute fasting neutrophil metabolic state in vivo and was associated with tissue-specific changes in neutrophil effector functions. Fasting also altered bacterial virulence programs, including increased expression of the staphylococcal toxin psmA in the heart. Consistent with this, infection of a strain lacking PSM improved survival in the fasted mice, identifying a potential mechanism contributing to fasting-associated mortality. Together, these data identify acute fasting as a rapidly induced and previously underappreciated state that suppresses neutrophil immunometabolism, alters host-pathogen interactions, and heightens vulnerability to severe infection.</p>
Room 413ABC	Session 4: Moderator Dr. Lindsey Burcham

<p>PD_06</p>	<p>Integration competent HIV-1 replication complexes are formed in the cytoplasm of infected cells within hours after virus entry</p> <p>Rajasree Chakraborty^{1,2}, Prem Prakash^{1,2}, Shilpak Bele^{1,2}, Faben B Zeleke^{1,2}, Jiong Shi³, Christopher Aiken³, Muthukumar Balasubramaniam^{1,2}, and Chandranu Dash^{1,2} ¹Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN. ²Department of Microbiology, Immunology, and Physiology, Nashville, TN. ³Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN.</p> <p>HIV-1 infection is dependent on reverse transcription, nuclear import and viral DNA integration. The viral RNA genome is reverse transcribed into a double-stranded DNA by the reverse transcription complex (RTC). By an undefined time and unknown mechanism, the RTC transitions into a pre-integration complex (PIC), that contains the viral DNA, the viral integrase (IN) enzyme, and other factors necessary for integration. The molecular and biochemical details of reverse transcription, nuclear entry and integration have been studied extensively. However, it is unclear whether each of these steps occurs in an independent, sequential or overlapping manner. In T cells, reverse transcription may take up to 4-16 hours to complete, yet functional cytoplasmic PICs can be extracted as early as 3 hours post-infection (hpi). Importantly, a functional PIC requires 3'-end processed viral DNA ends and there is evidence that this process is completed in the cytoplasm. Based on these observations we suggest a model that formation of functional PICs is not dependent on the completion of reverse transcription. To test this model, we are meticulously studying the kinetics of HIV-1 reverse transcription, nuclear entry and integration in parallel with the extraction and biochemical analysis of functional PICs. Our results show that cytoplasmic PIC-associated viral DNA accumulates rapidly within 3 hpi and continues through 18-24 hpi. Parallely, in vitro PIC-associated integration activity becomes detectable at 3 hpi and increases gradually thereafter. Viral DNA integration into the host chromosomes showed delayed kinetics relative to reverse transcription and peaked after the onset of measurable PIC activity. Together, these data suggest that functional PIC assembly in an infected cell occurs much earlier than the previously reported timeline (~16 hpi) for reverse transcription completion. To further clarify our model, we are conducting kinetic analysis of nuclear import and analyzing capsid protein levels in the PICs.</p>
<p>PD_07</p>	<p>Living in a Lactobacillus World: Group B Streptococcus in the Vaginal Microenvironment</p> <p>Jéssica da Conceição Mendonça¹, Natalie Heaton¹, Reese Saho¹, Zachary Burcham¹, Lindsey R. Burcham^{1,2}. ¹Department of Microbiology, University of Tennessee, Knoxville, TN; ²Department of Obstetrics and Gynecology, University of Tennessee Health Sciences Center College of Medicine, Knoxville, TN.</p> <p>Microorganisms are vital for human functions and interactions with the host and other microbes contribute to shaping dynamic and diverse communities. In the vaginal tract, the composition of the microbiota is intrinsically associated with maternal and neonatal health during pregnancy and after birth. Protective microorganisms, such as Lactobacillus species are thought to limit growth of the opportunistic pathogen Group B Streptococcus (GBS); however, we have observed co-existence of these species in murine models and clinical studies performed in collaboration with the University of Tennessee Medical Center. In this work, we aim to investigate the molecular mechanisms that impact co-existence of GBS and species of the Lactobacillus genera found in the vaginal mucosa. Our results indicate that the Lactobacillus secretome impacts GBS organization, induces GBS aggregation, and alters hemolytic activity, suggesting co-occurrence may attenuate GBS virulence. In coculture GBS viability is significantly diminished in a contact-dependent manner, though subpopulations of GBS survive and adapt to this stress. Expansion of recovered tolerant GBS cells through sequential co-culture passage enrich for an adapted population capable of growing unaffected in the presence of Lactobacillus. Whole-genome sequencing of the experimentally evolved subpopulation and GBS transposon mutagenesis screening will provide insight into the genetic basis of this tolerance and identify adaptive mutations that promote GBS survival in Lactobacillus-dominant communities. Together, these findings significantly expand our knowledge of microbial interactions within the vaginal environment.</p>
<p>PD_08</p>	<p>Active immunization with a recombinant arthropod protein impacts the transmission of rickettsial pathogen from ticks to a vertebrate host</p> <p>P.P. Mahesh, Prachi Namjoshi, Hameeda Sultana and Girish Neelakanta. Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.</p> <p>Background: Human anaplasmosis, caused by Anaplasma phagocytophilum, is a common vector-borne disease transmitted by the black-legged tick Ixodes scapularis. An anti-tick vaccine could impair arthropod blood feeding and/or transmission of single or multiple pathogens from ticks to the hosts. Our laboratory has characterized IsoATP4056, an arthropod Organic Anion Transport Polypeptide, OATP, as a potent anti-tick vaccine candidate. In our previous study, we showed that passive immunization with an affinity purified antibody targeting a short peptide in Extracellular Loop-6 (EL-6) of IsoATP4056 impairs transmission of A. phagocytophilum from ticks to mice. The same study showed decreased molting of ticks upon feeding on an EL-6 antibody-immunized mice. In addition, we noted increased cell death in tick cells upon treatment with EL-6 antibody.</p> <p>Methods: We used protein purification, qRT-PCR, immunoblot, ELISA, RNAi, TUNEL assay and immunization of mice in this study.</p> <p>Results: In the present study, we purified the entire EL-6 fragment with a GST tag (rGST-EL6) and actively immunized the mice with this purified recombinant protein. Similar to the observation noted in passive immunization study, we found impaired transmission of the bacteria from ticks to mice and reduced molting in fed ticks in this active immunization study. In addition, we noted increased apoptosis in tick cells, as measured by TUNEL assay, upon treatment with anti-rGST-EL6 serum compared to the treatment with control anti-GST serum. Furthermore, we found increased transcript levels of caspase-3 in tick cells upon RNAi-mediated silencing of isoatp4056 expression.</p> <p>Conclusions: Our study provides another evidence for IsoATP4056-based anti-tick vaccine strategy to target transmission of A. phagocytophilum from ticks to the host. Also, it shows that the anti-tick serum can cause apoptosis in tick cells.</p>

F_O2	<p>Prevalence of Phenotypic Multidrug-Resistant Enterobacteriales in Kentucky (USA) Surface Waters and Evaluation of Sentinel Antibiotics for One Health Surveillance</p> <p>Jason W. Marion, S. Travis Altheide, Eastern Kentucky University</p> <p>Antimicrobial resistance (AMR) remains a major global health threat, with increasing concern over environmental reservoirs that facilitate its persistence and spread. This study evaluated phenotypic AMR and multidrug resistance (MDR) in <i>Escherichia coli</i> and other Enterobacteriales isolated from surface waters in Central and East-Central Kentucky, and assessed whether tetracycline (TE)-supplemented media enhances recovery of MDR isolates. A total of 151 isolates were obtained from 160 water samples collected across multiple watersheds using a Most Probable Number (MPN) approach with and without TE selection. Antibiotic susceptibility testing across nine classes revealed that 19.5% of isolates were MDR, with substantially higher MDR prevalence among Enterobacter spp. (78%) compared to <i>E. coli</i> (15%). TE resistance was common (48% overall), but TE-supplemented media did not significantly increase MDR recovery relative to non-selective media.</p> <p>Water quality analysis indicated widespread fecal contamination, with most samples exceeding recreational standards; however, MDR prevalence was not significantly associated with <i>E. coli</i> density. TE-treated media enriched for resistance to several clinically relevant antibiotics, including third- and fourth-generation cephalosporins, aztreonam, and trimethoprim-sulfamethoxazole. Diagnostic analyses identified ampicillin and cefazolin non-susceptibility as strong predictors of MDR (AUC > 0.90), whereas TE non-susceptibility showed poor predictive performance under selection conditions.</p> <p>Findings highlight the utility of culture-based environmental surveillance for detecting AMR patterns and identifying sentinel indicators of MDR. While TE resistance remains widespread, its use as a selective screening tool for MDR is limited. The study also underscores the importance of accurate species identification, as misclassification of Enterobacter spp. as <i>E. coli</i> may inflate MDR estimates. Integrating environmental monitoring into One Health frameworks is critical for understanding AMR dynamics and informing mitigation strategies.</p>
F_O3	<p>Evolutionary indicators of viral cooperative behavior are significant predictors of progression to SAIDS</p> <p>Andrea S. Ramirez-Mata^{1,2}, Marco Salemi^{1,2}, Brittany Rife Magalis^{3,4}. ¹Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA ²Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, USA ³Department of Biochemistry and Molecular Genetics, University of Louisville School of Medicine, Louisville, KY, USA ⁴Center for Predictive Medicine, University of Louisville School of Medicine, Louisville, KY, USA</p> <p>Background: Growing evidence suggests viral cooperative interactions may influence adaptation and persistence. For example, hepatitis C virus (HCV) is hypothesized to exhibit antigenic cooperation (AC), a kind of altruistic behavior enabling immune escape of specific variant populations. The AC model raises the question as to whether other chronic viral infections can be better understood through a social framework? For example, cooperative behavioral dynamics within measurably evolving organisms are often disrupted by mutation, requiring compensatory mutations (i.e., co-evolution) to restore homeostasis. Because HIV is one of the most rapidly mutating infectious viruses, compensatory mutations within the host may occur with sufficient frequency to detect the presence of cooperation and inform the interactions responsible. Yet, no computational tool exists that can distinguish population-level from individual genome co-evolution to investigate the putative role of viral cooperative interactions in disease.</p> <p>Methods: We developed the Graphical mOdel of Social Interactions using Phylogenies (GOSIP), capable of capturing significantly co-evolving sites across branches of simulated viral populations (98% accuracy). We applied this tool to S[imian]IV envelope sequences sampled longitudinally from multiple tissues within two cohorts of untreated macaques – one undergoing transient CD8+ cell depletion and rapid SAIDS onset, and the other allowed to naturally progress to SAIDS. The resulting co-evolutionary data were evaluated for tissue involvement, location within targeted immune epitopes, and potential role in disease progression.</p> <p>Results: The major finding of this study was the significant inverse correlation among all animals of the time to SAIDS onset with the rate of amino acid co-evolution (R²=0.83, p<0.001).</p> <p>Conclusion: Results point to viral population-level co-evolution as a more prominent biomarker of disease progression than prior markers, such as CD4+ T cell nadir or viral load set point. Determining whether the dependencies described can be explained by AC is a critical next step for developing an effective therapeutic regime.</p>
Room 406	<p>Session 5: Moderator Dr. Brad Binder</p>
Gb_O7	<p>Variability of Spontaneous Prophage Induction is Related to Plasmid Maintenance in Model Marine Bacterium <i>Sulfitobacter pontiacus</i></p> <p>Madeline McHugh, Dr. Frank May, Cameron Jackson, Dr. Alison Buchan University of Tennessee, Knoxville</p> <p>Mobile genetic elements (MGEs), including phages and plasmids, drive bacterial evolution and fitness. In natural systems, bacteria simultaneously encounter multiple MGEs, and interactions between them can have profound influence on host cells. <i>Sulfitobacter pontiacus</i> CB2047, an active member of the Roseobacteraceae family of marine bacteria, is emerging as an environmentally relevant model for dissecting the biological significance of plasmid-phage interplay. We have previously described a role for a native, large (171 kbp) low-copy plasmid (pS_{po}CB-1) in prophage stability in CB2407, where plasmid loss results in elevated rates of spontaneous prophage induction (SPI). In the few (<5) bacterial systems where SPI has been quantified, rates are quite low (0.2-3%), making this phenomenon inherently difficult to define in most systems. Here, we describe the development of a reporter plasmid for quantification of SPI in CB2047. When the integrated prophage initiates early lytic gene expression, a red fluorescent protein (RFP) is produced. This reporter plasmid has been deployed in both our wildtype and high-inducing CB2047 strain, which experiences elevated rates of SPI due to the loss of pS_{po}CB-1. Prophage induction across bacterial growth curves was measured in both strains through the quantification of RFP-expressing cells using flow cytometry. While rates of SPI were consistently low (<5%), induction in both strains shows a similar two-step pattern across growth curves. Induction rates in the high-inducing strain were 11-fold higher than the wildtype in early lag (1.41% vs 0.13%) and 1.5-fold higher in exponential phase (4.42% vs 2.93%). Induction rates increased again for both strains in late-stationary phase, with the high-inducing strain maintaining an elevated rate relative to the wildtype strain (5.12% vs 3.04). These data suggest that SPI rates demonstrate a growth phase-dependency, and future studies aim to identify the underlying mechanism of induction in plasmid-bearing/lacking strains and assess the ecological significance of these interactions.</p>

Gb_O6	<p>Multidrug-Resistant Salmonella enterica from Cattle: Integrated Phenotypic and Genomic Characterization</p> <p>Nada A. Fahmy and Yosra A. Helmy Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, Kentucky, USA</p> <p>Introduction: Non-typhoidal Salmonella enterica is a leading cause of foodborne illness worldwide and represents a significant One Health concern due to zoonotic transmission and the increasing emergence of multidrug-resistant (MDR) strains. Cattle serve as an important reservoir, facilitating dissemination through the food chain, farm environments, and trade networks. This study combined phenotypic and genomic approaches to characterize Salmonella isolates recovered from necropsied cattle.</p> <p>Method: A total of 1008 samples were collected, and confirmed isolates, identified by MALDI-TOF MS and serotyping, were subjected to phenotypic virulence characterization and antimicrobial susceptibility profiling. Whole-genome sequencing was used to determine sequence types, virulence genes, antimicrobial resistance genes (ARGs), and mobilome components. Results: Salmonella enterica was recovered from 27 necropsied cattle, with S. Dublin identified as the predominant serotype (29.6%). All isolates demonstrated biofilm-forming ability, while 96.3% exhibited both swimming and swarming motility. Antimicrobial susceptibility testing against 19 antibiotics revealed high resistance rates, particularly to macrolides (100%) and chloramphenicol (85.2%). All isolates were MDR, and five were extensively drug resistant (XDR). Genomic analysis identified 12 sequence types, with ST10 as the dominant lineage (25.9%), and detected 223 virulence genes and 1,148 antimicrobial resistance genes, many of which are mediated by efflux systems such as AcrAB-TolC. Plasmids were present in 77.8% of isolates, with integrons (11.1%), prophages, and insertion sequences contributing to a complex mobilome. Most resistance loci were chromosomal (63.5%), followed by prophage-associated (24.8%) and plasmid-associated (11.7%) regions, with plasmid-borne genes showing stronger association with insertion sequences, consistent with transposition-mediated mobility. Pan-genome analysis revealed an open genomic architecture dominated by accessory genes (77.6%), and phylogenetic analysis demonstrated clustering of cattle and food-associated isolates, suggesting ongoing transmission.</p> <p>Conclusion: These findings highlight the role of mobile genetic elements in shaping antimicrobial resistance and supporting the need for enhanced surveillance and prudent antimicrobial use in livestock systems.</p>
Gb_O7	<p>Temperature histories of host and phage influences infection of Microcystis aeruginosa</p> <p>Kennedi M. Hambrick, David Talmy, Steven W. Wilhelm, Erik R. Zinser Department of Microbiology The University of Tennessee, Knoxville</p> <p>Background: Microcystis aeruginosa is a freshwater cyanobacterium that contributes to the formation of toxic harmful algal blooms. The global prevalence of these blooms has sparked research into factors which could initiate bloom collapse. Of these, a less well characterized factor is cyanophage. Bloom seasons in Lake Erie, USA, span from early summer (~19° C) in which there are lower cyanobacterial and cyanophage abundances to late summer (~26° C) in which abundances of both populations increase. This study examined how temperature history of both the host M. aeruginosa strain (NIES-298) and a cyanophage that infects it (Ma-LMM01) influences infection outcomes.</p> <p>Methods: Batch cultured, axenic M. aeruginosa NIES-298 was pre-acclimated to both 26° C and 19° C without phage exposure. Once stationary phase was reached, samples were taken and challenged with cyanophage propagated initially at either 26° C or 19° C. For lysate propagation, batch cultures of mid-log phase M. aeruginosa NIES-298 pre-acclimated to both 26° C and 19° C were inoculated with a previous stock of Ma-LMM01 lysate (propagated from 26° C Microcystis). The resulting lysates were filter sterilized with a 0.22-µm syringe filter with a PVDF membrane. Host cell abundance and total viral particles were measured throughout infection by flow cytometry.</p> <p>Results: M. aeruginosa pre-acclimated to 26° C was susceptible to both the 26° C and 19° C propagated phage. In contrast, M. aeruginosa pre-acclimated to 19° C was susceptible to lytic infection by 26° C propagated phage but not to lytic infection by 19° C propagated phage.</p> <p>Conclusion: Overall, our results indicate that the temperature history of both M. aeruginosa NIES 298 and its lytic cyanophage shapes infection dynamics. Ongoing work aims to elucidate the resistance mechanism observed under the 19° C pre acclimation and 19° C phage propagation conditions through metabolomics and DNA sequencing of both host and phage.</p>
Gb_O8	<p>Microplastic type determines soil microbial restructuring and nutrient cycling dynamics in agroecosystems</p> <p>Toyosi N. Ajide-Bamigboye, Lois S. Taylor, Avisshesh Neupane, Sindhu Jagadamma, Anton Astner, Doug Hayes, Amanda L. May, Jennifer M. DeBruyn University of Tennessee Institute of Agriculture, Knoxville</p> <p>Microplastic accumulation in agricultural soils is an emerging environmental concern, yet the divergent ecological consequences of conventional versus biodegradable polymers remain poorly resolved. We conducted a 24-week greenhouse study using field-realistic fragments of low-density polyethylene (LDPE) and poly (butylene adipate-co-terephthalate) (PBAT) to evaluate their impacts on soil biogeochemistry, enzyme activity, metabolomics, and microbial community dynamics.</p> <p>Polymer-specific effects were pronounced and driven by distinct mechanisms. LDPE primarily induced physicochemical modifications, characterized by sustained ammonium accumulation (up to 4-fold higher than controls) and prolonged Beta-glucosidase activity, suggesting enhanced nutrient retention and structural stabilization of soil organic carbon. In contrast, PBAT functioned as a transient biochemical substrate, stimulating an immediate "metabolic pulse" with elevated CO₂ efflux (144% above control) and microbial biomass carbon enrichment (~99%). This was followed by metabolic exhaustion and severe nitrate suppression (up to 98% reduction), indicating intense nitrogen immobilization.</p> <p>While Alpha-diversity remained stable, Beta-diversity analyses revealed significant, polymer-dependent restructuring. PBAT rapidly and persistently shifted fungal and bacterial compositions within two weeks, enriching pioneer degraders including Purpureocillium, Streptomyces, and Pseudonocardia. Untargeted metabolomics confirmed PBAT depolymerization through the quantifiable accumulation of terephthalic acid and shifts in glucosamine and purine metabolism. In contrast, LDPE-associated shifts were weaker and transient, with communities converging toward controls over time. Collectively, these findings demonstrate that polymer chemistry, not biodegradability alone, governs soil ecological outcomes. While PBAT reduces physical persistence, it destabilizes nitrogen cycling and microbial metabolism; conversely, LDPE promotes nutrient retention but contributes to long-term physical contamination.</p>
Ga_O6	<p>Colorful Competition: The co-culture dynamics and violacein modulation of Raoultella planticola with Chromobacterium violaceum</p> <p>Katelyn Miller, Dr. Sean J. Fox Department of Biomedical Health Sciences, East Tennessee State University</p> <p>Raoultella planticola, formerly named Klebsiella planticola, shares a near-identical genotype and phenotype with the infamous multi-drug resistant pathogen Klebsiella pneumoniae. R. planticola has been increasingly misdiagnosed as K. pneumoniae infections in recent years, yet its pathogenicity and antibiotic resistance profile remain largely unexplored. C. violaceum produces the bis-indole secondary metabolite called violacein. Violacein is a compound with antimicrobial and signaling properties, which has potential in antibiotic treatments. This project aims to characterize the interactions between C. violaceum and R. planticola which could affect the survival of R. planticola or modulate violacein production. Current data from growth curves, biofilms, Kirby Bauer, CFUs, violacein extractions and MIC assays, show an increase in both growth and violacein production under conditions involving varying cephalosporin antibiotic concentrations, particularly cefotaxime, in co-cultures, suggesting potential metabolic or quorum-sensing interactions. Research in-progress will explore in-vivo effects with C. elegans models, violacein spot-test assays against common pathogens, CFUs from cultures containing co-cultures with antibiotic concentrations of interest, and plasmid screening to explore potential horizontal gene transfer and resistance exchange. By exploring these interactions, this research aims to better understand how emerging Raoultella species interact with other microbes, as well as the conditions producing violacein and its potential implications in polymicrobial systems, and how such systems may influence the persistence and evolution of antibiotic resistance.</p>

Gb_09	<p>Cross-kingdom ethylene signaling through bacterial receptors regulates biofilm formation, root colonization, and metabolic wiring</p> <p>Mahbuba Ferdous, Scott Carlew, Gladys Alexandre, Brad M. Binder. University of Tennessee-Knoxville</p> <p>Ethylene is a phytohormone involved in plant growth, development, and responses to biotic and abiotic stresses. The roles of ethylene have mostly been explored in plants. However, putative ethylene receptors are found in many species of bacteria. We show that the rhizospheric, α-proteobacterium <i>Azospirillum brasilense</i> contains a functional ethylene receptor that we call <i>Azospirillum</i> Ethylene Response1 (AzoEtr1) after the nomenclature used in plants. <i>A. brasilense</i> cells have saturable ethylene-binding sites and heterologous expression of AzoEtr1 shows that it directly binds ethylene. Treating cells with very low levels of ethylene reduces biofilm formation and colonization of plant root surfaces. Biochemically, application of ethylene causes widespread metabolic changes to enhance carbon metabolism and reduce nitrogen metabolism in the bacteria. One result of this is the accumulation of the carbon storage molecule poly-hydroxybutyrate. Our data supports a model in which ethylene in the environment, perhaps from host plants, alters the density of colonization by <i>A. brasilense</i> and re-wires its metabolism.</p>
Room 413ABC	<p>Session 6: Moderator Dr. Heidi Goodrich-Blair</p>
KN2	<p>Dr. Shaun Brinsmade: Lean on me (for lipids): Metabolic synergy between <i>Staphylococcus aureus</i> and <i>Enterococcus faecalis</i> during polymicrobial infection</p>
F_O4	<p>Identification of putative binding sites in tail fibres of tailocins from animal-associated <i>Xenorhabdus</i> bacteria</p> <p>Ryan M. Awori, Emmanuel C. Allwell, Heidi Goodrich-Blair Microbiology Department, University of Tennessee, Knoxville</p> <p>Background: <i>Xenorhabdus</i> bacteria utilize virus-like nanoparticles called tailocins to kill kin that threaten mutualistic relationships with their natural nematode hosts. The specificity, efficacy and diversity of these tailocins, makes them ideal for modification into the precision therapies needed to mitigate today's global antimicrobial resistance crisis. Yet, molecular insights into how <i>Xenorhabdus</i> tailocins specify receptors on target cell surfaces are scarce. Therefore, we determined putative binding sites in tail fibres of tailocins from three strains of <i>Xenorhabdus</i>.</p> <p>Methods: Six tailocins were categorised into those did or did not strongly inhibit <i>X. nematophila</i> ATTC19061. From their respective tailocin-encoding loci, all tail fibre genes were identified, translated, and protein sequences fragmented into 5-7mer amino acid motifs. Presence/absence of a motif was matched with strong inhibition/no inhibition of <i>X. nematophila</i> ATTC19061. Motifs that were associated with killing of ATTC19061 were identified in other strains, whose tailocins were then tested for inhibition of ATTC19061. AlphaFold-predicted model structures of <i>Xenorhabdus</i> tail fibres were compared to those of determined structures of tailocin tail fibres.</p> <p>Results: The protein sequence GESYTKSES was associated with killing of ATTC19061. This motif was also in tailocin tail fibres of <i>Xenorhabdus</i> sp. TH1, and upon testing, its tailocin-containing lysates killed ATTC19061. GDSYTKSES, and GESYTKLES were encoded by <i>X. bovienii</i> ML and <i>X. poinarii</i> G6 tailocin tail fibre genes, respectively, but their tailocin-containing lysates did not inhibit ATTC19061. In model structures, the GESYTKSES motif was in the Knob2 subdomain that has been associated with receptor binding.</p> <p>Conclusions: The GESYTKSES motif is a strong predictor of tailocin killing of <i>X. nematophila</i> ATTC19061 and its polar amino acids, glutamate and serine are key to this activity. The motif is likely located in the Knob2 subdomain. Taken together, this motif is a probable binding site and therefore key to the tailored transformation of tailocins into targeted therapies.</p>
F_O5	<p>Role of L,D-transpeptidases in the survival of <i>Legionella pneumophila</i></p> <p>Dipak Kathayat^{1, 2}, Yujia Huang², Joee Denis², Benjamin Rudoy², Hana Schwarz² ¹Lincoln Memorial University College of Veterinary Medicine, Harrogate, TN. ²Cornell University College of Veterinary Medicine, Ithaca, NY.</p> <p>Background Legionnaires' disease (LD), caused by the opportunistic intracellular bacterium <i>Legionella pneumophila</i>, is the leading cause of reported waterborne disease outbreaks in the United States and a major cause of severe pneumonia in humans. Treatment failure and mortality remain high in patients with LD despite receiving currently recommended antibiotic therapy, underscoring the need for more effective treatment strategies. Because of its biphasic life cycle, <i>L. pneumophila</i> has the ability to survive for prolonged periods within human lung macrophages and in environmental reservoirs such as human-made water systems. However, the molecular mechanisms underlying this survival remain incompletely understood.</p> <p>Methods Using glycoproteomics, we investigated cell envelope remodeling in <i>L. pneumophila</i>, particularly in the peptidoglycan (PG) layer, during its transition to a survival phase.</p> <p>Results and Conclusions We found significant enrichment of PG structures catalyzed by L,D-transpeptidases (LDTs), enzymes that mediate 3-3 cross-linking between PG peptide stems, when <i>L. pneumophila</i> survives in tap water, a primary habitat of this bacterium. Five LDTs (lpg1386, lpg2641, lpg1582, lpg0910, and lpg1336) were upregulated, and deletion of the LDT lpg1386 significantly reduced <i>L. pneumophila</i> survival. Under the same condition, we discovered a unique outer membrane (OM) long-chain fatty acid transporter (Lpg1810, FadL) tethered to the PG layer, likely mediated by LDT(s), suggesting a previously unrecognized role for this unique FadL in <i>L. pneumophila</i> survival. Collectively, these findings demonstrate that LDT-mediated cell envelope remodeling is a major determinant of <i>L. pneumophila</i> survival. This work has the potential to inform the rational design of new therapeutic regimens to treat LD that is refractory to current antibiotic therapy and to uncover molecular targets to disrupt <i>L. pneumophila</i> transmission from contaminated water systems to humans.</p>
F_O6	<p>Dietary lipids tune staphylococcal Geh-dependent control of NETosis</p> <p>Ashley Wise^{*1}, Eden TenBarge^{*1}, Ivan Acosta², William Beavers³, Francis Alonzo², Andrew Monteith¹ ¹ University of Tennessee-Knoxville ² University of Illinois-Chicago ³ Louisiana State University</p> <p><i>Staphylococcus aureus</i> is a major cause of invasive infection, but how host metabolic conditions shape bacterial immune evasion remains incompletely understood. Neutrophil extracellular traps (NETs) are an important part of host defense against <i>S. aureus</i>, yet they can also contribute to inflammatory tissue damage. In this work, we show that the secreted staphylococcal lipase Geh functions as a regulator of suicidal NETosis by reshaping the lipid signals encountered by neutrophils. Rather than acting as a fixed virulence factor, Geh-dependent immunomodulation is strongly influenced by the surrounding lipid environment. We find that saturated versus unsaturated lipid availability alters the ability of <i>S. aureus</i> to suppress NET formation, linking bacterial fatty acid metabolism to host innate immune signaling. These effects extend beyond in vitro systems, as dietary lipid context changes the extent to which Geh controls NET responses and tissue infection outcomes during systemic infection. Overall, our findings support a model in which host lipid composition is not simply a background feature of infection, but an active determinant of how <i>S. aureus</i> engages neutrophils and shapes NET-driven inflammation. This work highlights a broader principle that bacterial metabolism and host nutritional state intersect to control immune effector function during infection.</p>

Poster presentations (in alphabetical order by presenter)

UP1	<p>Hepatozoon spp. detected in foxes (<i>Vulpes vulpes</i> and <i>Urocyon cinereoargenteus</i>) and coyotes (<i>Canis latrans</i>) in Tennessee and Virginia</p> <p>Rebecca Adams, Emily Brown, Monica Mercurio, Lauren Hatfield, Bonnie Brenseke, and Kathryn Purple Lincoln Memorial University, Harrogate, TN and Campbell University School of Osteopathic Medicine, Lillington, NC</p> <p>Hepatozoon spp. are tick-borne protozoan parasites that are potentially fatal to domestic dogs and also infect a wide-range of wild canid hosts including coyotes and, less frequently, foxes. Reports including both histopathology and molecular confirmation of these parasites in wildlife in the U.S. remain limited. We performed necropsies on road-killed or hunter-harvested wildlife collected in Virginia and Tennessee, including 3 red foxes (<i>Vulpes vulpes</i>), 3 gray foxes (<i>Urocyon cinereoargenteus</i>), and 26 coyotes (<i>Canis latrans</i>). Samples of heart and spleen were frozen prior to DNA extraction and PCR targeting the 18S rRNA gene of <i>Hepatozoon</i> spp. Tissues including heart and spleen were preserved in 10% buffered-neutral formalin for histopathologic examination. One red fox had both histopathology of the spleen with <i>Hepatozoon</i> spp. meronts with a wheel-spoke arrangement and a PCR-positive heart and spleen. All remaining samples had no significant findings on histopathology, but 100% red foxes (2/2), 66% gray foxes (2/3), and 19.2% coyotes (5/26) were PCR-positive in either heart, spleen, or both tissues. We have found PCR-positive tissue in an additional gray fox, red fox, and coyote and histopathology is pending. We plan on sequencing all PCR-positive samples for molecular confirmation and phylogenetic analysis. Our results represent only the second documentation of <i>Hepatozoon</i> spp. in a gray fox and the second red fox with both histopathological and molecular identification. These data will contribute to our understanding of the distribution and transmission of <i>Hepatozoon</i> spp. in southeastern U.S. wildlife.</p>
UP2	<p>Distribution of Inteins across Archaeal and Bacterial Genomes</p> <p>Tia M. Ariagno, Jonathan D. Winkelman, Christopher W. Lennon Murray State University</p> <p>Background Intervening proteins, or inteins, are mobile genetic elements characterized by their ability to rearrange peptide bonds in a post-translational, self-splicing reaction. A prior investigation found that the distribution of inteins across archaea and bacteria was biased, with archaea being more saturated with inteins than bacteria.</p> <p>Methods Using the prior study as a foundation, our expansion and subsequent analysis reinforce the previous findings and provide a closer examination of mini-intein distribution across the two domains.</p> <p>Results & Conclusions The analysis of intein invasion among protein functional groups finds similar results to those of the previous study as well, as inteins are primarily found in DNA replication, recombination, and repair (RRR) proteins. Exploring the distribution of mini-inteins across archaea and bacteria shows that bacterial inteins are more likely to have lost their HEN domain than archaeal inteins, offering a potential explanation regarding the lack of inteins throughout bacteria relative to archaea. Following this with an examination of mini-intein abundance within protein functional groups finds that certain classes of bacterial intein-containing proteins mostly contain mini-inteins instead of full-length inteins, indicating a possible link between diminished function of the proteins within the categories and the presence of a HEN domain that, in turn, facilitates a high amount of HEN loss once inteins have invaded these bacterial proteins.</p>
UP3	<p>Persisting in the Saltiest Pond on Earth - Strategies for Extremophile Survival</p> <p>William Armstrong University of Tennessee, Knoxville</p> <p>Polyextremophile microorganisms are adapted to multiple environmental stressors, and utilize a variety of adaptive strategies to survive. The McMurdo Dry Valleys in Antarctica, which hosts some of the most extreme conditions on Earth, is the site of Don Juan Pond (DJP), a hypersaline brine. DJP is among the world's most saline natural bodies of water, with ~40% salt by mass, ~90% of which being CaCl₂. The high salt concentration and Antarctic temperatures as low as -50 °C pose a distinct challenge to the persistence of life. Here we characterize the genotype and phenotype of a bacterium (strain <i>Aquibacillus</i> sp. DJP33) isolated from DJP sediments. The associated genome was annotated to identify known cold-adapted genes. AntiSmash 8.0 was used to determine secondary metabolite biosynthesis potential. Cells of DJP33 were grown in marine broth at 4°C and 19°C for motility assays in 0.4% marine agar. Endospores were observed using the Schaeffer-Fulton staining method. DJP33 is revealed to be a potentially novel, gram positive, motile, rod-shaped, endospore-forming bacterium of the phylum Firmicutes. Comparative genomic analysis using BLAST and TYGS, tools used to compare genomic similarity, indicates strain DJP33 has the highest 16S rRNA gene (96.90%) sequence identity to a <i>Aquibacillus halophilus</i> strain isolated from a hypersaline lake in Iran, with a dDDH of 21.5% indicating DJP33 is potentially a novel species. Biosynthetic gene clusters associated with secondary metabolite production of ectoine and terpene were present, as well as post-translationally modified peptides most similar to the lassopeptide paenodiol. Motility was exhibited in both incubation temperatures and flagellar propulsion was observed microscopically. The combination of these adaptations and yet uncovered strategies allow DJP33 to manage multiple stressors. How life survives in environments with multiple extremes provides valuable insight into the limitations of life on Earth and beyond.</p>
UP4	<p>Creating genetic tools in <i>Finnegoldia magna</i></p> <p>Seth A. Brewer, Shannon R. West, Aanuoluwa E. Adekoya, and Carolyn B. Ibberson Department of Microbiology, University of Tennessee, Knoxville, USA</p> <p>Chronic wounds (CWs) are persistent, treatment-resistant infections that affect millions of people annually and impose substantial healthcare and economic burdens. These wounds are typically polymicrobial environments where a multitude of microbial species are present, which contribute to delayed healing and prolonged treatment. Polymicrobial interactions within CWs are largely understudied, especially amongst pathogens and commensals. A highly prevalent CW commensal and opportunistic pathogen is <i>Finnegoldia magna</i>, a Gram-positive, anaerobic coccus with an undefined role within the CW microenvironment. However, studying interactions between <i>F. magna</i> and co-infecting microbes within CWs has been limited by a lack of reliable genetic tools. Although <i>F. magna</i> naturally contains a large, low-copy extrachromosomal plasmid pFMC and has been shown to maintain foreign plasmids long enough to undergo homologous recombination, no studies have been published showing the maintenance of an introduced, foreign plasmid. Therefore, we are attempting to introduce and maintain plasmids in <i>F. magna</i> utilizing the POSSUM Toolkit. We hypothesize that this modular plasmid system with a viable origin of replication and selectable marker, paired with an effective delivery method, could make <i>F. magna</i> genetically tractable. To test this idea, we began by establishing antibiotic selection conditions and then utilized POSSUM plasmid assemblies. We are exploring two routes for introducing these plasmids: natural transformation and electroporation. While we are still in the process of establishing this system, plasmid-based genetic tools for <i>F. magna</i> will enable direct functional tests of its microbial interactions in the CW microenvironment and provide a broader framework for investigating non-model microbes in chronic infections.</p>

UP5	<p>Analysis of off-target mutations in a CRISPR/Cas9 cytosine base edited phytopathogen <i>Pectobacterium versatile</i></p> <p>Elli Chumley, Jeanne Seibel, Dan Chang, Joe Deweese and Caleb Kersey Freed-Hardeman University</p> <p>Genetic engineering by CRISPR/Cas systems have become valuable tools in investigating genetic mechanisms responsible for phenotypic features. While traditional CRISPR/Cas editing relies on double stranded breaks by Cas9 nuclease, Cas platforms have been recently utilized with nickase and deactivated variants of Cas proteins enabling single base editing. We used a CRISPR/Cas 9 cytosine base editing (CBE) construct that induces cytosine to thymine transitions to create a premature stop codon in the quorum sensing gene <i>hslI</i> of the phytopathogen <i>Pectobacterium versatile</i>. While the target nucleotide was edited as desired, it was of interest to evaluate the remainder of the genome for potential off-site nucleotide changes, as CRISPR/Cas systems have been associated with varying degrees of editing efficiencies. Whole genome sequencing on two different CBE <i>hslI</i> mutants and wildtype <i>P. versatile</i> were performed using Oxford Nanopore Technologies. Geneious Prime software was subsequently used to provide assessments of nucleotide variation. There were 315 and 227 off-site mutations in the two <i>hslI</i> mutants respectively, with all edits reflecting cytosine to thymine changes. When comparing the off-site mutations between the two mutants, there were 13 loci that were mutated in identical places, while all the other off-site mutations were unique. Of those 13 matching off-site mutations, two were a few base pairs away from the target nucleotide in the <i>hslI</i> gene, while five others were found in genes coding proteins and six in non-coding regions. The five protein coding genes included the gene <i>corC</i>, <i>secD</i>, <i>TolC_2</i>, <i>metQ_2</i> and one gene encoding a protein with unknown function. To inquire if these off-site mutations had any sequence similarity with the <i>hslI</i> target sequence, a 30-base pair sequence starting at the base mutated in each of these genes was aligned with the target <i>hslI</i> gene sequence. All five genes shared identical DNA sequence matching to the GAAAG...TATTCT sequence found with the target <i>hslI</i> gene, as well as a probable PAM sequence downstream of the mutation site. The sequences of the six non-coding loci mutated in both <i>hslI</i> CBE mutants did not share matching sequence to the target gene. To analyze genes that showed offsite mutations that were not shared between the two <i>hslI</i> CBE mutants, four uniquely mutated genes in the mutant with 315 off-site mutations were compared with the target sequence of <i>hslI</i>. These showed no similarity to the target sequence. These data suggest that the use of CRISPR/Cas CBE requires increased optimization and the development of more stringent efficiency if it is to be a viable option as a genetic engineering tool in the study of <i>P. versatile</i>.</p>
UP6	<p>Chryseobacterium and Stenotrophomonas as models to understand pathogen induced dysbiosis of the microbiome.</p> <p>Crick, JE*; Dallas, JW; Walker, DM, Department of Biology, Middle Tennessee University</p> <p>Background: Snake fungal disease (SFD) is an emerging infectious disease caused by <i>Ophidiomyces ophidiicola</i> that affects snake species and causes dysbiosis (disruption) of the skin microbiome. Previous studies have identified certain bacterial genera, including <i>Chryseobacterium</i> and <i>Stenotrophomonas</i>, as important members of the skin microbiome and predictors of disease state. By exploring model taxa in the skin microbiome, this study advances fundamental knowledge on the ecology and evolution of the skin microbiome in response to fungal pathogen induced dysbiosis.</p> <p>Methods: I isolated <i>Chryseobacterium</i>, <i>Stenotrophomonas</i>, and other bacterial genera from samples taken from disease positive and negative wild <i>Crotalus horridus</i>, and enriched communities from disease negative <i>Nerodia erythrogaster</i>. Samples were grown on and isolated from 1% keratin or R2A plates before performing DNA extraction and Sanger sequencing to determine identity.</p> <p>Results: Among all sample types, 19 distinct genera were identified, including <i>Stenotrophomonas</i>, <i>Providencia</i>, <i>Pseudomonas</i>, <i>Serratia</i>, <i>Achromobacter</i>, <i>Shinella</i>, <i>Bruceella</i>, <i>Cedecea</i>, <i>Sphingobacterium</i>, <i>Microbacterium</i>, <i>Chryseobacterium</i>, <i>Massilia</i>, <i>Acinetobacter</i>, <i>Paenarthrobacter</i>, <i>Agrobacterium</i>, <i>Pantoea</i>, <i>Exiguobacterium</i>, <i>Paracoccus</i>, and <i>Epilithonimonas</i>. <i>Chryseobacterium</i> and <i>Stenotrophomonas</i> were present across disease states, and diversity differed among samples.</p> <p>Conclusions: The results of this project indicate that there are differences in microbial taxa that are culturable between host species (<i>C. horridus</i> and <i>N. erythrogaster</i>), sample type (wild vs enriched), and disease state (<i>O. ophidiicola</i> positive vs negative). Results suggest a potential change in culturable microbes in response to <i>O. ophidiicola</i> and may help to inform which bacterial genera may be important as an extension of the innate immune system in resisting SFD. Additionally, the data generated during this project will enable future studies comparing the genomes of important members of the snake skin microbiome, increasing knowledge of the ecology and evolution of the microbiome in response to <i>O. ophidiicola</i>.</p>
UP7	<p>Effect of Anti-HIV drug Dolutegravir on DHFR (Dihydrofolate reductase)</p> <p>Debasmita Dikshit 1 Center for AIDS Health Disparities Research 2 Department of Microbiology, Immunology, & Physiology, School of Medicine, Meharry Medical College, Nashville, TN.</p> <p>Background: Children born to mothers with or at risk of human immunodeficiency virus type-1 (HIV-1) infection are decreasing globally due to affordable access to antiretroviral therapy (ART). DTG is one of the most prescribed antiretroviral drugs for treating people with HIV infection, including women of child-bearing potential or pregnant. Dolutegravir (DTG) may induce potential risk of neural tube defects (NTDs) associated with periconceptional usage of dolutegravir (DTG) in Botswana, first recorded during 2018. Early reports suggested that, probably in relation to folic acid (FA) shortage, DTG may induce neural tube defects in infants born to women taking the drug during pregnancy. Dihydrofolate reductase (DHFR) is a key enzyme to regulate folate metabolism, however folate/DHFR activity in neuronal development has not been fully understood.</p> <p>Methods : We used HEPG2 (human hepatocellular carcinoma-derived line) cells in this study. HEPG2 cells serve as a valuable model for studying liver-specific metabolic functions and are widely used to investigate how drugs impact metabolic pathways, including DHFR-mediated metabolism. Cells exposed to clinically relevant concentrations of dolutegravir (DTG) and selected ART combinations recommended for use during pregnancy. Control groups included untreated cells and DMSO vehicle control. HEPG2 cells were treated with Dolutegravir for 24, 72 and 120 hours. DHFR Protein and gene expression were analyzed by western blot and qPCR respectively. DHFR activity was measured concurrently.</p> <p>Results : Our results show a significant reduction in DHFR protein expression in both 72 and 120 hours of DTG treatment and reduction in mRNA expression in higher concentration of DTG. Interestingly, such increase was not observed with RAL treatment. Additionally, biochemical activity measurements indicate a concurrent reduction in DHFR enzyme activity in the lysates of DTG-treated cells.</p> <p>Conclusions: Findings show DTG may inhibit DHFR function. Currently, we are investigating DTG's effect on DHFR using a mouse model.</p>
UP8	<p>From Soil to Antibiotic Resistance: Investigating Interactions between <i>Sporosarcina</i> BK-6 and <i>Bacillus Subtilis</i></p> <p>Haley Farinato, Dr. Sean Fox East Tennessee State University</p> <p>Antibiotic resistance is becoming an increasing global health threat as many bacterial pathogens are becoming resistant to commonly used drugs. Antibiotic resistance takes place when bacterium develop mechanisms that prevent antibiotics from killing or inhibiting their growth, which make infections harder to treat. This poses as a threat to modern antibiotics and shows the importance of studying bacterium from all different environments. Exploring biodiverse environments, like the Appalachian Mountains, can lead to the discovery of new bacteria and novel antimicrobials that are capable of being used for advancements in medicine. The interactions between BK-6, a gram-positive bacterium, and <i>Bacillus subtilis</i>, a common gram-positive bacteria found in soil, are examined in this study. The bacterium BK-6 was officially identified as <i>Sporosarcina</i>, which produces endospores allowing it to survive in harsh conditions. A Kirby-Bauer test was performed which showed that the bacterium was resistant to Kanamycin (K-30), an antibiotic used to treat short-term bacterial infections. Additionally, planktonic co-culture and biofilm growth was performed to evaluate the inhibitory effect of BK-6 on <i>Bacillus</i> species. The results from these experiments may help to identify how the two bacteria interact with each other. Understanding the interactions can reveal potential novel targets or treatments for <i>Bacillus</i> infections.</p>

UP9	<p>The Crosstalk between Ethylene Signaling and Auxin Synthesis in <i>Azospirillum brasilense</i></p> <p>Josephine Galehouse, Ishita Banerjee, Gladys Alexandre University of Tennessee, Knoxville</p> <p>Background: The rhizosphere, which is the soil surrounding plant roots, is influenced by root exudates and microorganisms. Conditions in the rhizosphere affect microbial activity. Phytohormones and microbially produced hormones, which can alter plant physiology, further modulate rhizosphere interactions. The rhizosphere is thus a competitive environment in which microbes and plant roots compete for resources. Bacteria have evolved strategies to adapt to the competitive environment of the rhizosphere, including through sensing of phytohormones and nitrogen fixation. Notably, it synthesizes a well-studied plant hormone, auxin, through a tryptophan-dependent pathway with the help of pyruvate decarboxylase (ipdC gene encoded) enzyme. Previous studies show that <i>Azospirillum brasilense</i> has a functional ethylene receptor, named <i>Azospirillum Ethylene Response 1</i> (AzoEtr1), which binds ethylene with high affinity. Ethylene is a plant hormone that regulates plant growth, development, and stress responses. While its role in plant-microbe interactions has been studied mainly from the plant's perspective, little is known about its function in microbes. The overarching goal of this research is to identify the role of ethylene-auxin signaling crosstalk in <i>A. brasilense</i> to make cellular decisions to adapt to the changing environment.</p> <p>Methods: Using transcriptional reporter assays, we characterized the promoter activity of the ipdC gene, which is important for auxin production, in the absence and in the presence of ethylene. Additionally, we have measured auxin accumulation in ethylene-treated cells using GC-MS with ESI.</p> <p>Result: We have observed that ethylene exposure significantly increased the promoter activity of ipdC under nitrogen-fixing conditions. In addition, we found auxin in ethylene-treated cells, especially in ΔipdC cells.</p> <p>Conclusion: Our data suggest a model in which ethylene exposure induces the cells to make more auxin in the ipdC mutant through an unknown pathway.</p>
UP10	<p>Investigating the Prevalence of Environmental Antibiotic Resistance within the Nolichucky Watershed</p> <p>Caleigh Greene, Brynna K. Johnson, & Shannon D. Elliott Tusculum University</p> <p>Antibiotic resistance (AR) is a critical issue with far-reaching implications for global health, which is attributed to complex interactions between humans, animals, and the environment. Natural water systems and riparian zones can act as reservoirs for AR pathogenic species, facilitating transmission between both human and animal hosts. Antibiotics act as selective pressure within these environments, promoting transfer of antibiotic resistance genes (ARGs) among bacterial species. This study investigates the prevalence of environmental antibiotic resistance within natural waterways and riparian soils of the Nolichucky Watershed of Greene County, TN using media-based and molecular methods. Water samples were collected from 11 sites, serially diluted, and coliforms isolated on MacConkey tetracycline (MAC tet) agars at concentrations of 3 ug/mL and 30 ug/mL to determine the average CFU/mL and total average % resistance. We observed the total average % resistance for tet 3 ug/mL between all sites was 2.55%, with individual sites ranging from 0% to 7.6%. At tet 30 ug/uL, total average % resistance of all sites was 0.85%, with individual sites ranging from 0%-3.13%. To identify possible attributing ARGs, environmental DNA (eDNA) from riparian soil samples was screened via PCR for tetracycline-resistance genes (tetM, tetB, tetA), which was then expanded to include beta-lactam ARGs (bla-TEM, bla-SHV, ampR). We detected the presence of tetM at 3 of 11 locations, with bla-TEM at 1 location, but conclusive detection of environmental ARGs remains difficult due to insufficient eDNA yields by current extraction methods. However, taken together, the results of this small study suggest the prevalence of ARGs, such as those attributed to tetracycline resistance, are potentially widespread within these waterways and riparian zones, highlighting the need for greater surveillance and interventions to prevent further spread of ARGs between potentially pathogenic bacteria.</p>
UP11	<p>The Role of <i>Lactobacillus</i> spp. in Modulating Group B <i>Streptococcus</i> Colonization</p> <p>Natalie Heaton, Jéssica da Conceição Mendonça, Lindsey Burcham University of Tennessee, Knoxville</p> <p>Interactions between microbes in host-associated communities play a significant role in maintaining human health, but the mechanisms by which microbes survive and colonize diverse niches are yet to be defined. In the vaginal tract, commensal <i>Lactobacillus</i> species are dominant and typically prevent pathogen colonization; however, they fail to completely inhibit colonization of opportunistic pathogens such as Group B <i>Streptococcus</i> (GBS). In this study, we aim to understand the impact of <i>Lactobacillus</i> spp. on GBS growth and virulence. Confocal microscopy imaging revealed structural changes in GBS chain formation when grown with <i>Lactobacillus</i> spp. in a spatially-separated co-culture system. To investigate their coexistence <i>in vitro</i>, we used a dual species biofilm model cultured in 5% CO₂ or anaerobically. We observed that GBS growth was reduced in aerobic co-cultures, but GBS growth was completely inhibited in anoxic co-cultures, suggesting that oxygen and carbon dioxide may play a role in this interaction. Due to limited genetic tools available in vaginal <i>lactobacilli</i> species, we are optimizing electroporation protocols in <i>Lactobacillus gasseri</i>, towards a long-term goal of generating a transposon insertion mutant library. Altogether, these findings broaden our limited understanding of how <i>Lactobacillus</i> and GBS interact within the vaginal tract.</p>
UP12	<p>From Soil to Solution: Antibiotic Potential of Soil Bacteria from Appalachian Mountains</p> <p>Abigail Johnston & Dr. Sean Fox Department of Biomedical Health Sciences at East Tennessee State University</p> <p>Antibiotic resistance is a growing global health concern characterized by the ability of bacteria to modify themselves to withstand the effects of drugs designed to eradicate them. According to a study published by the World Health Organization (WHO) in October of 2025, antibiotic resistance is expected to increase between 5%-15% annually, which poses a serious threat to the number of successful therapeutic treatments that are available to patients. This growing concern highlights the need to advocate for responsible antibiotic use and continued research to discover alternative therapies. To achieve this, a soil sample was collected in the Appalachian Mountains, and any existing microbes were isolated for identification. A gram stain of the chosen unknown bacteria was performed, showcasing a gram-positive coccobacillus morphology with endospores, suggestive of a <i>Bacillus</i> species. The genome of the selected bacteria was then entered into the National Library of Medicine's (NIH) database and matched with high similarity to a <i>Brevibacillus</i> species. A spot test was then performed to identify any zones of inhibition formed from <i>Brevibacillus</i> on the competing microbe, <i>Bacillus subtilis</i>. <i>Bacillus subtilis</i> is commonly found in the gastrointestinal tract of humans, usually acting as a beneficial probiotic, but it can also cause bloodstream infections and meningitis in immunosuppressed individuals. Planktonic co-cultures were then created to determine colony forming units for inhibition. To distinguish between the likely <i>Bacillus</i> species soil microbe and <i>Bacillus subtilis</i> in the co-cultures, a starch hydrolysis test was performed. <i>Bacillus subtilis</i> will hydrolyze starch due to its ability to produce the α-amylase enzyme necessary for breaking down starch into smaller, soluble molecules, while other <i>Bacillus</i> species do not possess this enzyme. Biofilm analyses were then performed to determine the microbes' ability to resist removal from surfaces, a characteristic crucial for testing antimicrobials. Collectively, these findings provide insight into microbial interactions and may contribute to the identification of novel antimicrobial compounds to combat antibiotic-resistant infections.</p>

UP13	<p>Harnessing Appalachian Tannins: Antimicrobial Impacts on <i>Candida albicans</i></p> <p>Kelsey Lawson, Tasha Phillips, Alex Hopke, Allison Scherer University of Virginia's College at Wise</p> <p>Background: <i>Candida albicans</i> is an opportunistic fungal pathogen responsible for mucosal and systemic infections, particularly in individuals with compromised immunity. Rising multidrug resistance underscores the urgent need for novel anti-fungal approaches. Recent studies show that individuals with type II diabetes are more susceptible to <i>Candida</i> infections, with a reported prevalence of 11.4% among diabetic patients. This research investigates the potential effects of tannins from <i>Rhus typhina</i> (staghorn sumac), a plant native to the Appalachian region, on <i>C. albicans</i> growth and host immune responses. Methods: We evaluated the effects of tannins on <i>C. albicans</i> using in vitro assays. Neutrophil-mediated killing was measured using a PrestoBlue killing assay following co-incubation with penta-O-galloyl-β-D-glucose (PGG), gallic acid (GA), DMSO, and Fluconazole. Phagocytosis and reactive oxygen species (ROS) production were measured using flow cytometry to quantify neutrophil uptake of <i>C. albicans</i>. Results: Both tannins (PGG and GA) demonstrated anti-fungal activity, though effects varied. Fluconazole treatment improved neutrophil killing, as expected, compared with DMSO, while PGG and GA showed variable effects. Neutrophils from healthy donors showed overall similar killing levels to those from diabetic donors, with GA effects more variable across samples. Notably, diabetic neutrophils exhibited greater variability in killing responses, especially at a higher multiplicity of infection (MOI 5). Complementary phagocytosis assays reveal that, while both groups generated reactive oxygen species (ROS) upon exposure to <i>Candida</i>, diabetic neutrophils exhibited increased, inconsistent phagocytosis events. Conclusions: These findings highlight the antimicrobial potential of Appalachian tannins and demonstrate how the host immune status can influence therapeutic outcomes. Although there was variability between human donors, tannins exhibited both direct growth inhibition and some modulation of neutrophil responses. Further work will examine <i>C. albicans</i> morphology and gene expression to further define the mechanisms of tannin-mediated fungal inhibition and immune modulation.</p>
UP14	<p>Fungal metabolism alters skin microbiome assembly</p> <p>Tatyana A. Martinez, Ian B. Wilson, Jason W. Dallas, Donald M. Walker Department of Biology, Middle Tennessee State University</p> <p>Background: <i>Ophidiomyces ophidicola</i> is a keratinophilic fungal pathogen and causative agent of ophidiomycosis, an emerging fungal disease affecting snake populations across multiple continents. The skin serves as a physical and chemical barrier to cutaneous pathogens, and harbors a unique microbiome that contributes innate host immunity. However, <i>O. ophidicola</i> exhibits strong keratinase activity, capable of altering the metabolic niche space available to resident microbial communities, resulting in pathogen-induced dysbiosis (PID) on wild snakes. Characterized by reduced bacterial richness and increased community dispersion of the microbiome, as PID intensifies with <i>O. ophidicola</i> loads, understanding how a gradient of fungal metabolized resources (i.e., niche space) and resulting fungal metabolites influence the microbiome assembly provides a crucial link between disease progression and the host microbiome. Methods: To assess how the microbial community responds across the progression of ophidiomycosis, we established a stable community on a keratin minimal media, reflective of the primary constituent of the snake epidermis, in the absence of fungal metabolism. We then exposed stabilized communities to <i>O. ophidicola</i> spent media representing degrees of resource use and metabolite production at 5 and 10 days. Communities were then sequenced with bacterial richness and β-diversity indices analyzed using models with fixed effects, including passage event, nutrient/metabolite type. Results: Family level abundances vary significantly among host replicates, with bacterial community dispersion and richness varying significantly across hosts ($p < 0.01$) and partitions of fungal spent media ($p < 0.01$). Conclusions: Taxonomic composition and measures of bacterial richness and community dispersion (β-diversity) in response to a gradient of fungal resource-use and exometabolite production result in consistent patterns of community structure that is largely host-specific. Partitions of fungal metabolism serve as significant predictors of community assembly between hosts and across passages.</p>
UP15	<p>Investigating the Role of Superoxide Dismutase in Group B <i>Streptococcus</i> Vaginal Colonization</p> <p>Ryan Mesler, Jéssica Da Conceição Mendonça, Justin A. Thornton, Lindsey R. Burcham University of Tennessee, Knoxville</p> <p><i>Streptococcus agalactiae</i> (or Group B <i>Streptococcus</i>, GBS) is a Gram-positive, opportunistic pathogen that is known to asymptotically colonize the gastrointestinal and vaginal tracts, and is known to cause adverse infection-induced pregnancy outcomes worldwide. To successfully colonize, GBS can utilize the enzyme superoxide dismutase (SOD) to overcome reactive oxygen species (ROS) stress produced by the host immune system. In the context of GBS colonization in the vaginal tract, the role of SodA is understudied. Following our observations of delayed growth within a <i>sodA</i> transposon mutant strain compared to our wild type (WT) strain, in vitro assays revealed significant reduction of GBS <i>sodA::tn</i> invasion and intracellular survival in vaginal and endocervical cells, supporting the hypothesis that SodA is necessary for successful host interactions during colonization. To investigate the critical role of manganese as a SodA cofactor, we induced superoxide stress using paraquat and our results indicated that GBS requires functional SodA co-factored with available manganese to successfully detoxify oxidative stress. Isothermal calorimetry (ITC) data support this finding, showing significantly greater binding affinity of recombinant SodA protein to manganese than iron, an inhibitory metal cofactor. Furthermore, we observed decreased vaginal colonization in the <i>sodA::tn</i> mutant strain when compared to WT, indicating the importance of this metalloenzyme in oxidatively challenging environments within the host. This study lays the framework to better understand the mechanisms GBS employs to persist within host environments.</p>
UP16	<p>Characterizing PhyR and the general stress response in <i>Azospirillum brasilense</i></p> <p>Om Patel, Hahley Wiltse and Gladys Alexandre Biochemistry & Cellular and Molecular biology Department The University of Tennessee Knoxville, TN</p> <p>Background: <i>Azospirillum brasilense</i> is a common plant-growth-promoting soil bacteria that has been shown to enhance the growth of many staple crops such as wheat, barley, sorghum, etc. It is important to study this bacterium's physiology to understand how to improve bio-inoculation for crops and because bacterial stress responses are essential for survival in the rhizosphere. An example of this is the general stress response pathway in <i>A. brasilense</i>. The purpose of this project is to characterize a member of the general stress response, PhyR. Methods: We combine computational tools such as gene synteny and phylogeny analyses, and wet lab techniques, such as promoter activity assays and Bacterial Two Hybrid (Ba2H) assay. Results: We found that reporter genes of the general stress responses depend on PhyR for activation under oxidative stress conditions. Compared to other alphaproteobacteria where PhyR has been characterized, the genomic organization of the general stress response in <i>A. brasilense</i> is significantly different. Conclusions: Our results suggest a model in which PhyR serves as a hub to receive signals of stress conditions and to mediate adaptive responses. The role of PhyR in <i>A. brasilense</i> appears to deviate from the established models studied to date.</p>

UP17	<p>Small Molecules to Inhibit Quorum Sensing and Biofilms in Salmonella</p> <p>Anish Penmecha, Bibek Lamichhane, Khaled A. Shaaban, Larissa V. Ponomareva, and Jon S. Thorson, Yosra A. Helmy</p> <p>Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, Kentucky, USA, Center for Pharmaceutical Research and Innovation, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536, United States</p> <p>Salmonella is a major cause of foodborne gastroenteritis in both humans and animals. It is commonly transmitted through food and water contaminated with feces, especially poultry and poultry products. Although infections are usually treated with antibiotics, the rapid rise of antibiotic-resistant Salmonella strains highlights the need for alternative treatments. Quorum sensing (QS) is a communication system used by bacteria, where signaling molecules (autoinducers like AI-2) help regulate virulence and other important functions. In this study, we screened approximately 2,500 small molecules at 10 μM to identify compounds that can inhibit QS/AI-2 without affecting bacterial growth, using a <i>Vibrio harveyi</i> bioluminescence assay. We identified 10 compounds that inhibited QS/AI-2 by more than 95% without impacting growth. Among these, six compounds were non-toxic and showed strong anti-biofilm activity (95-100% inhibition). These six compounds also significantly reduced intracellular survival of Salmonella in human intestinal (HT-29) cells, with two compounds achieving complete clearance. Future studies will focus on evaluating the effect of the SMs in the expression of genes associated with virulence factors such as biofilms, motility, colonization and quorum sensing.</p>
UP18	<p>Promoting Protein Splicing</p> <p>Christopher R Powell, John S Smetana, Tia M Ariagno, Hazel Thomas, Joel Weinberger II, Noy Bagdad, Eyal Gur, Christopher W Lennon.</p> <p>Murray State University Ben-Gurion University of the Negev.</p> <p>Background: Inteins, or intervening proteins, are translated within larger host proteins. Inteins remove themselves through a self-mediated splicing reaction that results in a complete, uninterrupted, host protein and a liberated intein. Inteins are found in many archaea and bacteria, often found within essential proteins for DNA replication, recombination, and repair. In <i>Mycobacterium smegmatis</i>, the helicase DnaB is one such protein, containing two unique inteins. While recent work has investigated environmental effects on intein splicing, factors within the cellular context remain largely unexplored. This project investigates the effects of the GroEL/ES chaperonin system on intein splicing. Chaperonins, a family of chaperones, aid with protein folding under normal and stress conditions.</p> <p>Methods: Using a Kanamycin-Intein-Splicing-Reporter (KISR), which gives splicing-dependent survival, we show in vivo that the GroEL system promotes splicing of a DnaB intein from <i>M. smegmatis</i>. Additionally, Western blotting techniques indicate that GroEL dramatically increases the amount of unspliced precursor in the cell while GroEL only modestly increases the amount of Kanamycin resistance protein in the inteinless controls.</p> <p>Results / Conclusions: Upon GroEL expression, KISR-containing cells show increased survival compared to empty vector controls. This increased survival was not observed when survival was not dependent on splicing, suggesting the interaction between GroEL and the intein is responsible for the improved survival. Our findings using KISR suggest the GroEL/ES system can promote protein splicing in vivo and Western blots show GroEL predominantly stabilizes the unspliced intein, giving it more opportunities to splice and thus leading to increased survival.</p>
UP19	<p>Under Pressure: Characterizing the general stress response in <i>Azospirillum brasilense</i></p> <p>Emma Scheibe, Hahley Wiltse, Dr. Gladys Alexandre</p> <p>Biochemistry & Cellular and Molecular biology Department The University of Tennessee Knoxville, TN</p> <p>Introduction: <i>Azospirillum brasilense</i> is a plant-associated soil bacterium that is able to survive fluctuating environmental conditions and stressors in the soil and rhizosphere using a variety of molecular mechanisms. The general stress response (GSR) pathway in <i>A. brasilense</i> is one such pathway. The GSR is regulated by the sigma factor RpoE6, anti-sigma factor Asf6, and anti-anti-sigma factor PhyrR, yet the pathway's functional role in stress tolerance remains incompletely characterized. This study aims to evaluate how disruptions to core GSR components affect bacterial survival under heat, oxidative, and osmotic stress.</p> <p>Methods: Wild-type and GSR mutant strains, including ΔrpoE6, ΔphyR, and Δasf6, were assessed using spot plate assays. A series of dilutions was created and spotted onto TY agar plates, grown overnight, and qualitatively compared between strains and stress conditions and were quantified by counting CFU.</p> <p>Results: Results obtained thus far show that Δasf6 mutants visually show a similar survival tolerance to that of the wild-type strain under stress, while ΔrpoE6 and ΔphyR qualitatively show decreased rates of survival under the same stress conditions. ΔrpoE6 and ΔphyR have also shown slower growth under conditions of osmotic stress.</p> <p>Discussion : Ultimately, these findings will contribute to a broader understanding of how conserved stress response pathways enable soil bacteria to thrive in dynamic environments, with potential implications for agricultural applications involving plant growth promoting bacteria.</p>
UP20	<p>Development of A New PCR Assay for Rapid Detection of Salmonella Enteritidis in Poultry</p> <p>Kaylea Simmons, Wei Wang, Christina Sigmon, Lin Walker, Xiang Li</p> <p>The University of Tennessee</p> <p>Abstract: Salmonella has been and continues to be a major concern in poultry production and food safety. There are more than 2,600 known Salmonella serotypes, and traditional culture-based methods used to identify them are labor intensive and time consuming. These methods can take five to seven days to produce results. While molecular methods such as polymerase chain reaction (PCR) allow for faster detection, the high genetic similarity between Salmonella serotypes can make it difficult to design highly specific PCR assays.</p> <p>The objective of this study was to develop and validate a PCR assay that specifically detects Salmonella Enteritidis, which is one of the most common serotypes associated with poultry and foodborne illness. To identify unique genomic targets, 300 whole-genome sequences of <i>S. Enteritidis</i> from the NCBI Pathogen Detection database were analyzed using comparative genomics. An Enteritidis specific PCR primer pair was designed and tested using standard PCR and gel electrophoresis protocols to confirm the expected amplicon size across a panel of diverse Salmonella strains sourced from North Carolina. Our newly developed PCR assay successfully amplified the target sequence in <i>S. Enteritidis</i> isolates while showing no cross-reactivity with other serotypes (<i>S. Heidelberg</i>, <i>S. Montevideo</i>, <i>S. Kentucky</i>, <i>S. Typhimurium</i>, and <i>S. Infantis</i>). Our assay showed 100% specificity against <i>S. Enteritidis</i> (tested in duplicates). This study provides a rapid and accurate method to identify <i>S. Enteritidis</i> and greatly reduces the time required for detection compared to traditional culture methods. This tool could help improve disease monitoring in poultry production and allow producers and researchers to respond more quickly to potential outbreaks. Future work will continue testing additional isolates and expand the assay to include other important Salmonella serotypes.</p>

UP21	<p>Host-Parasitoid Viral Association: Identification of a Novel Cripavirus in SVG Diachasmimorpha longicaudata</p> <p>Vanesa Torres, Charles Dawe, Kelsey A. Coffman Department of Entomology and Plant Pathology, University of Tennessee, Knoxville</p> <p>Parasitoid wasps are widely used in biological control programs due to their ability to develop within and ultimately kill arthropod hosts. Many parasitoid species harbor symbiotic viruses that enhance parasitism success by modulating host physiology and immunity. However, numerous viruses associated with these wasps remain uncharacterized, limiting our understanding of their ecological and applied significance. The braconid wasp <i>Diachasmimorpha longicaudata</i> is an important biocontrol agent of the oriental fruit fly, <i>Bactrocera dorsalis</i>, a major agricultural pest. Here, we examined the short venom gland (SVG) strain of <i>D. longicaudata</i> and characterized viral associations within this host-parasitoid system. Cripaviruses have previously been identified in <i>B. dorsalis</i>, raising the possibility of viral exchange within this host-parasitoid system.</p> <p>Using next-generation sequencing and viral discovery pipelines, we identified a novel cripavirus associated with <i>D. longicaudata</i>. Cripaviruses have previously been reported in <i>B. dorsalis</i>, and preliminary transcriptomic data revealed viral RNA reads in parasitoid wasps, suggesting possible cross-species association. RNA extracted from flies and wasps was screened using PCR and qPCR, confirming viral presence in both organisms. Viral loads were substantially higher in flies than in wasps, supporting the fly as the primary host while indicating potential transmission through parasitoid development or oviposition.</p> <p>Detection of viral RNA in both host and parasitoid raises the possibility of trophic or vertical exchange within this multitrophic system. Because viral infections can influence parasitoid fitness, colony stability, and mass-rearing efficiency, understanding viral dynamics in this system is essential for maintaining consistent and effective fruit fly biological control programs.</p>
UP22	<p>Recombination Contributes to Diversification of the Tailocin Locus in <i>Xenorhabdus</i></p> <p>Matthew T. Traywick, Emmanuel C. Allwell, Ryan M. Awori, Sarah J. Kauffman, Heidi Goodrich-Blair. University of Tennessee, Knoxville</p> <p>Tailocins are bacterially produced bacteriophage-tail-like particles that function as competitive weapons during interactions among closely related bacterial strains. Tailocins are composed of tail fibers, which are surface recognition structures that mediate binding to susceptible bacterial cells. Comparative genomic studies have revealed extensive diversification within tailocin encoding loci, particularly with respect to tail fiber receptor binding domains (RBDs), which are predicted to mediate specificity for target strains. However, it remains unclear whether diversification arises solely through long-term evolutionary processes (e.g., inter-strain DNA exchange) or can occur dynamically within a population. To help address this question, this study examined whether dynamic, recombination-mediated genomic rearrangements occur within the locus of the bacterium <i>Xenorhabdus nematophila</i> ATCC19061 during laboratory growth. Cultures were sampled prior to induction of tailocin production and across a temporal series following this induction, and genomic DNA was purified for analysis. The region encoding the tail fiber RBD sequences was amplified using polymerase chain reaction (PCR) and analyzed by gel electrophoresis and sequencing. These analyses revealed that after overnight growth, the bacterial population consisted of multiple distinct genomic architectures of the tail fiber region, derived from recombination-mediated rearrangements. These findings suggest that genetic rearrangements within tailocin loci may occur over short timescales, providing a mechanism for rapid diversification of expressed tailocin RBD, and therefore altered target specificity, within a clonal bacterial population. Together, these results provide molecular support for models in which recombination within phage-derived antimicrobial systems promotes adaptive diversification during bacterial competition.</p>
UP23	<p>Identification of Chemical Compounds to Reduce Desiccation Survival of <i>Acinetobacter baumannii</i></p> <p>Molly Williams, Gabrielle Shipstone, Garrett Reynolds, Erik Petersen East Tennessee State University</p> <p><i>Acinetobacter baumannii</i> is a multidrug-resistant pathogen known for its ability to remain on dry surfaces, contributing to its prevalence within healthcare environments and infections in immunocompromised patients. The objective of this project was to identify chemical compounds capable of limiting desiccation survival of <i>A. baumannii</i> on plastic surfaces. Nutrient-type compounds were chosen to evaluate signaling-associated responses rather than testing for bactericidal effects from pure chemical treatments, as this should aid in both reducing the generation of resistance as well as decrease associated toxicity. A library of ~400 nutrient-type chemicals was selected for testing of desiccation inhibition. Fifteen different chemicals were selected from the initial screen for further testing. After standardizing bacterial density, exposing cultures to serial dilutions, and drying under controlled conditions, survival of treated bacteria was monitored using a growth curve analysis. The data indicates that specific chemicals reduce desiccation survival compared to controls. These findings suggest that chemical exposure may influence stress tolerance mechanisms and could potentially limit environmental persistence. Future studies will further assess chemical compounds as well as genetic approaches that examine the mechanisms that contribute to desiccation tolerance.</p>
GaP1	<p>Pangenome-based identification of probiotic and non-probiotic <i>Bifidobacterium</i></p> <p>Bharosha Adhikari, Anjula Swathi Sagam, Md Sifat Habib Siam, Gajender Aleti Tennessee State University</p> <p><i>Bifidobacterium</i> are key members of the human gut microbiota and are widely recognized for their probiotic functions, including host colonization, immune modulation, and carbohydrate metabolism. However, the genomic determinants underlying these probiotic traits, and how they differ from non-probiotic strains, remain poorly defined.</p> <p>In this study, we employed a pangenome-based approach to identify gene-level differences between probiotic and non-probiotic <i>Bifidobacterium</i> strains. Representative strains were analyzed. Functional categories relevant to probiotic functions, including adhesion (gut colonization), carbohydrate metabolism, and stress tolerance (bile and acid resistance), were systematically compared across strains. Core gene clusters associated with these functions were conserved across all strains, including Sortase A (srA), β-galactosidase (lacZ), galactokinase (galK), galactose-1-phosphate uridylyltransferase (galT), and alkyl hydroperoxide reductase (ahpC). However, substantial variation was observed within the accessory genome, reflecting functional diversification among strains. Notably, probiotic strains exhibited distinct genomic features compared to non-probiotic counterparts.</p> <p>Overall, our findings demonstrate that probiotic functionality in <i>Bifidobacterium</i> is highly strain-specific and largely driven by variation in the accessory genome. This study provides a genomic framework for the identification and selection of functionally robust probiotic candidates.</p>

GaP2	<p>Relic DNA Differentially Affects Source Attribution in Soil and Human Touch Samples</p> <p>Emily Cantrell B.S., Savannah Truan B.S., Julia Erie Tait B.S., Reese Saho B.S., Alison Buchan Ph.D., Giovanna Vidoli Ph.D., Zachary Burcham Ph.D. Department of Microbiology, University of Tennessee, Knoxville</p> <p>The forensic microbiome has become increasingly investigated as microbial trace evidence, however, its reliability for source connectivity remains unclear. One potential source of influence on microbiome diversity calculations is relic DNA, which is extracellular DNA that persists in the environment. We hypothesized that the presence of relic DNA could have a negative impact on forensic analyses. To test this, we generated environmental and human touch trace evidence samples to train machine learning models to predict source attribution. Environmental soil samples were collected from various locations using cotton swabs, cotton t-shirts, and rubber-soled shoes. Human touch samples were collected by simulating grasp to transfer evidence, and then swabbing the donor's hand and the grasped PVC. Samples were processed in paired replicates, creating a treatment relic-DNA-removal group and a control group. DNA was extracted and processed for 16S rRNA gene sequencing. Sequences were then analyzed for changes in microbiome community diversity. For soil samples, results show that relic DNA depletion affects community structure, however it was not the strongest driver of community composition. Alpha diversity and beta diversity metrics showed that location had the strongest influence on microbiome composition, over sample type or treatment group. Machine learning models for soil samples achieved high accuracy for source attribution and remained strong across processing conditions. In contrast, microbial community diversity from human touch samples was more sensitive to relic DNA removal. Diversity metrics showed lower variation amongst samples, indicating lower community complexity. Results of these analyses show that community variation is largely donor specific. When used in machine learning models, treated touch samples showed reduced accuracy for source attribution when compared against controls. Overall, the results of this study show that microbial signatures can be reliable for source attribution, though the accuracy of these predictions can differ based on evidence type and processing conditions.</p>
GaP3	<p>You are what you eat: Short-term western diet rewires neutrophil metabolism and enables Staphylococcus aureus to suppress NETosis.</p> <p>Aliyah Collins, Morgan Hetzel, Bailey Holder, Calleigh Reber, Jadan Zavestovski, Eden TenBarge, Ashley Wise, William Bever, Andrew Monteith. Department of Microbiology, University of Tennessee, Knoxville</p> <p>Immunometabolism ties diet to frontline antibacterial defense. Neutrophils rely on glycolysis to power the oxidative burst and on mitochondrial metabolism to drive neutrophil extracellular trap (NET) release. We asked whether brief exposure to a high-fat, high-calorie western diet (WD), in the absence of obesity or diabetes, is sufficient to alter neutrophil function during Staphylococcus aureus infection. In a 4-day systemic S. aureus model, short-term WD alone worsened infection outcomes, with higher bacterial burdens in the liver. Ex vivo, fatty acid oxidation (FAO) supports NETosis, indicating that fatty acid availability can fuel this effector program. However, despite increased fatty acid availability in mice on a western diet, NETosis was decreased because S. aureus exploits WD-derived fatty acids to generate or modify lipoproteins that actively suppress NET release. In addition, neutrophils from WD-fed mice display a diminished oxidative burst, consistent with Randle cycle-driven substrate competition that diverts metabolism away from glycolysis-dependent NADPH oxidase activity. Together, these findings reveal a rapid, diet-driven vulnerability in innate immunity that S. aureus can leverage. Rather than fueling NETosis through FAO, the same fatty acids are used by S. aureus to blunt NET release, while the Randle cycle dampens ROS generation. Defining how diet shifts neutrophil fuel choice and how S. aureus capitalizes on that shift points to metabolic and nutritional strategies to restore NETosis and oxidative burst during infection.</p>
GaP4	<p>Multidrug-Resistant Salmonella enterica from Cattle: Integrated Phenotypic and Genomic Characterization</p> <p>Nada A. Fahmy and Yosra A. Helmy* 1 Gluck Equine Research Center, Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, Kentucky, USA</p> <p>Introduction: Non-typhoidal Salmonella enterica is a leading cause of foodborne illness worldwide and represents a significant One Health concern due to zoonotic transmission and the increasing emergence of multidrug-resistant (MDR) strains. Cattle serve as an important reservoir, facilitating dissemination through the food chain, farm environments, and trade networks. This study combined phenotypic and genomic approaches to characterize Salmonella isolates recovered from necropsied cattle.</p> <p>Method: A total of 1008 samples were collected, and confirmed isolates, identified by MALDI-TOF MS and serotyping, were subjected to phenotypic virulence characterization and antimicrobial susceptibility profiling. Whole-genome sequencing was used to determine sequence types, virulence genes, antimicrobial resistance genes (ARGs), and mobilome components. Results: Salmonella enterica was recovered from 27 necropsied cattle, with S. Dublin identified as the predominant serotype (29.6%). All isolates demonstrated biofilm-forming ability, while 96.3% exhibited both swimming and swarming motility. Antimicrobial susceptibility testing against 19 antibiotics revealed high resistance rates, particularly to macrolides (100%) and chloramphenicol (85.2%). All isolates were MDR, and five were extensively drug resistant (XDR). Genomic analysis identified 12 sequence types, with ST10 as the dominant lineage (25.9%), and detected 223 virulence genes and 1,148 antimicrobial resistance genes, many of which are mediated by efflux systems such as AcrAB-TolC. Plasmids were present in 77.8% of isolates, with integrons (11.1%), prophages, and insertion sequences contributing to a complex mobilome. Most resistance loci were chromosomal (63.5%), followed by prophage-associated (24.8%) and plasmid-associated (11.7%) regions, with plasmid-borne genes showing stronger association with insertion sequences, consistent with transposition-mediated mobility. Pan-genome analysis revealed an open genomic architecture dominated by accessory genes (77.6%), and phylogenetic analysis demonstrated clustering of cattle and food-associated isolates, suggesting ongoing transmission.</p> <p>Conclusion: These findings highlight the role of mobile genetic elements in shaping antimicrobial resistance and supporting the need for enhanced surveillance and prudent antimicrobial use in livestock systems.</p>

GaP5	<p>Genomic Insights into Antagonism of <i>Escherichia coli</i> O157:H7 by <i>Ligilactobacillus animalis</i> NP51</p> <p>K.P Feldmann, J.E. Beever, P.R. Myer Department of Animal Science, University of Tennessee, Knoxville, TN</p> <p>Background: Growing concerns over antimicrobial resistance in livestock production have increased the need for alternatives to antibiotics, such as probiotics. The probiotic <i>Ligilactobacillus animalis</i> NP51 (LANP51) has been extensively investigated in feedlot cattle as a pre-harvest mitigation strategy due to its antagonistic activity against <i>Escherichia coli</i> O157:H7 (O157:H7). Although LANP51 consistently reduces O157:H7 shedding, the underlying mechanism remains unclear. One potential mechanism is the production of antagonistic metabolites; however, the biosynthetic potential of this strain has not been fully characterized due to the lack of a high-quality genome. Therefore, the objective of this study was to generate a complete genome assembly of LANP51 and evaluate its secondary metabolite biosynthesis potential.</p> <p>Methods: Whole-genome sequencing was performed using Oxford Nanopore Technologies (ONT) long-read platform. High-quality genomic DNA was extracted and purified using SPRI bead cleanup following ONT recommendations. Libraries were prepared using the Ligation Sequencing Kit v14 (ONT, UK) and sequenced on a MinION Mk1D with Flongle adapter. Base calling, assembly, and polishing were conducted using ONT workflows with default parameters. Gene prediction and annotation were performed with the NCBI Prokaryotic Genome Annotation Pipeline. Biosynthetic potential was assessed using AntiSMASH.</p> <p>Results: Sequencing produced a complete genome that is 1,881,978 bp long. A total of 1,925 genes were annotated, including 1,777 protein-coding sequences, 18 rRNA genes, 64 tRNA genes, 3 ncRNA genes, 62 pseudogenes, and 1 CRISPR array. Notably, AntiSMASH analysis identified a putative Type III polyketide synthase (T3PKS) biosynthetic gene cluster, suggesting previously uncharacterized secondary metabolite potential in LANP51.</p> <p>Conclusions: The complete genome assembly of LANP51 supports future work elucidating mechanisms underlying its probiotic activity. Identification of a putative T3PKS biosynthetic gene cluster highlights the potential for secondary metabolite production and warrants further investigation into its role in O157:H7 antagonism, with implications for improving pre-harvest food safety strategies in beef production systems.</p>
GaP6	<p>Microbial Drivers of Methane Emissions and Proxy-Based Feed Efficiency in Forage-Based Beef Cattle Operations</p> <p>R.N. Flinchum1, K.M. Mason1, T.N. Rowan1, P.R. Myer1 1Department of Animal Science, University of Tennessee, Knoxville, TN</p> <p>The rumen microbiome enables cattle to convert human-indigestible plant biomass into edible protein, but this process is accompanied by energetic losses in the form of metabolic heat and gaseous emissions. Methane (CH₄) production represents both a biological inefficiency and an environmental concern, particularly in forage-based systems where intake is difficult to quantify. Improving feed efficiency is therefore critical; however, the microbial mechanisms underlying variation in residual heat production (RHP), a physiological metric of feed efficiency, and CH₄ emissions among individuals remain poorly characterized under grazing conditions. This study aimed to evaluate relationships among rumen microbial composition, RHP, and CH₄ emissions in forage-based beef cattle. Fifty (n = 50) Angus heifers grazing cool-season perennial forages (average starting weight 292.6 ± 36.9 kg) were enrolled in a 203-day greenhouse gas emissions trial. Methane, CO₂, and O₂ fluxes were individually measured up to three times daily using a C-Lock GreenFeed system (C-Lock Inc., Rapid City, SD). Heat production was calculated using Brouwer's equation and regressed on metabolic body weight and average daily gain to generate RHP values. Animals were categorized into low, mid, and high RHP and CH₄ emission groups based on herd quintiles. Rumen fluid samples were collected on day 203 via gastro-esophageal tubing, and microbial community composition was characterized using 16S rRNA and ITS gene amplicon sequencing. Methane emissions were positively correlated with RHP at the animal level (r = 0.52, P = 6.9 × 10⁻⁴). Microbial relative abundances were summarized at the genus level and assessed for differential abundance across RHP and CH₄ emission categories using ANCOM-BC2 in RStudio. Numerous bacterial genera differed significantly among RHP and CH₄ groupings (P < 0.05), with directional shifts in abundance indicated by positive or negative β coefficients. Collectively, these findings demonstrate that variation in RHP and CH₄ emissions is associated with distinct rumen microbial profiles in forage-based beef cattle and highlight the value of integrating microbial signatures with precision phenotyping technologies such as GreenFeed to identify biologically efficient animals where individual feed intake cannot be directly measured.</p>
GaP7	<p><i>Escherichia coli</i> Persister Formation through Controlled Toxin Induction</p> <p>Mohamad Hashemi(1), Dacheng Ren(1,2), 1) Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN, USA 2) Department of Biomedical Engineering, University of Tennessee, Knoxville, TN, USA</p> <p>Bacteria are well known to enter a dormant stage and form persister cells under stress conditions. Persister cells resume growth after the stress is removed and thus play important roles in chronic infections. Despite the significance, bacterial persister cells only exist as a small subpopulation in each culture. To better understand persister formation and develop new persister control strategies, it is important to develop a robust experimental system to obtain consistent and high-level persister formation. To achieve this goal, we focused on the HipA7 toxin, a variant of the HipA toxin known for its high persistence phenotype. Unlike the wild-type HipA that has broad range of targets, the HipA7 variant is non-toxic yet still induces high levels of persistence. We cloned the hipA7 gene under the pBAD promoter and induced its expression in multiple <i>Escherichia coli</i> strains using either blue light or arabinose. The highest persister levels were observed after 16 hours of blue-light induction (8.1%) and 22 hours of arabinose induction (67%) in the BW29655ΔhipBA strain. In contrast, only 1.0% and 2.2% of the uninduced cultures are persister cells at the same time points, respectively. The remaining non-persister population of the induced samples consisted of both normal culturable cells and viable but non-culturable (VBNC) cells. These findings demonstrate that controlled hipA7 induction by arabinose can generate high persister fractions while minimizing toxic effects on growth, providing a promising system for studying bacterial persistence and developing new control agents.</p>
GaP8	<p>Biotinylated Probes Increase RNA Sequencing Specificity for <i>Clostridioides difficile</i></p> <p>Lily Hernandez, Michelle Chua, James Collins, University of Louisville</p> <p>Background: <i>Clostridioides difficile</i> is an opportunistic pathogen and the leading cause of hospital-associated diarrhea. Traditionally, it has been assumed that patients acquire <i>C. difficile</i> in hospitals following broad-spectrum antibiotic use. However, recent research has shown that a significant number of healthy adults carry <i>C. difficile</i> asymptotically, and that carriers can develop symptomatic disease following antibiotic use. During severe infection, <i>C. difficile</i> accounts for 1-2% of the gut microbiota, and in asymptomatic carriers, <i>C. difficile</i> abundance is even lower (~0.1%). While RNA sequencing can be used to understand the mechanisms of asymptomatic colonization of the host, it is limited by the read depth with ~99.9% of reads coming from other microbes. Here, we present data on an enrichment method that outperforms standard RNA-seq and may help shed light on the silent persistence of this important pathogen.</p> <p>Methods: In a pilot study, we designed 100 biotinylated probes targeting the first 20% or 200 nt (whichever is greater) of 24 <i>C. difficile</i> genes. <i>C. difficile</i> RNA was mixed with whole RNA extracted from mouse stool at known ratios to generate cDNA libraries. The cDNA was incubated with or without the biotinylated probes, extracted, and following cleanup the libraries were sequenced using the Illumina platform. Data were analyzed using Kallisto and Deseq2.</p> <p>Results: Using 100% <i>C. difficile</i> RNA as our control, we found that traditional RNA-seq failed to detect genes that were expressed at a low level when <i>C. difficile</i> RNA was spiked into total RNA at 0.2%. In contrast, probe enrichment led to the detection of all targeted genes and was highly correlated to the control sample. Further, gene length or GC content did not affect probe efficacy.</p> <p>Conclusion: The use of enrichment probes may enable us to examine RNA expression levels of asymptotically colonizing <i>C. difficile</i> within a complex gut microbiota.</p>

GaP9	<p>Sulfolipid Substitution as a Marker of Phosphorus Availability in Lake Erie Blooms</p> <p>Kelly E. Joyce¹, Robbie M. Martin², David J. Niknejad², Katarina A. Jones³, Malcolm A. Barnard⁴, Justin D. Chaffin⁵, Hans W. Paerl⁶, Shawn R. Campagna³, Steven W. Wilhelm²</p> <p>¹Department of Genome Science & Technology, University of Tennessee at Knoxville, Knoxville, TN, USA ²Department of Microbiology, University of Tennessee at Knoxville, Knoxville, TN, USA ³Department of Chemistry, University of Tennessee at Knoxville, Knoxville, TN, USA ⁴Department of Chemistry, University of Texas at Austin, Austin, TX, USA ⁵F. T. Stone Laboratory, Ohio Sea Grant, and The Ohio State University, Put-in-Bay, OH, USA ⁶Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC, USA</p> <p>Background: Cyanobacteria-dominated freshwater harmful algal blooms (HABs) disrupt ecosystem services and threaten drinking water resources and human health. Nutrient enrichment of nitrogen (N) and phosphorus (P) are linked to the formation of HABs. Understanding how phytoplankton communities respond to both large and small changes in nutrient availability is ultimately necessary for developing strategies to manage bloom formation. To this end, we investigated the effects of nutrient decreases (through dilution) and increases (through amendments) on the physiology of a <i>Microcystis</i>-dominated phytoplankton community. We monitored community transcriptional profiles and sulfolipid substitution ratios as response indicators.</p> <p>Methods: A microcosm study was conducted in 4-L Cubitainers using surface water communities collected from Lake Erie that were diluted to 40% concentration using ionically-balanced filtered lab water. Amendments of either N, P, or N+P were added in a series of increasing concentrations for treatments. Unamended bottles were included as controls. The design resulted in a total of 17 treatments, all conducted in triplicate. Bottles were incubated in situ for 72 h. Samples were collected at 0, 24, 48, and 72 h. Total community RNA was extracted for sequencing and total lipids were extracted for lipid ratio analysis.</p> <p>Results: The sulfolipid ratio was inversely correlated to dissolved P concentration, consistent with predictions. Surprisingly, there were no changes in community composition across the transcription profiles due to treatments. We observed changes in gene expression of functional genes between the lowest and highest amendments of the +N and +P series.</p> <p>Conclusions: Even against the backdrop of abnormally high dissolved nutrient concentrations, the sulfolipid ratio was responsive to slight changes in P concentrations, demonstrating that the sulfolipid ratio is a sensitive indicator of P-availability within <i>Microcystis</i>-dominated phytoplankton communities. Ongoing analysis will determine associations between gene expression and lipid ratios.</p>
GaP10	<p>Excision decisions: Characterization of Group B <i>Streptococcus</i> prophage excision dynamics and implications within the vaginal mucosa</p> <p>Emily F. Landolt¹, Lindsey R. Burcham¹</p> <p>¹ Department of Microbiology, University of Tennessee, Knoxville,</p> <p>Group B <i>Streptococcus</i> (GBS) is a Gram-positive commensal of the gastrointestinal tract and colonizes the vaginal tract of 25-30% of healthy women. GBS colonization is a contributing factor to preterm birth and complications can arise during birth for both the pregnant person and their fetus. Analysis of the GBS A909 genome by PHASTEST revealed the presence of two structurally complete prophages, Javan 7 and Javan 8, and one incomplete satellite prophage. Transcriptomics in the A909ΔperR background showed a downregulation of nearly all genes encoded by Javan 7. PerR is a transcriptional regulator with implications for metal homeostasis and infection dynamics, and downregulation suggests an important role of the prophage in cellular physiology and disease outcomes. To explore this, we characterized excision dynamics utilizing semi-quantitative PCR in steady state and under pressure of antibiotics known to induce prophage. We found that Javan 7 readily excises in steady state, whereas Javan 8 and the satellite do not, even under inducing conditions. To understand the role of this prophage in the vaginal tract, we utilized a murine vaginal colonization model and found that a Javan 7 cured strain colonized to wild type levels but had a clear tissue invasion defect. However, in a co-colonization this phenotype is less stark, suggesting the importance of community level prophage carriage. Taken together, these data suggest that Javan 7 is important for GBS associated disease in the vaginal mucosa. We plan to use a dual-pronged approach incorporating host and bacterial factors to further elucidate this interaction.</p>
GaP11	<p>Iron Metabolism as a Key Regulator of Group B <i>Streptococcus</i>-Host Interactions</p> <p>Matthew R. Lopez, Stephen W. Lumsdaine, Jéssica da Conceição Mendonça, Lindsey R. Burcham</p> <p>Department of Microbiology, University of Tennessee, Knoxville, TN, 37996</p> <p>Group B <i>Streptococcus</i> (GBS) is a leading cause of neonatal sepsis and meningitis. While colonization of the female reproductive tract is typically asymptomatic, GBS can pose a serious threat to pregnant people and the developing fetus. During infection, hosts employ nutritional immunity strategies to restrict metals from invading pathogens. The mechanism by which GBS overcomes host metal restriction remains poorly understood. Iron homeostasis in bacteria is commonly regulated by ferric uptake regulator (Fur), which is lacking in the GBS genome. BLAST analysis revealed a Fur-family homolog the peroxide resistance regulator PerR, which is known to maintain iron homeostasis in Group A <i>Streptococcus</i>. The perR mutant exhibited impaired growth under iron chelation conditions, which suggests an important role in GBS response to iron starvation. Transcriptomic analysis of the perR mutant compared to wildtype revealed differential expression in metal homeostasis machinery, including fetAB, VIT, and mtsABC. These results seem to indicate that PerR coordinates the transcriptional response of diverse metal transporters. The physiological relevance of host nutritional immunity in constraining GBS pathogenesis was demonstrated in vivo, where mice deficient in lipocalin-2, an innate immune protein that restricts iron availability, exhibited significantly higher bacterial burdens and succumbed to infection faster than wildtype mice in a systemic infection model. Together these findings reveal alterations in GBS metal transport as a response to host iron restriction and highlight the central role of nutritional immunity in host-pathogen interactions.</p>
GaP12	<p>Defining Microbial Community Subtypes in Chronic Wounds to Inform Healing Therapies</p> <p>Shannon A. Manning¹, Aanuoluwa Adekoya¹ and Carolyn B. Ibberson¹</p> <p>¹Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA</p> <p>Chronic wounds (CW) are characterized by complex polymicrobial communities whose structure and functional activity remain poorly understood. While prior studies have demonstrated multiple community types in CWs using 16S rRNA sequencing and metagenomics, how these community types lead to differences in wound outcomes, healing vs. non-healing, remains a key knowledge gap. Preliminary analysis of CW metagenomic and metatranscriptomic data confirms three distinct community clusters dominated by: (A) <i>Staphylococcus</i> and <i>Corynebacterium</i>, (B) anaerobes, and (C) Gram-negative microbes. Further investigation of Cluster A at the species level showed two potential community subtypes, one dominated by <i>Corynebacterium</i> and coagulase-negative staphylococci (CoNS) and one dominated by <i>S. aureus</i>. We hypothesize that the CW subtype comprised mostly of CoNS and <i>Corynebacterium</i> resembles healthy skin microbial communities, leading to a healing wound phenotype. We further hypothesize that functional dynamics within these CW subtypes represent a microbial signature that contributes to wound outcome, either healing or non-healing. Therefore, we aimed to determine if the healing wound microbial community is similar to healthy skin. We did this by quantifying microbial relative abundances and prevalence using MetaPhlan and HUMAnN within 58 metatranscriptomic samples of healthy skin swabs. From this, we generated plots of healthy skin microbial community cumulative abundance and prevalence for comparison with the proposed healing wound subtype. These results show that healthy skin is dominated by <i>Corynebacterium</i> and CoNS, which closely aligns with the proposed healing wound subtype. Future work will leverage these findings to build and test these proposed different CW subtypes using in vivo and in vitro model systems. Defining CW subtypes using model systems can inform wound healing outcomes, how the interactions among dominant species shape community stability, and which taxa may drive wound persistence. Integrating multi-omic data enables accurate modeling of multiple community types to establish a framework for developing microbiome-informed therapeutic strategies.</p>

GaP13	<p>Masters of Microbial Mischief: How Klebsiella Stays One Step Ahead</p> <p>Olivia May East Tennessee State University</p> <p>The discovery of antibiotics transformed medicine by providing therapeutics that could kill or inhibit the growth of bacteria. However, the overprescription and misuse of antibiotics have allowed bacteria to quickly evolve and become resistant to their effects. Antimicrobial resistance is rapidly becoming a leading threat to global health, making the urgent development of novel strategies to combat these deadly, multidrug-resistant pathogens absolutely vital. Our laboratory previously found that the supernatant of a particular strain of <i>Klebsiella pneumoniae</i> produces a potential plasmid-mediated bacteriocin that effectively inhibits the growth of certain bacterial genera. This includes members of the highly drug-resistant Enterobacteriaceae, such as <i>Enterobacter</i>, <i>Citrobacter</i>, and other <i>Klebsiella</i> species. My current research has utilized a transposon insertion library screen to identify loss-of-function mutants, followed by plasmid sequencing to pinpoint the essential genetic drivers of this inhibitory activity. Future research will test the bacteriocin's therapeutic efficacy using the nematode <i>C. elegans</i> as an infection model. Our lab plans to infect the <i>C. elegans</i> with the target pathogen and subsequently attempt to treat the nematode using the bacteriocin. Further studies will explore the bacteriocin's potential ability to enhance host defense by triggering neutrophil swarming. This work aims to validate the plasmid-mediated bacteriocin as a viable, novel strategy for developing future therapeutic treatments against multidrug-resistant pathogens.</p>
GaP14	<p>Activation of the Staphylococcus aureus SaeRS System by chronic wound GPAC</p> <p>Kathleen Medina-Rodríguez¹, Aanuoluwa Adekoya¹, and Carolyn Ibberson¹ ¹Department of Microbiology, University of Tennessee, Knoxville, TN</p> <p>Chronic wounds (CW) are wounds that fail to successfully heal and clear pathogens within six to eight weeks post-treatment intervention and result in billions of annual healthcare costs. These are often infected by polymicrobial communities dominated by <i>Staphylococcus aureus</i>, a canonical pathogen. Studies have shown that Gram-positive anaerobic cocci (GPAC), such as <i>Anaerococcus hydrogenalis</i> and <i>Finegoldia magna</i>, co-exist with <i>S. aureus</i> and are associated with an increased disease severity. We hypothesize that synergistic interactions between <i>S. aureus</i> and GPAC, mediated by secreted metabolites, are key drivers of CW severity. Using transcriptomics, we found that in co-culture with <i>F. magna</i> in wound-like media, <i>S. aureus</i> significantly increased expression of the SaeRS, a two-component system (TCS), a global regulator of virulence factors, particularly toxins. Using an SaeRS transcriptional reporter, we measured activity of the <i>S. aureus</i> SaeRS TCS and observed activation in the presence of cell-free media (CFM) from <i>F. magna</i>. <i>F. magna</i> is a member of the GPAC group, therefore we next tested whether the activity observed was conserved among GPAC species. We found CFM from <i>A. hydrogenalis</i>, another GPAC species frequently found in wound infections, also activated the SaeRS system, and that the <i>A. hydrogenalis</i> activation of the SaeRS was increased and more stable compared to that of <i>F. magna</i>. Therefore, using <i>A. hydrogenalis</i>, we aimed to identify the secreted factor responsible for SaeRS activation in <i>S. aureus</i>. Biochemical analyses, including proteinase K digestion and size fractionation, suggest that the secreted factor is a small peptide with a molecular weight of less than 3 kDa. Future work will employ molecular and bioinformatic analyses to fully characterize this interaction. Altogether, our study aims to understand the molecular mechanisms of microbial interactions that drive wound severity and understand microbial synergies that lead to CW infections.</p>
GaP15	<p>Salmonella Growth Inhibition on Surfaces Using Nutrient-Type Compounds</p> <p>Mackenzie Nicholas, Erik Petersen East Tennessee State University</p> <p><i>Salmonella enterica</i> is the causative agent behind the majority of foodborne illnesses. With the ability to survive desiccation, <i>S. enterica</i> can be found on many surfaces in agricultural facilities, including the produce that is sold to consumers, causing illness when eaten. Because of the prevalence of infection, most facilities use toxic chemicals as disinfectants, which could be harmful to both the inhabitants of the facilities and the consumers. To combat the issue of toxicity, this study seeks to assess the effectiveness of ten different naturally occurring nutrient-type compounds in inhibiting <i>Salmonella</i> growth in a desiccation context. These compounds were tested against <i>Salmonella enterica</i> serovar typhimurium on a plastic surface in varying concentrations and manners of application. It was determined that sorbic acid, itaconic acid, glyoxylic acid, salicylic acid, and guanine were effective in inhibiting <i>S. enterica</i>. When the chemicals were added to a buffer solution to neutralize the pH, it was found that sorbic acid, glyoxylic acid, and salicylic acid retained the ability to inhibit <i>Salmonella</i> growth, showing that the mechanism of action is not dependent upon the chemical's acidity. Additionally, the results of this study show that some chemicals were more effective when applied to the surface before the bacteria and used as a preventative measure, while others were more effective when added after the bacteria as a treatment. Overall, these results suggest that nutrient-type chemicals could be a potent inhibitor of <i>Salmonella</i> growth. Further research could be done with the eventual goal of creating an effective and non-toxic disinfectant for agricultural facilities.</p>
GaP16	<p>Exploitation of the fire ant (<i>Solenopsis invicta</i> Buren) gut microbiome for potential biological control agents against plant pathogens</p> <p>Joshua Obeng, Tyler Buxton, Mohammad Fazel Soltani Gishini, Peter Prestwich, Ali Taheri and Korsi Dumenyo Department of Ag Sci & Engineering, Tennessee State University, 3500 John A. Merritt Blvd, Nashville, TN.</p> <p>The gut microbiome of fire ants offers a promising platform for developing biological control agents to combat plant pathogens through competition for space and nutrients, mycoparasitism, and production of antimicrobial compounds that either inhibit or suppress pathogens' growth and development. This innovative project seeks to harness the diverse microbial communities present within the digestive systems of fire ants (<i>Solenopsis invicta</i> Buren). This community has evolved to thrive in a unique ecological niche. This study aims to evaluate the biological control activities of bacterial isolates from the gut of fire ants and characterize them using molecular techniques. Fifty-five (55) bacterial isolates, part of the microbial collection at the Plant Bacteriology Laboratory at Tennessee State University, were screened against 11 fungal phytopathogens using dual-plate culture and cell-free plate culture methods for a possible inhibition of fungal growth. Ten promising bacterial isolates (antagonists) were selected based on the formation of clear zones of inhibition between each antagonist and the pathogen. A universal 16S/18S primer pair was used to amplify the 16S rRNA region, and the PCR products were sequenced using the forward primer. All isolates belong to the genus <i>Bacillus</i> but have not yet been classified into species. This strategy not only offers a sustainable alternative to chemical fungicides but also enhances our understanding of the intricate relationships between insects, their microbiomes, and pathogen dynamics. Through rigorous testing and analysis, the goal is to develop effective biocontrol solutions that can protect plants from diseases caused by biotic organisms while minimizing environmental impact.</p>

GaP17	<p>Ancestral Maintenance or Lineage-Specific Loss and Gain? Evolutionary Dynamics of Type III Effectors hopW1 and xopE5 in <i>Xanthomonas translucens</i></p> <p>Oladele F. Temitope, Oduokpaha, Godwin Edet, Uzair, Muhammad, Merfa E Silva Marcus Vinicius University of Tennessee, Knoxville</p> <p>Background: While pathogen host range is shaped by multiple complex factors, the composition of Type III Secretion System (T3SS) effector repertoires often plays a critical role in determining host specificity. In the cereal pathogen <i>Xanthomonas translucens</i>, closely related pathovars exhibit drastically different niches: <i>translucens</i> (Xtt) is a barley specialist, while <i>undulosa</i> (Xtu) is a broad-range generalist. Genomic profiling indicates that Xtt encodes unique T3SS effectors, including hopW1 and xopE5, which are absent in Xtu. This study investigates the specific evolutionary mechanisms that shape these distinct effector repertoires to drive host adaptation.</p> <p>Objective: To reconstruct the evolutionary history and genomic synteny of hopW1 and xopE5 across <i>X. translucens</i> to determine the evolutionary mechanism of effector divergence between Xtt and Xtu.</p> <p>Methods: A comprehensive comparative genomics pipeline was developed. Genomes were annotated via Prokka, and core-genome phylogenomics were constructed using Panaroo and IQ-TREE. Orthologs were identified via Ortho Finder. To assess evolutionary synteny, whole-genome alignments were performed using progressive Mauve. Specific loci, including upstream and downstream flanking regions, were extracted via Benchling, submitted to Clinker for comparative gene cluster visualization, and phylogenetic trees were built using IQTree.</p> <p>Results: Phylogenetic reconstruction revealed that hopW1 and xopE5 homologs are present in the early-diverging Clade I outgroup, <i>X. hyacinthi</i> (CFBP 1156). Clinker visualizations demonstrated that among Xtt strains, the xopE5 and hopW1 loci are highly conserved and syntenic, while absence is observed for Xtu. Overall, results collectively demonstrated that these two effector genes were lost in the generalist Xtu lineage.</p> <p>Conclusion: The presence of hopW1 and xopE5 in outgroups and their syntenic conservation in Xtt indicate that these genes were present in the common ancestor with Xtu but were lost in the generalist lineage. This validates the paradigm that shedding specialized effectors is a primary evolutionary driver for pathogens transitioning to a broad-host generalist lifestyle.</p>
GaP18	<p>Fungal Foundries: Differential Nutrient Uptake Across the Fungal Kingdom</p> <p>Jack Orebaugh1*, Keaton Tremble2, Miguel Rodríguez Jr.1, Rytas Vilgalyš2, Christopher Schadt1, Madhavi Z. Martin1, Hunter B. Andrews3, Tomás A. Rush1 1Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, U.S.A.</p> <p>Within the fungal kingdom, differential lifestyles, genetics, morphotypes, and environmental conditions confer different nutrient uptake strategies for survival. While much has been studied on fungal nutrient exchange for applications in agriculture, little has been done to understand the overall differential elemental uptake across the fungal kingdom. To help address this knowledge gap, we assessed elemental uptake in more than thirty fungi from across the fungal kingdom in three common growth media formulations, one defined and two undefined, representing a gradient of growth conditions. To investigate the fungal elemental uptake, laser-induced breakdown spectroscopy (LIBS) was employed to determine the elemental profiles of these fungi, for comparison across media, species, lifestyle, morphotype, and taxonomy. A "core ionome" was found to be composed of Na, P, K, Fe, H, and C based on PCA biplot analysis, however, these elements had little ability to provide clustering or classification of traits. While there is no defining characteristic to explain all fungal nutrient profiles, certain fungal lifestyles (mutualist, pathogen, saprotrophs) take up some elements in higher relative quantities. This data provides evidence for different nutrient affinities by different lifestyles that cannot be easily explained by traditional evolutionary classifications, and no one fungal trait has high predictive power of elemental patterns. Future experiments will be needed to further investigate the roles of the elemental partitioning patterns may play within their traditional ecological or evolutionary niches as well as how different growth conditions may affect their patterns. Our results show mutualist fungi having a lower elemental uptake trend when compared to pathogens and saprotrophs. More research is needed to further investigate the role that different nutrients play within fungal systems, as well as how different conditions may influence these profiles. Finally, with the crucial symbioses that fungi form with plants, understanding how differential nutrient uptake and accumulation strategies may provide some insights into the exchange of nutrients between fungi and their host plants.</p>
GaP19	<p>Jekyll & Hyde: Modeling DMSP-Induced Algicidal Switching in <i>Emiliania huxleyi</i>-<i>Sulfitobacter</i> Interactions</p> <p>Jackson Page-Roth, David Talmy Department of microbiology, University of Tennessee, Knoxville</p> <p>Background: Phytoplankton are central to the global ocean carbon cycle. Their role in marine organic matter cycling has been extensively studied and incorporated into ecosystem models. However, influences of heterotrophic bacteria on elemental flows remain underrepresented in models. <i>Emiliania huxleyi</i> is a bloom-forming, calcifying microalga that exudes dimethylsulfoniopropionate (DMSP) and plays a key role in global sulfur and carbon cycling. <i>E. huxleyi</i> blooms are believed to be moderated by <i>Sulfitobacter</i> D7, a <i>Roseobacter</i> strain, whose pathogenicity toward <i>E. huxleyi</i> is induced by DMSP concentration.</p> <p>Methods: As a step toward representing bacterial pathogenicity in marine ecosystem models, we developed a mathematical model of population dynamics using a series of ordinary differential equations (ODEs) to describe the interactions between <i>E. huxleyi</i> and D7. We present a system of equations that capture dynamics of live cell counts, SYTOX stained dead cells, and measured DMSP concentration from <i>E. huxleyi</i> and D7 co-culture growth experiments.</p> <p>Results: Initially, <i>E. huxleyi</i> grows in co-culture similarly to pure culture, but as it enters stationary phase, the population collapses. We struggled to fit both the <i>E. huxleyi</i> and D7 growth curves in co-culture, as an increased abundance of D7 resulted in a premature decline in <i>E. huxleyi</i> population. We solved this by modulating pathogenicity as a function of estimated DMSP concentration in solution, which delayed <i>E. huxleyi</i> death and dramatically increased model accuracy. Additionally, we found that by using separate parameters for natural <i>E. huxleyi</i> death and D7-induced death, we could incorporate SYTOX stained cells into the model.</p> <p>Conclusion: This model provides a framework for modeling metabolite-initiated pathogenicity in microbial interactions. On larger scales, this framework could be incorporated into ecosystem models to characterize impacts of pathogenic bacteria on algal blooms and their demise.</p>
GaP20	<p>Novel sphingomyelinase inhibitor DPTIP inhibits tick-borne Langat virus replication in medically important ticks</p> <p>Naveen Pant, Durga Neupane, Girish Neelakanta, and Hameeda Sultana Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA</p> <p>Tick-borne flaviviral diseases poses severe public health concerns, yet the molecular mechanisms underlying the flaviviral replication in ticks largely remains unknown. Our current study investigated the effects of two sphingomyelinase inhibitors, GW4869 (a commercially available product) and DPTIP (a novel modified product), potent, cell-permeable, non-competitive pharmacological agents that blocks the production and release of exosome/extracellular vesicles. Langat virus (LGTV, a tick-borne flavivirus) readily infects and replicates in <i>Ixodes scapularis</i> derived ISE6 tick cells and in nymphs. Cell viability and cytotoxicity of GW4869 and DPTIP treatments (at tested dose of 5 µM) assessed using the LDH and MTT classical assays showed no differences in ISE6 cells morphology and viability. Simultaneous treatment and LGTV infection in ISE6 cells significantly reduced LGTV loads compared to the uninfected controls. In <i>in vivo</i> experiments with simultaneous treatment and LGTV infection or pretreatment and LGTV infection in nymphal ticks showed reduction of LGTV loads in DPTIP treated ticks when compared to the uninfected controls and previously proven GW4869 treatments. Our results suggests that DPTIP could be an enhanced and potential therapeutic agent for controlling tick-borne diseases.</p>

GaP21	<p>Next-Generation Probiotics to Combat Clostridium difficile: A One Health Strategy to Address Antimicrobial Resistance</p> <p>Madhav Paudel and Yosra A. Helmy* Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky.</p> <p>Clostridium difficile infection (CDI) is a leading cause of antibiotic-associated diarrhea (ASD) and in severe cases, can lead to pseudomembranous colitis and toxic megacolon in both humans and animals. Although antibiotics remain the primary treatment strategy, their efficacy is increasingly compromised by the emerging multidrug resistance strains, persistent spore reservoirs, and disruption of the native gut microbiota, underscoring the need for safe, gut-friendly alternatives such as next-generation probiotics (NGPs). Unlike conventional probiotics, NGPs are specifically selected and optimized for their ability to target specific pathogens, modulate host immunity, and inhibit microbial virulence.</p> <p>In this study, 46 candidate NGP strains were screened for anti-C. difficile activity using agar well diffusion assays. Twenty-three strains (50%) exhibited measurable inhibition, with zones of inhibition (ZOI) ranging from 13 to 22 mm. Five strains (P3, P7, P14, P32, and P33) demonstrated the strongest activity in whole-culture assays, with mean ZOI values of 20.5 ± 0.5 mm, 19.3 ± 0.8 mm, 15.0 ± 1.0 mm, 20.0 ± 1.0 mm, and 19.5 ± 0.5 mm, respectively. Four strains (P3, P7, P32, and P33) retained inhibitory activity across cell-free supernatant and washed cells, suggesting the involvement of both secreted antimicrobial compounds and contact-dependent mechanisms.</p> <p>These findings highlight the potential of these NGP candidates as biotherapeutic alternatives to conventional antibiotics. Ongoing studies will evaluate their effects on C. difficile virulence traits, stability within the gastrointestinal environment, and their effect on epithelial immune responses and barrier integrity using in vitro models. Within a One Health framework, this work supports the development of microbiome-based interventions to reduce CDI burden and antimicrobial resistance in both animals and humans.</p>
GaP22	<p>ONGOING VIRAL REPLICATION REVEALED IN MULTIPLE TISSUES DURING SUPPRESSIVE COMBINED ANTIRETROVIRAL THERAPY IN THE MACAQUE MODEL OF HIV INFECTION</p> <p>Faezeh Rafiei¹, Melanie N Cash^{2,3}, Jose L, Estrada ^{2,3}, Helen Bai⁴, Elizabeth Patrick⁵, Joseph Nidhiry⁶, Marco Salemi^{2,3}, Brittany Rife Magalis^{1,2,6*}</p> <p>¹Department of Biochemistry and Molecular Genetics, University of Louisville School of Medicine, Louisville, KY, USA; ²Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA; ³Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; ⁴DuPont Manual High School, Louisville, KY, USA; ⁵Health Science Academy, Atherton High School, Louisville, KY, USA; ⁶Center for Precision Medicine, University of Louisville, Louisville, KY, USA</p> <p>Background: Although combination antiretroviral therapy (cART) reduces HIV to undetectable levels in blood, whether viral replication persists in deep tissues during treatment remains unclear. Prior evolutionary studies have reported negligible viral genetic divergence over the course of treatment, suggesting HIV persists through clonal expansion of long-lived infected cells rather than replication; however, these studies have been constrained by limited sequencing depth and breadth from the blood compartment. We hypothesized that the use of near-full-length viral DNA genomes from high-throughput single-cell DNA sequencing (scDNA-seq) of multi-tissue sampling would reveal ongoing viral replication during cART that previous datasets failed to detect.</p> <p>Methods: The S[imian]IV-infected macaque model recapitulates HIV infection and treatment and permits longitudinal sampling of multiple anatomical sites for viral RNA and DNA sequencing. Capitalizing on this model, seven animals underwent SIV infection, cART, and cART interruption. Viral RNA was isolated from longitudinal plasma samples and sequenced using single-genome Sanger sequencing, whereas viral DNA was isolated from peripheral blood mononuclear cells (PBMCs) and tissue using scDNA-seq. Longitudinal tissue sampling included lymph node and lamina propria lymphocytes (gut). Additionally, spleen and brain were sampled at necropsy. Plasma RNA sequences (n=72) from one animal (JA41) were used to calibrate the viral evolutionary rate in BEAST and to estimate the date of origin of DNA sequences (n=1394) using tip date sampling. Estimation was performed across 14 replicates of randomly sampled (with replacement) DNA sequences (n=100) to assess robustness, with effective sample sizes (ESS>200) confirming reliable estimates.</p> <p>Result: In PBMCs and tissue, up to 100% of post-cART lineages were estimated to emerge during cART. Date estimates were consistent across replicates (median range = 1.24 days).</p> <p>Conclusions: These results support ongoing HIV replication during cART, challenging clonal expansion models, though additional animals and evolutionary modeling are necessary to reliably characterize HIV persistence and refine cure strategies.</p>
GaP23	<p>Analysis of Siderophore(s) from Pseudomonas mosselii</p> <p>Kemper Rasnake and Dr. Ranjan Chakraborty East Tennessee State University</p> <p>Iron is an essential nutrient for many organisms. It is an important cofactor in metabolic processes such as the Krebs' cycle and electron transport chain. Therefore, it is necessary for bacteria to obtain iron for their metabolic needs. Yet, in anoxic environments iron is in its insoluble ferric (Fe³⁺) form so bacteria have evolved mechanisms to overcome this. Most prevalent mechanism is by the secretion of low-molecular weight high-affinity iron chelators termed siderophores. Siderophores are secreted in low iron environments to bind iron and bring it back to the bacterial cell.</p> <p>In our work, we collected soil samples from northeastern Tennessee and screened for siderophore-producing colonies on Chrome Azurol S (CAS) agar. Siderophore producing strains were sent for sequencing to identify bacterial species. Pseudomonas mosselii was selected for further studies due to the lack of research on the siderophores it produces. Growth conditions were optimized for increased production of siderophores; then purification is conducted by size exclusion and High-Pressure Liquid Chromatography (HPLC). After purification, structural analysis was conducted with mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.</p> <p>Characterization of novel siderophores will provide a better understanding of siderophores and their potential applications. Siderophores have been used in a "trojan horse" method to combat antibiotic resistance by the conjugation of antibiotic with siderophore, allowing for entry into the cell. Additionally, they have been used for better iron acquisition for crops such as tomatoes. Therefore, this research may contribute to the development of more effective therapeutic strategies, improved clinical outcomes, and better agricultural applications.</p>

GaP24	<p>Development of a CRISPRi Platform to Investigate Root Colonization in <i>Bacillus velezensis</i> GBO3</p> <p>Pallavi Ramaswamy, Ilene Del Valle, Carrie Eckert Biosciences Division, Oak Ridge National Laboratory Bredesen Center for Interdisciplinary Research, Graduate School of Genome Science and Technology, University of Tennessee, Knoxville</p> <p>Background <i>Bacillus</i> is a Gram-positive rhizosphere bacterial genus, with <i>B. velezensis</i> GBO3 widely used as a commercial root inoculant and foliar spray to promote plant growth and suppress disease. Its growth-promoting effects rely on successful root colonization, a process influenced by root exudates such as sucrose that enhance solid surface motility. However, GBO3 inoculants often fail to establish or produce sustained effects in complex soil environments, highlighting a need to better understand the genetic basis of rhizosphere establishment.</p> <p>Methods We developed a CRISPR interference (CRISPRi) system to enable high-throughput, temporal and inducible gene knockdown in <i>B. velezensis</i> GBO3 for genotype-to-phenotype mapping. dCas9 was genomically integrated (strain GED10) under the control of an inducible quorum sensing system (RpaI/RpaR, induced by p-coumaroyl-homoserine lactone [pC-HSL]). A synthetic guide RNA (gRNA) library (~40,000 guides; ~10 guides per gene and 400 non-targeting controls) was constructed. Methylation motifs were modified to avoid restriction by native nucleases and losing library coverage while passing through a GBO3 methylating <i>E. coli</i> strain. The library was amplified in NEB high-efficiency <i>E. coli</i>, electroporated into GED10, and screened under sucrose-selective conditions prior to Illumina sequencing to uncover genotypes that were better suited at sucrose utilization.</p> <p>Results Sequencing revealed that the recovered gRNA population was dominated by a small subset of guides, with most gRNAs absent from the dCas9-integrated strain. This skewed representation indicates a strong founder effect, suggesting that only a limited fraction of the original library successfully established in GED10.</p> <p>Conclusions Adequate representation of the ~40,000-member gRNA library ($\geq 100\times$ coverage) requires a transformation efficiency of $\sim 4 \times 10^6$ cfu/μg, which exceeds the observed electroporation efficiency of GED10 (10^4–10^5 cfu/μg). The loss of library diversity is therefore likely driven by inefficient transformation. To address this, future work will focus on integrating the gRNA library into the GED10 chromosome via natural competency to improve library coverage.</p>
GaP25	<p>Genomic and Phenotypic Characterization of Multidrug-resistant <i>Staphylococcus aureus</i> Across Interconnected One Health Reservoirs</p> <p>Rosbelly Rios, Ajran Kabir, Mohamed Saleh, and Yosra A. Helmy* Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky.</p> <p><i>Staphylococcus aureus</i> is an opportunistic pathogen that affects both humans and animals. Its ability to acquire antimicrobial resistance (AMR), including methicillin resistance, makes it a major One Health concern. We investigated the prevalence, antimicrobial resistance, and genomic characteristics of <i>S. aureus</i> across animal, food, and environmental reservoirs in Kentucky. A total of 1,756 samples were collected from dogs (n = 511), horses (n = 420), retail meats (n = 217), and wastewater (n = 608). Samples were enriched, cultured on selective media, and isolates were confirmed as <i>S. aureus</i> by PCR targeting the nuc gene. Methicillin resistance was determined by mecA detection and cefoxitin disk diffusion. <i>S. aureus</i> was detected in 5.1% (89/1,756) of samples, with 28.1% (25/89) identified as MRSA. A subset of isolates (n = 38) underwent antimicrobial susceptibility testing against eight antibiotic classes. All tested isolates were multidrug-resistant, showing universal resistance to azithromycin and high resistance across β-lactams, aminoglycosides, fluoroquinolones, lincosamides, tetracyclines, rifamycins, and phenicols. All isolates produced biofilm, with 25.6% classified as strong producers. Whole-genome sequencing of a representative subset (n = 15) revealed genomes averaging ~2.78 Mb with GC content of 32–33%. Multiple sequence types (ST) were identified, with ST-1153 most prevalent, and one novel ST was detected. Genomes contained extensive virulence repertoires (~50–65 genes), including determinants associated with adhesion, capsule formation, and biofilm development. AMR genes were detected in all genomes, with β-lactam resistance genes (mecA, blaZ) most common alongside efflux pumps (norA, mepA). Fifteen plasmid replicon types were identified, with rep16 being the most frequent. Phylogenetic analysis placed study isolates within established <i>S. aureus</i> lineages and among international reference strains. These findings demonstrate the circulation of multidrug-resistant <i>S. aureus</i> across interconnected One Health reservoirs, highlighting the importance of integrated phenotypic and genomic surveillance to understand AMR dissemination beyond clinical settings.</p>
GaP26	<p>Dietary fiber reprograms gut microbial effects on lipid accumulation.</p> <p>Anjula Swathi Sagam, Bharosha Adhikari, Md Sifat Habib Siam, Gajender Aleti Tennessee State University</p> <p>The relationship between diet, the gut microbiome, and host metabolism is central to obesity, yet consistent microbial signatures and their functional effects remain poorly understood. Here, we performed a meta-analysis across >200 publicly available microbiome studies to identify taxa consistently associated with obesity. This revealed consistent patterns of microbial enrichment and depletion across cohorts, highlighting candidate taxa associated with obesity or health. To functionally validate these associations, representative bacterial isolates were cultured under low- and high-fiber simulated dietary conditions. Cell-free supernatants were then applied to differentiated adipocytes to assess lipid accumulation. We observed a strong fiber-dependent shift in microbial function. Supernatants derived from low-fiber conditions promoted lipid accumulation, whereas those from high-fiber conditions reduced lipid storage. Importantly, these responses were not strictly taxon-dependent: microbes typically associated with health exhibited pro-lipogenic effects when grown under low-fiber conditions, indicating that dietary context can override canonical taxonomic associations. Together, these findings demonstrate that microbial function, rather than taxonomy alone, governs host metabolic outcomes and that fiber availability is a key determinant of this functional shift. This work provides a mechanistic framework linking diet quality, microbial metabolism, and host lipid accumulation, with implications for microbiome-targeted strategies in obesity prevention.</p>

GaP27	<p>Nutritional Regulation of Microbial Community Assembly and Interactions in the Black Soldier Fly Larval Gut Microbiome</p> <p>Reese Saho, Amber MacInnis, Jeff Tomberlin, Zach Burcham University of Tennessee-Knoxville Texas A&M University</p> <p>Microbial symbionts support host macroorganism growth and development through metabolic cross-feeding, niche partitioning, and competitive exclusion. In the black soldier fly larva (<i>Hermetia illucens</i>; BSFL), the gut microbiome is thought to support generalist decomposition behavior by rapidly responding to diverse nutrient inputs, increasing host metabolic range. However, the ecological processes driving these diet-specific microbiome responses remain unclear, limiting our general understanding of microbiome plasticity under resource change. Here, we tested how low-complexity (single-component) versus high-complexity (multi-component) diets spanning contrasting macronutrient sources shape BSFL gut community assembly, taxon persistence, and putative microbial interactions. We reared BSFL on a complex grain-based diet for 5 days, shifted cohorts to distinct secondary diets for 14 days, dissected guts, extracted DNA, and profiled gut bacterial communities. We found that the secondary diet fed to the larvae explained substantial variation in community composition (PERMANOVA $R^2 = 0.6349$ for unweighted UniFrac; $R^2 = 0.8855$ for weighted UniFrac; $p < 0.001$). Yet, phylogenetic null-model analyses indicated that deterministic assembly processes did not drive deep phylogenetic turnover across diets, suggesting that selection is acting at finer taxonomic resolution while reshaping community relative abundance structure. Next, we tested whether taxa were consistently retained across diets by classifying ASVs by prevalence as "absolute core" (100%), "shell core" (80–99%), or "non-core" (0–79%). Seventeen absolute-core ASVs occurred in all diets and dominated low-complexity diets (>50% mean relative abundance). High-complexity diets were more diverse and included greater contributions from shell-core and non-core taxa, although high-prevalence taxa (absolute + shell core) still comprised the majority of the community. Finally, we investigated whether diet alters the associations among community members. Co-occurrence network inference revealed diet-dependent rewiring of putative interactions where core taxa clustered into distinct modules and association patterns across diets, suggesting that microbiome plasticity may arise through interaction restructuring around core community members. Together, these findings show that deterministic effects of resource environments can emerge as within-lineage sorting and interaction-network rewiring rather than deep phylogenetic turnover, highlighting fine-scale taxonomic resolution and interaction structure as key features to examine when detecting ecological responses to resource change.</p>
GaP28	<p>A chemoreceptor for aerotaxis in <i>Azospirillum brasilense</i></p> <p>Suparna Sarkar, Gladys Alexandre Biochemistry & Cellular and Molecular Biology department, The University of Tennessee, Knoxville</p> <p>Background: <i>Azospirillum brasilense</i> is a plant growth promoting microaerophilic rhizobacterium able to sense and respond to environmental cues through chemotaxis. In <i>A. brasilense</i>, chemotaxis is mediated by two major signaling pathways (Che1 and Che4) and multiple chemoreceptors that coordinate signaling to modulate bacterial motility responses and contribute to plant root surface colonization. A cytosolic chemoreceptor, named Tlp68, possesses a unique domain organization; consisting of an N-terminal protoglobin domain predicted to sense oxygen, a chemotaxis signaling domain followed by a PilZ domain for cyclic-di-GMP binding, and a C-terminal Zinc-binding CZB domain. This domain organization suggests that the chemoreceptor could integrate multiple intracellular and environmental signals to coordinate cellular motility with the physiological state. Here, we aim to characterize the role of Tlp68 in bacterial chemotaxis and its impact of cellular physiology.</p> <p>Methods: We determined the growth pattern of Δtlp68 mutant compared to the parent strain under different aeration and nutritional status. Aerotaxis assays in a spatial gradient of air were conducted to determine if the chemoreceptor contributes to sensing and moving in air gradients, as predicted from its domain organization.</p> <p>Results: Cells lacking the Tlp68 chemoreceptor display an altered growth behavior characterized by a longer lag phase compared to wild-type cells. The Δtlp68 mutant cells exhibit a delayed response and an impaired aerotactic band formation in response to spatial air gradients.</p> <p>Conclusion: These findings suggest that Tlp68 functions in aerotaxis but may also impact growth, though whether the effects are direct, remain to be determined.</p>
GaP29	<p>Characterization of wastewater-derived bacteriophages reveals broad host range activity against multiple <i>Salmonella</i> serovars</p> <p>Eshita Shahanaz¹, Daniel W. Bryan¹, Harleen K. Chaggar¹, Lauren K. Hudson¹, Thomas G. Denes¹</p> <p>Background: Environmental sources contain wide and largely unexplored bacteriophage diversity. Characterized phages can therefore be used to develop rapid diagnostic tools or biosensors for detecting pathogens such as <i>Salmonella</i> in water, food, or environmental samples. This study aimed to characterize genomic features, and host range diversity of newly isolated <i>Salmonella</i> phages from local wastewater.</p> <p>Methodology: Samples for bacteriophage isolation were collected from primary effluent at a wastewater treatment plant in Knoxville, Tennessee. Samples were screened for phage activity against 26 <i>Salmonella</i> strains using the double agar overlay method. For strains that did not produce plaques by direct plating, samples were enriched in liquid culture. Isolates obtained from direct plating or enrichment were plaque purified three times, followed by amplification using the plate lysate method. Host range was assessed using a modified efficiency of plaquing (EOP) assay. A subset of 22 isolates exhibiting distinct host range profiles were selected for Nanopore sequencing, genome assembly, and further characterization.</p> <p>Results: A total of 31 bacteriophage isolates were recovered using the <i>Salmonella</i> host strains, with at least one phage isolated from each host. The EO assay showed that multiple phages exhibited activity against a broad range of <i>Salmonella</i> serovars. Strong phage lysis activity was observed against strains of some <i>Salmonella</i> serovars, including Enteritidis, Heidelberg, Cerro, Dublin, Kentucky, Saintpaul, Panama, and Stanley. Preliminary genomic analysis of the sequenced subset resulted in multiple complete phages with similarity to known <i>Salmonella</i> phages, including <i>Salmonella</i> phages SE131, VSe13, vB_Sen-E22, and vB_SalS_ABTLsp9.</p> <p>Conclusion: These preliminary results from our ongoing <i>Salmonella</i> phage study demonstrate the successful isolation of a diverse collection of <i>Salmonella</i> bacteriophages from wastewater, exhibiting varied host ranges and distinct genomic characteristics.</p>
GaP30	<p>Dietary fiber-driven differences in gut microbiome interactions with per- and polyfluoroalkyl substances (PFAS)</p> <p>Md Sifat Habib Siam, Anjula Swathi Sagam, Bharosha Adhikari, Gajender Aleti Tennessee State University</p> <p>Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants associated with adverse metabolic, immune, and developmental outcomes. Although individual gut bacterial strains have been shown to bioaccumulate PFAS, how complex human gut microbial communities with distinct dietary adaptations interact with PFAS remains poorly understood. Diet quality, particularly fiber intake, strongly shapes gut microbial structure and function and may therefore modulate PFAS retention, transformation, or removal in the gut. Here, we examine PFAS–microbiome interactions using human stool microbiota adapted to low and high-fiber dietary patterns. The most abundant and environmentally relevant PFAS are introduced into <i>in vitro</i> fermentation systems inoculated with these distinct microbial communities. Changes in microbial composition, functional potential, and PFAS distribution are assessed using shotgun metagenomics and mass spectrometry-based metabolomics. PFAS bioaccumulation and partitioning are quantified within microbial biomass and fermentation supernatants, together with associated changes in microbial taxa previously implicated in PFAS binding and transport. Comparative analyses of low- and high-fiber–adapted microbial communities evaluate whether dietary background influences PFAS retention, microbial tolerance, or potential removal. Microbial metabolic activity, including short-chain fatty acid production, is measured to assess functional responses to PFAS exposure. These findings provide new insight into how diet shapes gut microbiome interactions with persistent environmental contaminants and advance our understanding of PFAS–microbiome interactions in human microbial communities, potentially informing microbiome-based dietary or bioremediation strategies to mitigate PFAS exposure and toxicity.</p>

GaP31	<p>Characterization of Cyanophage Resistance in a Microcystis Isolate</p> <p>Smith, L. E. 1, Martin, R. M. 1, Truchon, A. R. 1, Stark, G. F. 1, Wei, B. 2, Boyer, G. L. 2, and Wilhelm, S. W. 1 1Department of Microbiology, University of Tennessee 2Department of Chemistry, State University of New York College of Environmental Science and Forestry</p> <p>Background: Harmful algal blooms are an increasing concern in freshwater ecosystems. <i>Microcystis aeruginosa</i> is a prevalent cyanobacterium in these events and can disrupt both ecological function and local economies. Viral predation significantly influences algal growth and evolutionary dynamics. Methods: To elucidate mechanisms of viral resistance within these populations, we used <i>M. aeruginosa</i> strain NIES-298 and its phage, Ma-LMM01. We selected a resistant mutant (NIES-298φR-B) in continuous culture chemostats, which was characterized, sequenced, and compared to the wildtype strain. Results: NIES-298φR-B displayed a lack of virus particle adsorption to the surface, reduced buoyancy, increased cell volume, and reduced toxin quota, along with 20 discrete genetic disparities. One of the mutations disrupts a putative surface protein, so we hypothesize this protein may be needed for viral adsorption. Conclusion: In contrast to the traditional "cost of resistance" paradigm, this phenotype appears competitively robust, suggesting that the associated mutations may not be detrimental but instead represent neutral "hitchhiking" changes. Continued characterization of this system will help clarify the mechanisms underlying host-phage interactions in algal bloom dynamics.</p>
GaP32	<p>Mulch-Derived Microplastics & Millipede Microbiomes</p> <p>Cyric Stults,¹ Ernest Bernard,² Andrea Valdyke,² Jennifer DeBruyn¹ 1: Department of Microbiology, University of Tennessee, Knoxville; 2: Department of Entomology and Plant Pathology, University of Tennessee, Knoxville</p> <p>Background Commercial agriculture relies heavily on plastic products. Mulch film, one such plastic product, increases crop yield by stifling weed growth, retaining moisture, and preventing soil erosion. Most mulch films are composed of polyethylene (PE), although some alternative biodegradable materials exist, such as polybutylene adipate terephthalate (PBAT). Despite being discarded at the end of the growing season, these films inevitably leave behind microplastics. These microplastics may be ingested by soil-dwelling detritivores, which play critical ecological roles as nutrient cyclers. The impacts of microplastics, biodegradable or otherwise, on the gut microbiomes of these organisms is unclear. Will consumption of mulch-derived PBAT microplastics drive changes in community composition of millipede gut microbiomes? Methods A four-month feeding experiment was conducted in which American giant millipedes (<i>Narceus americanus</i>) were exposed to PBAT plastic fragments. DNA was extracted from millipede fecal pellets and gut samples, then sequenced using amplicon sequencing targeting 16S and ITS regions to capture bacterial and fungal diversity, respectively. Reads were partitioned using DADA2. Statistical analysis is ongoing. Results and Conclusions Fecal samples differ from gut samples in terms of both alpha and beta diversity. While there was no significant difference in bacterial alpha diversity detected between fecal samples, there appears to be a potential decrease in species richness in gut samples from millipedes exposed to PBAT microplastics. Differential abundance analyses are ongoing and may shed light on potential microbial community shifts between treatments.</p>
GaP33	<p>Single-cell transcriptomic profiling of peripheral blood mononuclear cells in <i>Haemonchus contortus</i>-resistant and susceptible sheep</p> <p>Hannah G. Teddleton, Taylor B. Seay, Trevor F. Freeman, Jonathan Beever, and Elizabeth Shepherd Department of Animal Science, University of Tennessee, Knoxville</p> <p>Background: Grazing ruminants are subject to severe pathologies from gastrointestinal nematode <i>Haemonchus contortus</i>, where infection in young or susceptible animals can cause extreme anemia, and in some cases, death. Resistance to <i>H. contortus</i> infection is characterized by a robust and early T-helper 2 (Th2) response, resulting in reduced worm burdens and increased survivability. Parasite-resistant St. Croix sheep (STC) generate strong Th2 responses resulting in a rapid clearance of <i>H. contortus</i>, while parasite-susceptible Suffolk sheep (SUF) have delayed responses. While rapid Th2 responses are implicated in parasite resistance, the frequency and diversity of immune cell populations in sheep remain uncharacterized. Therefore, the objective of this study was to investigate the cellular and transcriptional landscape of peripheral blood, utilizing a model of parasite susceptibility and resistance in sheep. Methods: To address this, peripheral blood mononuclear cells (PBMCs) were isolated from parasite naive and <i>H. contortus</i> primed SUF and STC for single cell RNA sequencing. Library preparation was generated using the Evercode™ split-pool combinatorial barcoding and data were processed using the Parse Biosciences Pipeline (v1.3.1, Parse Biosciences) and analyzed using Seurat v5 (Satija Lab). Cell clustering, annotation, and differential gene expression analysis were performed to identify transcriptomes associated with phenotypic differences between infection status and between breeds. Results: In total, 15 cell types were identified, including T cells, B cells, monocytes, and dendritic cells. Additionally, rare cell types such as low-density eosinophils and neutrophils were identified, revealing the novel characteristics of the ovine immune cell type signature. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) identified key transcriptional pathways separating primed SUF and STC PBMCs. Conclusions: Together, identification of the ovine-specific immune cell landscape associated with parasite resistance provides critical insights into the cellular basis of host-parasite dynamics, informing improved parasite control strategies in susceptible livestock.</p>
GaP34	<p>Exploring <i>Proteus mirabilis</i>-specific bacteriophage from blow flies (Diptera: Calliphoridae) in Knoxville, Tennessee</p> <p>Makhali S. Voss*, MS1; Daniel Bryan2; Thomas Denes, PhD2; Charity G. Owings, PhD, M-ABFE1 1 The University of Tennessee Department of Entomology and Plant Pathology 2 The University of Tennessee Department of Food Science</p> <p>Blow flies are cosmopolitan necrophages that exhibit complex microbial interactions on and around carrion and other microbe-laden environments. Some bacteria, such as <i>Proteus mirabilis</i>, can mediate blow fly behavior (i.e., attraction, egg-laying) through quorum sensing and volatile organic compound production. However, other microbial associations remain vastly understudied, including potential tri-trophic interactions between blow flies, bacteria, and bacteriophage (i.e., "phage"; ubiquitous bacteria-killing viruses). Although phage have been isolated from filth flies previously, no published studies describe phage isolation and characterization from blow flies specifically, though such knowledge could be informative and valuable for agricultural and biomedical applications. Therefore, this research focuses on the isolation of <i>P. mirabilis</i>-specific phage from wild-caught blow flies. Fly homogenates were filtered and subjected to pass-through enrichment for three consecutive days prior to plating. Distinct plaques were selected based on general morphology and purified. Four <i>P. mirabilis</i>-specific phage were isolated: two from mixed-species fly samples and two from single-species fly samples of the secondary screwworm, <i>Cochliomyia macellaria</i> (Fabricius). As <i>C. macellaria</i> is the closest genetic relative to the New World screwworm, <i>Cochliomyia hominivorax</i> (Coquerel), an obligate parasite of vertebrates, these findings may have broader implications for future suppression strategies of this pest. Isolated phage from one sample have putatively been identified as Podovirus via transmission electron microscopy and whole genome sequencing. Myovirus and Siphovirus are present in the sample as well, potentially indicative of prophage. Future directions involve further characterization of isolated phage, as well as controlled bioassays to evaluate the potential of phage in suppressing fly-bacterial communication.</p>

GaP35	<p>Inclusion of synthetic protein diversity using biophysical constraints increases AI resilience of DNA synthesis screening</p> <p>Zoey Wexler, Brittany Rife-Magalis, PhD. University of Louisville</p> <p>Background: DNA synthesis order screening aims to protect against biological threats and commonly relies on comparing orders against databases of known sequences of concern (SoC). One growing area in Artificial Intelligence (AI) poses a threat to this screening, which includes the generation of novel protein sequences with anticipated function. Commonly used sequence-based screening concepts, such as profile hidden Markov models (HMM), have recently demonstrated to be somewhat limited in terms of AI resilience, requiring novel ways of thinking to improve screening against AI threats.</p> <p>Methods: A directed evolution (DE) algorithm was implemented to introduce unseen diversity into HMMs to determine its performance against filtered DNA fragments generated from the Protein Message Passing Neural Network (MPNN) model. Naturally occurring ricin sequences were gathered from multiple toxin databases and used in the DE approach, which calculates standard biophysical rules for existing sequences to generate new amino acids (among those permissible) at each site. Added sequences were verified structurally using AlphaFold. The extent of added sequence diversity was calculated and evaluated to determine sufficient diversity required to improve HMM detection of various MPNN fragment sizes.</p> <p>Results: The DE approach has the capability of expanding sequence diversity up to 4.5-fold over naturally occurring ricin (10.39%, SD = 0.1143). AlphaFold characterization of randomly selected DE sequences with the highest diversity revealed 100% structural consistency, either characterized as ricin (23.3%) or a ricin-based ribosome inactivating protein (76.7%). The addition of 50 to 125 DE sequences (35.54-43.23 % increased diversity) increased flagged AI sequences of 100 nucleotides (nt) in length by 9%; however, remaining fragment sizes (50, 75, 125, 150, 175, 200 nt) remained unchanged.</p> <p>Conclusion: Directed evolution based on known biophysical constraints enhances diversity used for HMMs, while maintaining predicted protein structure, and may provide a promising tool for boosting AI resilience of DNA synthesis screening.</p>
Gbp1	<p>Glycosyltransferases Encoded within the Tailocin Locus Governs Tailocin Resistance in <i>Xenorhabdus</i> Bacteria</p> <p>Emmanuel. C. Allwell 1 , Sarah Kauffman 1 , Micear Podar 3 , Heidi Goodrich-Blair 1 ; 1 Microbiology, The University of Tennessee-Knoxville - UNITED STATES, TN, Knoxville, TN, United</p> <p>Increasing resistance to antibiotics is a top global health issue, and bacterial tailocins are one potential alternative to antibiotics. Tailocins, resembling P2 phage-like contractile structures, mediate inter-microbial competition by eliminating target cells through membrane depolarization. Structurally, tailocins resemble phage tails: a contractile sheath wrapped around an inner tube, capped by a baseplate, and extended by the tail fiber. The tail fibers comprise a DUF3751 domain mediating attachment to the baseplate and a C-terminal receptor-binding domain (RBD), crucial for binding to the target bacterial cell surface. <i>Xenorhabdus nematophila</i>, is an insect-pathogenic bacterium that lives in mutualism with nematodes, and all analyzed strains encode a tailocin, each with a main tail fiber that varies across strains in the RBD, with the predicted consequence of varying target strain specificity. Each <i>X. nematophila</i> strain tailocin locus also contains a variable region, termed the intervening sequence (IVS), between the genes encoding the main tail fiber and the tail sheath. IVS genes include those predicted to encode RBD without the DUF3751 domain and those predicted to encode glycosyl transferases (GT). The role of IVS-encoded genes in tailocin sensitivity and resistance is unknown. We hypothesize that the IVS-encoded RBD may serve as defensive molecules by binding to the producer cell surface and blocking binding by enemy tailocins with the same RBD. We further hypothesize that IVS-encoded GT may confer tailocin resistance by modifying cell surface receptors. To test these hypotheses, whole-IVS deletion mutants were created in two <i>X. nematophila</i> strains (ATCC19061 and F1). These mutants displayed increased sensitivity to tailocins compared to their wild-type counterparts, suggesting a potential role in immunity against tailocin-mediated killing. To determine which IVS-associated genes confer this protection, we deleted each IVS-associated RBD and GT. While RBD mutants retained wild-type levels of tailocin resistance, the GT mutant exhibited increased sensitivity to tailocins, phenocopying the whole-IVS deletion phenotype. Complementation with the GT restored resistance to wildtype levels. Also, purified lipopolysaccharides (LPS) from GT mutants but not the wild type strains blocked tailocin activity. This suggest that the IVS-encoded GT confers tailocin resistance, likely through modification of cell surface LPS, which have been implicated as potential receptors for tailocins in other bacterial systems.</p>
Gbp2	<p>Ethylene signaling represses nitrogen fixation in <i>Azospirillum brasilense</i></p> <p>Ishita Banerjee, Gladys Alexandre Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville</p> <p>Background: The rhizosphere, the soil surrounding plant roots, is influenced by root exudates and microorganisms. Conditions in the rhizosphere affect microbial activity. Phytohormones and microbially produced hormones, which can alter plant physiology, further modulate rhizosphere interactions. Bacteria have evolved strategies to adapt to the competitive rhizosphere environment, including sensing phytohormones and nitrogen fixation. Ethylene is a plant hormone that regulates plant growth, development, and stress responses. While its role in plant-microbe interactions has been studied mainly from the plant's perspective, little is known about its function in microbes. Previous study showed that the plant-growth-promoting rhizobacterium <i>Azospirillum brasilense</i> possesses a functional ethylene receptor, named <i>Azospirillum Ethylene Receptor 1</i> (AzoEtr1), which binds ethylene with high affinity, causing changes in metabolism, as well as reducing biofilm formation and root colonization. The goal of this project is to characterize mechanisms by which ethylene affects nitrogen fixation, which is critical for the beneficial effects of <i>A. brasilense</i> on plants.</p> <p>Methods: Using transcriptional reporter assays in the WT and the <i>etr1</i> mutant backgrounds, we characterized promoter activity of selected genes encoding proteins that regulate nitrogen fixation in the absence and in the presence of ethylene. Additionally, untargeted metabolomics under the same conditions was used to test hypotheses generated by promoter activity analyses.</p> <p>Results: In nitrogen-fixing conditions, ethylene exposure suppressed promoter activity of <i>glnB</i>, <i>rpoN</i>, and <i>nifH</i> in both the WT background and in <i>etr1</i> mutant, consistent with a previously proposed model in which AzoEtr1 functions as a reverse agonist (i.e., ethylene binding to AzoEtr1 turns off signalling). Untargeted metabolomics linked ethylene signalling via AzoEtr1 to changes in specific metabolism that are caused by increased glutamine levels.</p> <p>Conclusion: Our data suggest a model in which sensing ethylene via AzoEtr1 reduces nitrogen fixation with accumulation of glutamine, a likely key metabolite regulating these effects, since glutamine directly regulates <i>GlnB</i>, which is required for induction of nitrogen fixation gene expression and nitrogenase activity.</p>

Gbp3	<p>Dengue virus induced endothelial hyperpermeability by altering the expression of upstream regulatory factors of genes involving vascularization</p> <p>Swarnendu Basak, Girish Neelakanta, and Hameeda Sultana University of Tennessee, Knoxville</p> <p>Dengue virus (DENV) infection constitutes a significant global health burden, with an estimated 390 million infections annually and considerable associated morbidity and mortality. The most severe clinical manifestations—dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are distinguished by pathological vascular leakage resulting from pronounced endothelial hyperpermeability. Integrity of the endothelial barrier is maintained by complex intercellular junctional complexes, including tight and adherens junctions, with claudin family members and occludin serving as pivotal determinants of paracellular permeability and junctional stability. Notably, recent studies implicate the flavivirus nonstructural protein 1 (NS1) as an effector that precipitates endothelial dysfunction and promotes plasma extravasation in vivo. Despite these insights, the precise molecular cascades that orchestrate junctional disruption during severe DENV infection remain incompletely characterized.</p> <p>In this study, we have validated expression of upstream regulatory factors that are significantly modulated upon DENV infection. Based on these results, we will next interrogate the functional activity of the identified regulators in the context of viral infection by using trans endothelial electrical resistance (TEER) assays, loss- and gain-of-function approaches, and complementing biochemical analyses, we will determine whether these upstream effectors are responsible for the emergence of endothelial hyperpermeability. Further mechanistic dissection will focus on how these regulators influence in altering the expression of pore forming as well as barrier forming claudins and occluding proteins, thereby compromising junctional integrity. Elucidation of these pathways not only refine our mechanistic understanding of dengue-associated vascular leakage but also facilitate identification of early biomarkers predictive of barrier dysfunction and inform the rational design of targeted therapeutics to mitigate severe disease.</p>
Gbp4	<p>ICAM-1 Mediated Regulation of the Immune Response and Barrier Integrity in Group B Streptococcal Vaginal Colonization</p> <p>Abbey E. Behler, N. Smith, M. Hetzel, A. Monteith, and Lindsey R. Burcham Department of Microbiology, University of Tennessee, Knoxville</p> <p>Streptococcus agalactiae or Group B Streptococcus (GBS) is a Gram-positive, opportunistic pathogen that resides in the gastrointestinal and reproductive tracts of roughly 25-30% of adults. GBS ascending infection during pregnancy can elicit a heightened inflammatory response, making GBS a leading cause of infection-induced preterm birth globally. One potential marker of preterm birth is elevated levels of soluble intercellular adhesion molecule 1 (sICAM-1), though typically expressed on the surface of host cells. ICAM-1 is primarily associated with leukocyte recruitment to infected tissue. However, little is known about how ICAM-1 expression impacts bacterial ascension and the onset of preterm labor. Challenge of human endocervical cells with pathogens associated with preterm labor including GBS and Escherichia coli resulted in induction of ICAM-1 expression while exposure to non-pathogenic microbes such as Lactobacillus gasseri and Enterococcus faecalis failed to induce ICAM-1 expression. We developed an in vitro 3D model of the endocervical epithelial barrier growing columnar endocervical at the air liquid interface on transwells, and found that GBS infection induced barrier damage detectable through FITC-dextran passage and transepithelial resistance. Female C57BL/6 mice challenged intravaginally with GBS showed a marked induction of ICAM-1 production in reproductive tissues compared to naïve controls. GBS vaginal colonization of WT C57BL/6 animals revealed a robust influx of myeloid cells in reproductive tissues that was reduced in ICAM-1^{-/-} animals, suggesting ICAM-1 plays a crucial role in driving the proinflammatory response to GBS in the reproductive tract. Altogether, these findings provide insight as to how the host response to GBS promotes inflammation, potentially contributing to adverse health outcomes associated with GBS carriage.</p>
Gbp5	<p>Hydrogen peroxide exposure elevates nutrient stress response in Prochlorococcus grown under nitrogen limitation.</p> <p>Emily C. Bowden, Erik R. Zinser Department of Microbiology, University of Tennessee, Knoxville</p> <p>Background: The marine cyanobacterium Prochlorococcus is the most abundant photosynthetic organism on Earth and is responsible for fixing four gigatons of carbon annually. Notably, Prochlorococcus is extremely vulnerable to hydrogen peroxide (H₂O₂), present in the ocean's surface where these cells reside. Despite its ecological importance, our understanding of Prochlorococcus response to H₂O₂-mediated stress is limited. While Prochlorococcus inhabits oligotrophic waters, previous studies have been conducted in unrealistic, nutrient replete conditions. Because nutrient stress has been shown to increase sensitivity to oxidative stress in some phytoplankton, we proposed work to examine how H₂O₂ exposure alters the physiological state of Prochlorococcus when cultured under nitrogen limitation.</p> <p>Methods: Prochlorococcus strain MIT9215 was cultured in nitrogen limited chemostats at low cell abundances. Control cultures received no amendments, while treatment cultures were challenged with multiple pulses of ~200 nM H₂O₂ over the course of multiple days. Samples were taken for photophysiological measurements, transcriptomics, and intracellular metabolomics.</p> <p>Results: Photosynthetic efficiency of control cultures remained steady, while efficiency decreased in cultures experiencing H₂O₂ amendments. Cells exposed to H₂O₂ displayed increased expression of genes involved in nitrogen stress response, photosynthesis, and protein turnover compared to those cells not exposed to H₂O₂. Intracellular metabolomics showed in H₂O₂-exposed cells a decrease in nucleosides and several amino acids, especially those containing sulfur, perhaps suggesting the funneling of resources into biosynthesis for cellular repair during oxidative damage.</p> <p>Conclusions: These experiments demonstrate that Prochlorococcus growing in the ocean surface can face elevated levels of nutrient stress when also exposed to H₂O₂, a link that was not previously appreciated. Continuing to study Prochlorococcus under oligotrophic conditions combined with additional environmental stressors will improve our understanding of Prochlorococcus ecology and its role as a primary producer.</p>

Gbp6	<p>PtsN1 links chemotaxis signaling to nitrogen fixation and other phenotypes in <i>Azospirillum brasilense</i></p> <p>Tasneem Ehsan, Emily Beaird and Gladys Alexandre Biochemistry & Cellular and Molecular Biology Department The University of Tennessee, Knoxville, TN, USA</p> <p>Background : <i>Azospirillum brasilense</i> are motile and chemotactic soil bacteria that colonize a wide array of plant species. <i>A. brasilense</i> utilizes two chemotaxis systems, named Che1 and Che4 to direct flagellar-dependent chemotaxis. Mutations that abolish these chemotaxis systems affect non-chemotaxis phenotypes such as nitrogen metabolism and flocculation through unknown mechanisms. Pulldown and Bacterial two-hybrid assays (BacTH) identified a non-chemotaxis interactor PtsN1 for both histidine kinases CheA1 and CheA4 and scaffolding proteins CheW1 and CheW4. PtsN1 encodes the output regulator EIINtr of nitrogen related phosphotransferase system (PTS_{Ntr}) which exist and function in both phosphorylated and dephosphorylated states depending on cellular metabolism. These results suggest PtsN1 interacts with chemotaxis signaling array base plate to potentially integrate chemotaxis and metabolic signaling.</p> <p>Methods: A combination of mutation and overexpression constructs altering PtsN1 activity were used to characterize its role in <i>A. brasilense</i>. These strains and constructs were characterized for chemotaxis by tracking swimming velocity and reversals; nitrogen fixation by calculating colony-forming units, promoter activity and BacTH; and flocculation at high aeration and carbon: nitrogen conditions.</p> <p>Results: Compared to the wild-type strain, a ΔptsN1 strain exhibited altered growth phenotypes with distinct nitrogen sources similar to defects previously observed in ΔcheA1 and ΔcheA4 strains and consistent with interactions between these proteins. We found that PtsN1 regulates nitrogen fixation and identify the underlying molecular mechanism for this role. A ΔptsN1 strain flocculated slower and produced larger polyhydroxybutyrates (PHB) granules compared to the wild type. Overexpressing PtsN1 and/or its phosphomimetic or phosphonull derivatives in the ΔcheA1 and the ΔcheA1cheA4 mutants rescued the flocculation defect of these mutant strains, suggesting their flocculation phenotype is related to interaction with PtsN1. We also discovered that PtsN1 and its derivatives affected motility (but not flagellation) motility when expressed in the wild type and chemotaxis mutant backgrounds, suggesting integration with other, yet uncharacterized regulatory networks impacting motility.</p> <p>Conclusions : Our results suggest a model in which PtsN1 interaction with chemotaxis signaling arrays coordinates multiple regulatory network that link chemotaxis, motility and metabolism. Given that similar interactions between PtsN and CheA homologs have been identified in other bacteria, we hypothesize such signal integration is widespread.</p>
Gbp7	<p>Active Vitamin D Reduces SARS-CoV-2 Infection in the Salivary Gland</p> <p>Denny Gao; Erika L. Figgins; Gill Diamond University of Louisville</p> <p>Background: SARS-CoV-2, which causes COVID-19, primarily targets the respiratory tract to cause disease. However, SARS-CoV-2 can infect multiple areas in the body, including the oral cavity, a potential reservoir of infection. SARS-CoV-2 also rapidly mutates and causes breakthrough infections, making the development of alternative therapeutic and prophylactic drugs a pressing concern. We have previously observed that pretreatment with the hormonally active form of vitamin D, 1,25(OH)₂D₃, reduces SARS-CoV-2 infection in the lung adenocarcinoma Calu-3 cell line. We have built upon our previous work to examine the impact of vitamin D against SARS-CoV-2 infection in the salivary gland.</p> <p>Methods: We used the human salivary gland carcinoma cell line A253 for our experiments. A253 cells were treated with 0nM (control) or 100nM 1,25(OH)₂D₃ for 24hr. To assess viral infection, pre-treated cells were then mock-infected or infected with SARS-CoV-2 at an MOI of 0.5 for 48hr. Cells and cell supernatants were harvested to assess viral infection and gene expression, as well as protein levels of ACE2 in cell lysates and on the cell surface.</p> <p>Results: We determined that pretreatment of A253 cells with active vitamin D increases production of CAMP to produce LL-37, and that vitamin D pretreatment significantly reduces SARS-CoV-2 infection. This reduction was attenuated by changing the media directly before SARS-CoV-2 infection, or by adding CAMP-specific siRNA during pretreatment. Furthermore, active vitamin D also significantly reduces ACE2 protein levels in cell lysate and on the surface of the cell, which may further reduce SARS-CoV-2 infection. Vitamin D pretreatment also decreased mRNA levels of ACE2 and TMPRSS2.</p> <p>Conclusions: Vitamin D utilizes multiple mechanisms, including inducing LL-37 production and reducing ACE2 surface levels, to reduce SARS-CoV-2 infection in the salivary glands, supporting its use as a potential prophylactic drug against SARS-CoV-2.</p>
Gbp8	<p>Host factors alter membrane organization and drug sensitivity of <i>Enterococcus faecalis</i></p> <p>Troy Getty, Liz Fozo Department of Microbiology, University of Tennessee Knoxville</p> <p><i>Enterococcus faecalis</i> is a Gram-positive commensal bacterium of the mammalian intestine that can become an opportunistic pathogen, resulting in bacteremia and endocarditis. In the gastrointestinal tract, <i>E. faecalis</i> is exposed to bile, and during extraintestinal infections, it encounters serum. Both bile and serum contain host-derived fatty acids that the bacterium incorporate into its membrane, resulting in increased tolerance to membrane-damaging agents. Given this alteration in membrane content, we examined whether the organization of fluidmembrane regions also changed, as this can correlate with resistance to certain membranetargeting antibiotics. In control cultures, the entire membrane stained evenly with DiIc-12, which preferentially inserts into fluid membrane regions. Growth with either bile or sera resulted in regions of high staining, appearing as bright puncta. Previous work indicated that the membrane protective effects of bile or sera can be mimicked by providing the host-derived unsaturated fatty acid oleic acid <i>E. faecalis</i>. We observed similar patterns of DiIc12 staining to bile or sera supplemented cells when provided oleic acid alone; this suggests that oleic acid is a primary driver for membrane organization in bile or sera exposed cells. Given that oleic acid, bile or serum can induce tolerance to the membrane-targeting antibiotic daptomycin, we hypothesized that cells grown in these fatty acid sources would have altered interactions with the drug. Treatment with daptomycin resulted in significant alterations in the organization of membrane fluid regions. Localization of the drug varied depending on whether <i>E. faecalis</i> was grown in the presence or absence of oleic acid: oleic acid grown cells had the drug dispersed around the membrane while control cells showed localization of the drug near regions of cellular growth. Ongoing work is elucidating the bacterial factors that contribute to membrane domain formation. These findings, combined with others, suggest active membrane re-organization of <i>E. faecalis</i> is dependent upon environmental growth conditions. Further, this works provides a mechanistic basis for how host fluids can induce drug tolerance within <i>E. faecalis</i>.</p>

Gbp9	<p>EFFECTS OF RED CLOVER ISOFLAVONES ON VASCULAR FUNCTION AND RUMINAL MICROBIAL COMMUNITIES IN BEEF CATTLE</p> <p>Juan Fernando Cordero Larena¹, Madison T. Henninger¹, Kyle J. McLean¹, F. Neal Schrick¹, Gary Bates², and Phillip Myer^{1*} University of Tennessee</p> <p>Background: Tall fescue toxicosis negatively impacts cattle performance and physiology. Red clover isoflavones are phytoestrogens with the potential to mitigate these effects and modulate ruminal microbial ecology. However, the effects of isoflavones on host vascular responses and rumen microbial communities undergoing fescue toxicosis conditions remain unclear. The objective was to evaluate the impact of isoflavone supplementation on vascular function, animal performance, and ruminal bacterial communities in beef cattle undergoing fescue toxicosis.</p> <p>Methods: Thirty Angus steers were assigned to a grazing 45-day trial, consisting of a completely randomized design with three treatments: (1) tall fescue only (CON), (2) tall fescue + dried distillers' grains (DDG; used as carrier), or (3) tall fescue + DDG + isoflavones (ISO; 100 g/animal/day). Hemodynamics were assessed biweekly via color Doppler ultrasonography. Body weight and rectal temperature were recorded weekly for performance. Rumen contents were collected on day 45 for bacterial 16S analyses. Hemodynamics were analyzed in SAS (9.4) using PROC MIXED and PROC UNIVARIATE procedures. Read cleaning and taxonomic classification were performed with packages 'phyloseq' and 'DADA2'. Bacterial differential abundances were analyzed with 'DESeq2'. Diversity analyses were conducted using 'vegan'.</p> <p>Results: Isoflavone supplementation increased systolic peak velocity ($P = 0.04$) and numerically increased VolMAX ($P = 0.09$), VolMIN ($P = 0.07$), and arterial inner-diameter ($P = 0.13$). Rectal temperature was not affected by treatment ($P = 0.43$). Average daily gain was greater in DDG ($P = 0.04$). Firmicutes were greater in ISO ($P < 0.05$). Prevotella, Christensenellaceae, Rikenellaceae RC9, and Ruminococcus were greater in ISO ($P < 0.05$). No differences were detected in alpha- and beta-diversity.</p> <p>Conclusions: Isoflavone supplementation modulated host vascular responses and altered ruminal bacterial community composition in cattle grazing undergoing fescue toxicosis. These findings support a link between isoflavones, ruminal microbial ecology, and host physiological responses, with implications for mitigation of fescue toxicosis in grazing systems.</p>
Gbp10	<p>An Aromatic Affair: Pollutant-Membrane Interactions in a Model Marine Bacterium, Ruegeria pomeroyi DSS-3</p> <p>Saloni Gupta¹, Emily Campbell², Tessa Calhoun^{2,3} and Alison Buchan¹ Departments of Microbiology ¹, Chemistry ², and Biochemistry and Molecular Biology ³ at the University of Tennessee, Knoxville</p> <p>Aromatic compounds are widespread environmental pollutants with significant impacts on marine ecosystems. Polycyclic aromatic hydrocarbons (PAHs), derived from crude oil and fossil fuel combustion, and lignin-derived aromatic compounds (LDACs), released through paper pulp industry discharge, can accumulate in bacterial membranes due to their hydrophobicity. Although studied in only a few species, membrane partitioning of these compounds has been shown to disrupt lipid packing, alter membrane fluidity, and impair energy transduction, ultimately affecting cell viability and function. <i>Ruegeria pomeroyi</i> DSS-3, a model member of the environmentally relevant Roseobacteraceae family, can utilize LDACs as primary growth substrates and co-metabolically transform PAHs. However, the physiological consequences of these interactions remain poorly characterized, limiting our ability to predict bacterial tolerance and their bioremediation potential. We hypothesize that para-hydroxy benzoate (POB, an LDAC) and phenanthrene (a PAH) elicit distinct, concentration-dependent perturbations to membrane organization, fluidity, and outer membrane composition. These differences likely reflect variation in their chemical properties and modes of interaction, with downstream effects on cell viability. To test this, we are employing a suite of complementary approaches: i) second harmonic scattering (SHS) with the membrane-active dye, Di-4-ANEPPDHQ, to probe membrane potential and dynamics; ii) DPH fluorescence anisotropy to assess membrane fluidity; iii) viable plate counts to quantify toxicity; and iv) LPS profiling to evaluate outer membrane remodeling. Together, this work will define membrane-level stress responses in <i>R. pomeroyi</i> DSS-3 exposed to aromatic compounds, advancing understanding microbial tolerance and bioremediation in polluted coastal environments.</p>
Gbp11	<p>Controlling matric potential in microfluidics to examine microbial dynamics in unsaturated porous media</p> <p>Shane M. Franklin¹, Scott T. Retterer^{1,2}, Amber N. Bible¹, Jennifer L. Morrell-Falvey¹ 1Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA 2Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA</p> <p>The use of microfluidics for the study of soil microbial ecology is an emerging field. Most microfluidic studies of biological systems, however, have been performed under fully saturated conditions that are not representative of natural soil. Therefore, while microfluidics offer many unique capabilities that other methodologies cannot, they are not currently suited to address the effects of matric potential, an important variable defining the microbial moisture niche. Here, a methodology is presented that allows the user to control the aqueous conditions within microfluidic networks by manipulating matric potential using a hanging water column.</p>
Gbp12	<p>Fever as a Metabolic Regulator of Host Immunity and Staphylococcus aureus Physiology</p> <p>Bailey E. Holder¹, Eva Belevska¹, Eden G. TenBarge¹, Aliyah J. Collins¹, Callista P. Reber¹, Jadan Zavestoski¹, Ashley D. Wise¹, Andrew J. Monteith^{1,2} 1Department of Microbiology, University of Tennessee, Knoxville, TN 2Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN</p> <p>Background: Fever is a conserved component of the host response to infection, yet febrile temperature is rarely incorporated into experimental models of bacterial pathogenesis. During infection with <i>Staphylococcus aureus</i>, neutrophils mediate bacterial clearance through reactive oxygen species (ROS) production and neutrophil extracellular trap (NET) formation. Temperature also regulates bacterial metabolism and virulence gene expression. How febrile conditions influence both host immune cell function and bacterial physiology remains poorly defined.</p> <p>Methods: A murine model of invasive <i>S. aureus</i> infection was used to compare tissue specific neutrophils from mice housed at standard room temperature (23°C) or thermoneutral conditions (30°C), which permit a febrile response to infection. Neutrophil mitochondrial biomass, oxidative burst capacity, and NET formation were assessed. In parallel, <i>S. aureus</i> monocultures were grown in human-like media at 37°C or 39°C, followed by overnight incubation and short-term back dilution, to evaluate membrane potential and intracellular ATP levels.</p> <p>Results: Neutrophils from fever-permissive mice exhibited significantly increased mitochondrial biomass within infected cardiac tissue, consistent with metabolic reprogramming. This shift was accompanied by reduced oxidative burst capacity and enhanced NET release, indicating altered effector prioritization under febrile conditions. Independently, <i>S. aureus</i> cultured at 39°C demonstrated altered membrane polarization and decreased intracellular ATP levels compared to 37°C controls, suggesting temperature-driven perturbations in bacterial bioenergetics.</p> <p>Conclusions: These findings indicate that febrile temperature functions as a shared environmental cue that independently reprograms neutrophil metabolism and <i>S. aureus</i> energy homeostasis. Incorporating physiologically relevant thermal conditions into infection models is essential for accurately defining mechanisms that influence host-pathogen interactions and disease progression.</p>

Gbp13	<p>Environmental Desiccation Links Group B Streptococcus Persistence to Ascending Vaginal Colonization</p> <p>Tamanna Jafar¹, Lindsey Burcham¹ ¹University of Tennessee, Knoxville, TN</p> <p>Group B Streptococcus (GBS) is an opportunistic pathogen that colonizes the female reproductive tract. GBS also contributes to adverse outcomes such as stillbirth and preterm birth in pregnant mothers. To successfully colonize this complex niche, GBS must adapt to fluctuating nutrient availability and intense microbial competition, yet it is unclear whether GBS can also persist for extended periods in nutrient-poor environments. We investigated how GBS adaptation to nutrient starvation conditions shapes GBS physiology and host interactions. Our data show that GBS can survive desiccation conditions for up to 105 days and withstand low-temperature stress for 115 days in the complete absence of nutrients, demonstrating prolonged environmental persistence. When GBS desiccated for 30-days were inoculated into a murine vaginal colonization model, we observed significantly higher recovered CFU in vaginal, cervical, and uterine tissues than non-desiccated control GBS, suggesting more efficient ascension through the female reproductive tract. Increased biofilm formation was observed in 30-day desiccated GBS when compared with non-desiccated GBS, suggesting that desiccation induces a physiological state optimized for persistence. Prior studies in other bacteria, including <i>Salmonella enterica</i> and <i>Escherichia coli</i>, have shown that desiccated strains can tolerate several stresses such as heat, oxidants, and disinfectants however, we observed no significant differences between desiccated and non-desiccated GBS in tolerance to UV, ethanol, hydrogen peroxide, or sodium hypochlorite, indicating a potential unique adaptation to desiccation by GBS, that enhances colonization and biofilm phenotypes rather than classical stress resistance. To define the genetic basis, we will perform whole-genome sequencing of desiccated versus non-desiccated GBS isolates.</p>
Gbp14	<p>Genomic insights into the population structure, temporal dynamics, and antimicrobial resistance diversity of human clinical Salmonella isolates in Kentucky</p> <p>Ajran Kabir, Department of Veterinary Science, University of Kentucky Logan Burns, Kentucky Department for Public Health, Frankfort, Kentucky, Beth Johnson, Kentucky Department for Public Health, Frankfort, Kentucky Vaneet Arora, Kentucky Department for Public Health, Frankfort, Kentucky Yosra A. Helmy, Department of Veterinary Science, University of Kentucky</p> <p><i>Salmonella enterica</i> continues to represent a major foodborne threat and a persistent challenge to public health in the United States. The extensive genomic diversity of <i>Salmonella</i>, together with its diverse antimicrobial resistance (AMR) profiles and outbreak potential, has established it as a central organism for investigating population structure and evolutionary dynamics. This study aimed to examine the serotype diversity, and multi locus sequence type profiles of <i>Salmonella</i> lineages along with their AMR determinants associated with human clinical infections in Kentucky between 2017 and 2025. A total of 3,705 <i>Salmonella</i> isolates were collected during this period and underwent whole-genome sequencing followed by sequence typing, serotype prediction, and resistant gene prediction. On average, 411 genomes were analyzed per year, revealing substantial diversity, with a mean of 78 unique sequence types (STs) and 54 serovars detected annually. The highest genetic diversity was observed in 2022, during which 105 unique STs and 74 serovars were identified. ST-11 emerged as the most prevalent STs and was primarily associated with the most prevalent serotype Enteritidis. ST-19, largely corresponding to serovar Typhimurium, represented the second most prevalent lineage. However, multiple sequencetypes were detected among single serotypes. High diversity of aminoglycoside and beta lactam antibiotic resistance genes were detected. AMR gene diversity peaked in 2021 with 33 unique resistance determinants detected. Clinically significant ESBL determinants such as CTX-M-65 and CMY variants emerged intermittently, alongside increasing plasmid-mediated quinolone resistance toward later surveillance years. These findings demonstrate a stable but heterogeneous population structure of clinical <i>Salmonella</i> in Kentucky carrying the risk of ESBL. This longitudinal genomic analysis provides valuable insights for public health surveillance, outbreak detection, and risk-based intervention strategies.</p>
Gbp15	<p>Phylogeographic Analysis of Salmonella enterica Serovar Javiana in the Southeastern United States</p> <p>Harleen Kaur , Thomas G. Denes Department of Food Science, University of Tennessee, Knoxville, Tennessee, USA 37996</p> <p>Background. <i>Salmonella enterica</i> serovar Javiana (hereafter S. Javiana) is an important non-typhoidal <i>Salmonella enterica</i> serovar associated with numerous foodborne outbreaks in the United States, particularly in the southeastern region. S. Javiana caused 324 laboratory-confirmed infections in the FoodNet catchment area, resulting in 114 hospitalizations (35.2%), four deaths (1.2%), and 10 outbreak-related cases (3.1%) in 2023. Despite its public health importance, genomic studies examining the population structure and spatial epidemiology of this serovar remain limited. This study aims to characterize the phylogenetic structure and spatial distribution of clinical S. Javiana isolates in the southeastern USA. Methods. A total of 6,368 clinical S. Javiana isolates from six southeastern USA state public health laboratories (Arkansas, Georgia, Kentucky, Mississippi, North Carolina, and South Carolina) were analyzed. Reference-free single nucleotide polymorphism (SNP) detection was performed, and clades were identified using FastBAPS. County-level incidence risk (IR) was mapped using ArcGIS Pro, and spatial clustering was assessed using Moran's I with Empirical Bayes adjustment. Results. Phylogenetic analysis based on core SNPs identified ten clades (1-10). County-level IR mapping of clinical S. Javiana isolates revealed distinct geographical patterns across clades. Clades 4 and 9 were predominantly concentrated along the coastal regions of the Carolinas, whereas Clade 6 exhibited a contrasting spatial pattern, with higher incidence in western states such as Mississippi and Arkansas. Global spatial autocorrelation analysis showed significant clustering for the overall serovar (Moran's I = 0.503, P = 0.01). At the clade level, significant spatial clustering was observed for Clades 3 to 10 (Moran's I range: 0.025 to 0.519, P < 0.05), with clades 6 and 9 demonstrating the strongest clustering patterns. Conclusion. These findings reveal distinct phylogeographic distribution of S. Javiana across the southeastern United States and highlight the value of integrating genomic and spatial analyses to better understand regional transmission patterns.</p>

<p>GbP16</p>	<p>ZFP36L1 Suppresses Human Coronavirus OC43 and Rotavirus Replication by Targeting Viral Nucleocapsid and Capsid Transcripts</p> <p>Tooba Momin*, Malabika Bhowmik, Mrigendra Rajput Lincoln Memorial University</p> <p>Background: RNA viruses are characterized by high mutation rates, enabling rapid adaptation and cross-species transmission, and posing pandemic threats. Targeting conserved, host-dependent pathways essential for viral replication represents a promising antiviral strategy. ZFP36L1 is a CCCH-type zinc finger RNA-binding protein involved in mRNA turnover in cells and has been identified as a potential host restriction factor. The current study evaluated the antiviral activity of ZFP36L1 against human coronavirus OC43 (HCoV-OC43) and human rotavirus A (HRV-A) and investigated the underlying molecular mechanisms of its antiviral activity</p> <p>Methods: Wild-type (WT), ZFP36L1-overexpressing (OE), ZFP36L1 knockdown (KD), and scrambled shRNA-transduced HCT-8 cells were infected with HCoV-OC43, while MA-104 cells were infected with HRV-A, at a multiplicity of infection (MOI) of 0.1. The effects of ZFP36L1 expression on viral titers, viral transcripts, and cytopathic effects were assessed by TCID₅₀, quantitative PCR (qPCR), and microscopy, respectively. To determine whether ZFP36L1 mediates its antiviral activity through poly(A) tail deadenylation, CNOT1 was knocked down in ZFP36L1-OE cells, followed by viral titer analysis. Viral RNA-ZFP36L1 interaction was assessed by RNA immunoprecipitation (RIP). Viral RNA-protein interactions were predicted using RNA-protein homology modeling and subsequently validated by luciferase assays.</p> <p>Results: ZFP36L1 overexpression reduced, whereas knockdown increased titers for both viruses compared with WT and scrambled shRNA control cells (p < 0.05). HCoV-OC43 expression in ZFP36L1-OE cells persisted despite CNOT1 knockdown, indicating that ZFP36L1 suppresses viral replication independently of poly(A) tail deadenylation mechanisms. RIP and luciferase assays demonstrated direct interaction between ZFP36L1 and the HCoV-OC43 nucleocapsid transcript. Similarly, computational analyses predicted strong binding of ZFP36L1 to the VP6 transcript of HRV-A.</p> <p>Conclusions: Collectively, these findings define ZFP36L1 as a broad-spectrum antiviral host factor that inhibits coronavirus and rotavirus replication through direct interactions with viral transcripts, underscoring the potential of RNA-binding proteins as targets for host-directed antiviral interventions.</p>
<p>GbP17</p>	<p>Architecture of Chemotaxis Chemoreceptor Arrays in Rhizobium leguminosarum bv. viciae</p> <p>Elaine Nunan, Chris Tinkey, and Gladys Alexandre Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee</p> <p>Background: The soil is a harsh environment with wide fluctuations in many factors such as temperature, pH, salt concentrations, and moisture. To survive in such an environment, motile bacteria can sense physicochemical conditions in their surroundings and move towards conditions that promote their survival and away from deleterious ones, using chemotaxis. During chemotaxis, bacteria use allosterically coupled chemoreceptors to sense and relay information to cytoplasmic chemotaxis signal transduction proteins (CheA, CheW, CheY) to alter direction of flagellar rotation. The chemotaxis genes in Rhizobium leguminosarum bv. viciae, a plant growth promoting bacteria, are organized as two distinct chemotaxis operons (che1 and che2). Two additional chemotaxis genes encoding for CheW homologs are also found outside of the operons, which we refer to as orphan CheWs. Previous work showed that che1 is essential for chemotaxis toward host plants while che2 appears to contribute to chemotaxis in a minor role.</p> <p>Methods: We aim to characterize the subcellular organization of chemoreceptor arrays in R. leguminosarum using fluorescence microscopy to visualize the subcellular localization of fluorescently labelled chemotaxis proteins in the parent and chemotaxis mutant derivatives background as well as by characterizing chemotaxis protein interactions using BacTH assays. Results: Results indicate that CheA1 interacts with CheW1 and both orphan CheWs. Whereas CheA2 interacts with CheW2 and one of the orphan CheWs (CheW4). Data indicate that CheA1-YFP localizes at the cell poles, as expected. However, deletion of che1 or che2 operons or both are dispensable for CheA1-YFP polar localization, suggesting that proteins outside of che1 and che2 contribute to the location of CheA1-YFP. We are currently testing the role of the orphan CheW homologs.</p> <p>Conclusion: Collectively ongoing and current data will provide a working model for the organization of chemotaxis proteins in R. leguminosarum that will provide molecular mechanisms for chemotaxis signal transduction in this species.</p>
<p>GbP18</p>	<p>Genomic and Functional Characterization of the Endophytic Biocontrol Strain Bacillus amyloliquefaciens 143-EN04</p> <p>Gift M. Obunukwu and Margaret M. Mmbaga Tennessee State University, Nashville, Tennessee, USA</p> <p>Endophytic microorganisms offer sustainable alternatives to chemical pesticides for improving crop productivity and managing plant diseases. High-value vegetable crops such as pepper (Capsicum annuum) and tomato (Solanum lycopersicum) are severely affected by soil-borne pathogens including Phytophthora capsici, while chemical control strategies face increasing limitations due to resistance and environmental concerns. This study investigated the plant growth-promoting (PGP) and biocontrol potential of the endophytic strain 143-EN04 isolated from papaya roots. Whole-genome phylogenomic analysis identified the isolate as Bacillus amyloliquefaciens. Genome mining revealed numerous biosynthetic gene clusters (BGCs) associated with antimicrobial compound production, including conserved lipopeptides such as fengycin, surfactin, difficidin, bacillaene, and macrolactin, while several clusters showed low similarity to known metabolites, suggesting potential for novel bioactive compounds. The genome also contained genes associated with nutrient acquisition, hormone synthesis, volatile compound production, motility, and biofilm formation. Carbohydrate-active enzyme (CAZyme) analysis indicated strong potential for carbohydrate degradation and interactions with plant and fungal cell walls. Comparative pan-genome analysis placed strain 143-EN04 within the B. amyloliquefaciens-velezensis clade and revealed a highly conserved core genome among related endophytic strains. Functional assays supported genomic predictions, with strain 143-EN04 inhibiting fungal pathogens including Sclerotium rolfsii, Fusarium spp., and Phytophthora capsici through diffusible and volatile metabolites. Greenhouse experiments further demonstrated significant improvement in tomato and pepper growth following seed biopriming and root irrigation treatments. Together, these findings highlight Bacillus amyloliquefaciens 143-EN04 as a promising microbial bioagent with dual plant growth-promoting and disease-suppressive capabilities and support its potential development as a sustainable biopesticide or biostimulant for crop production.</p>
<p>GbP19</p>	<p>Urea Reshapes Growth and Photophysiology in Raphidiopsis raciborskii.</p> <p>Olavesen JL1, S Eck1, RM Martin1, KM Yoshimura1,2, DJ Weston3, D Talmy1 and SW Wilhelm1 1 Department of Microbiology, The University of Tennessee 2 Department of Biology, James Madison University</p> <p>Background: The widespread shift toward urea-based fertilizers has raised urgent questions about how nitrogen form alters cyanobacterial photophysiology and competitive dynamics during harmful algal blooms. Raphidiopsis raciborskii, an increasingly important but understudied bloom former, provides a useful model for addressing these questions.</p> <p>Methods: Batch-culture incubations were used to compare the effects of stoichiometrically equivalent nitrogen sources on the growth, phenotype, and photophysiology of R. raciborskii. Primary measurements included PAM fluorometry, in vivo fluorescence, and newly developed flow-cytometric quantification, supported by comparative transcriptomic analyses.</p> <p>Results: Under bloom-relevant conditions of elevated pH and low inorganic carbon, urea supported substantially higher growth than nitrate and other nitrogen forms. This advantage coincided with pronounced phenotypic reorganization, depressed Fv/Fm, and transcriptional downregulation of PSII alongside signatures of alternative photophysiology strategies.</p> <p>Conclusion: By resolving how R. raciborskii exploits urea, tolerates environmental stress, and gains a competitive advantage, this work will improve mechanistic forecasts of bloom initiation and severity. More broadly, it advances understanding of cyanobacterial metabolic flexibility, with implications for freshwater management and emerging biotechnologies.</p>

Gbp20	<p>Investigating genetic adaptability in long-term stationary phase in <i>Vibrio natriegens</i>.</p> <p>Luke Qualey, Chloe Hall, Erik Zinser Department of Microbiology, University of Tennessee, Knoxville</p> <p>Background: In natural environments, bacteria frequently encounter prolonged nutrient limitation, selecting for adaptive strategies that enhance survival. Long term stationary phase (LTSP) promotes the emergence of mutants exhibiting a growth advantage in stationary phase (GASP) phenotype, which has been well characterized in <i>E. coli</i> but remains less explored in alternative model organisms. Here, we use the fast-growing bacterium <i>Vibrio natriegens</i> to investigate how LTSP drives genetic and phenotypic changes.</p> <p>Methods: Cultures were incubated in LB + 3% NaCl under LTSP conditions, and isolates were collected after 10 and 30 days. Competition assays were performed between unaged and aged populations to assess GASP phenotypes. Whole-genome sequencing of a 30-day aged isolate was conducted using Illumina short-read technology. Reads were quality filtered, assembled, annotated, and mutations were identified by reference-based SNP calling against the ancestral strain.</p> <p>Results: Populations developed a clear GASP phenotype after 10 days in LTSP, with enhanced competitive fitness relative to unaged cells and the ability to invade from rare. Genomic analysis of the 30-day isolate identified four high-confidence mutations in coding regions: a missense mutation in the global regulator <i>lrp</i>, frameshift mutations in <i>pckA</i> and <i>kdgR</i>, and an in-frame insertion in <i>nusA</i>. These mutations are predicted to impact transcriptional regulation, carbon metabolism, and nutrient scavenging pathways. Collectively, the data indicate substantial genetic and regulatory rewiring during prolonged stationary phase.</p> <p>Conclusions: Our findings demonstrate that <i>V. natriegens</i> adapts to long-term nutrient limitation through multiple mutations affecting diverse cellular processes, consistent with broad physiological remodeling. This work establishes <i>V. natriegens</i> as a useful model for studying the genetic architecture of bacterial survival and evolution under prolonged stationary-phase conditions.</p>
Gbp21	<p>A Metabolic Arms Race: Neutrophil Reprogramming and Bacterial Adaptation in Diabetic <i>Staphylococcus aureus</i> Infection</p> <p>C. Reber, E. TenBarge, M. Hetzel, A. Collins, B. Holder, R. Ervin, E. Belevska, A. Wise, A. Monteith Department of Microbiology, University of Tennessee, Knoxville, TN, USA</p> <p>Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and impaired insulin regulation. While insulin therapy has prolonged the lifespan of diabetic patients, the altered metabolic state leads to major complications, including heightened susceptibility to bacterial infections, most commonly by <i>Staphylococcus aureus</i>. Neutrophils are essential for controlling <i>S. aureus</i> infection, yet how diabetes reshapes neutrophil antibacterial effector programs remains unclear. In the streptozotocin (STZ) model of diabetes, mice are highly susceptible to bacteremia, and unlike macrophages that fail to elicit a robust oxidative burst, neutrophils exhibit a hyperinflammatory state characterized by enhanced reactive oxygen species (ROS) production and neutrophil extracellular trap release (NETosis). Surprisingly, the heightened oxidative burst and NETosis are not explained by increased glucose availability, uptake, or utilization. This suggests that alternative metabolic substrates may fuel neutrophil effector functions in diabetes, despite the traditional view that the oxidative burst in neutrophils requires NADPH derived from glucose metabolism and the pentose phosphate pathway. We identify that STZ neutrophils are adapted to use glutamine, a key substrate for the tricarboxylic acid (TCA) cycle, to support elevated ROS and NETosis. Despite enhanced neutrophil effector functions, the hyperglycemic milieu simultaneously protects <i>S. aureus</i> by enabling glucose-dependent adaptation that promotes resistance to oxidative stress and NET-mediated killing. Together, these findings reveal a metabolic "arms race" in diabetes in which host neutrophils rewire substrate use to sustain inflammation while <i>S. aureus</i> exploits excess glucose to bolster stress resistance, providing a framework to target host-pathogen metabolism to restore antibacterial defense.</p>
Gbp22	<p>Novel Small Molecules Targeting Intracellular <i>Rhodococcus equi</i> Survival in Macrophages</p> <p>Mohamed Saleh, Bibek Lamichhane, Khaled A. Shaaban, Larissa V. Ponomareva, Jon Thorson, and Yosra A. Helmy University of Kentucky</p> <p><i>Rhodococcus equi</i> is an emerging zoonotic pathogen responsible for severe pulmonary and systemic disease in immunocompromised humans and is the primary cause of bronchopneumonia in foals under six months of age. Infection typically occurs through inhalation of dust contaminated with soil or manure on horse farms. Although rifampin-macrolide combinations remain the standard therapy, increasing antimicrobial resistance underscores the urgent need for alternative treatment strategies. This study sought to identify novel small molecules (SMs) with potent activity against multidrug resistant <i>R. equi</i>. A high throughput screen of ~2,500 SMs yielded ten compounds that completely inhibited bacterial growth, with minimum inhibitory and bactericidal concentrations as low as 0.078 μM and 0.156 μM. Five SMs fully inhibited or eradicated biofilms at concentrations ranging from 0.078 to 10 μM. In intracellular assays using murine and equine alveolar macrophages, all ten SMs significantly reduced intracellular <i>R. equi</i> survival at 5 μM, achieving >3 fold reductions ($p < 0.05$). Scanning electron microscopy revealed pronounced morphological damage, including cell deformation and membrane collapse. These findings highlight promising SM candidates for further evaluation of stability, safety, and potential use in preventing <i>R. equi</i> colonization in foals, providing a foundation for next generation therapeutics.</p>
Gbp23	<p><i>Rickettsia parkeri</i> modulates host Receptor Tyrosine kinases for its survival and infection in endothelial cells</p> <p>Jeremy W. Turck 1, Lichao Liu 1,2 Hameeda Sultana 1 and Girish Neelakanta 1 University of Tennessee Knoxville</p> <p><i>Rickettsia parkeri</i>, the causative agent of <i>R. parkeri</i> rickettsiosis, in humans is primarily transmitted by a Gulf Coast tick <i>Amblyomma maculatum</i>. This bacterium primarily infects and colonizes endothelial cells. Studies on this pathogen could lead us in understanding modulation of several pathways by spotted fever group <i>Rickettsia</i> to survive in mammalian cells. In this study, we performed a human protein array analysis to determine whether <i>R. parkeri</i> differentially affects phosphorylation of host proteins upon infection of endothelial cells. We noted that <i>R. parkeri</i> infection modulates phosphorylation of several receptor tyrosine kinases in human endothelial cells. The protein array analysis revealed increased phosphorylation of some of the receptor tyrosine kinases (RTK) including JAK2, Fyn and EphA7 and reduced phosphorylation of EphA8, EphA3 and EphB1. qRT-PCR analysis showed that <i>epha7</i> and <i>fyn</i> transcripts were also significantly ($P < 0.05$) upregulated upon <i>R. parkeri</i> infection of endothelial cells compared to the levels noted in uninfected control. Furthermore, we will be using GFP-expressing <i>R. parkeri</i> and siRNA-mediated silencing experiments to understand the role of some of these host proteins in <i>R. parkeri</i>-endothelial cells interactions. Taken together, this study provides evidence on the role of RTK proteins in rickettsial pathogen interactions with the endothelial cells.</p>

Gbp24	<p>Swimming in Stress: "Tails" of Resilience from <i>A. brasilense</i></p> <p>Hahley Wiltse, Dr. Gladys Alexandre Biochemistry & Cellular and Molecular Biology, The University of Tennessee, Knoxville</p> <p>Introduction: Flagella allow bacteria to navigate and therefore survive in their environment. Studies on the flagellar motor have revealed exquisite machinery, yet the flagellar filament is often overlooked. Understanding the protein that composes the filament (flagellin) is essential for elucidating the characteristics of filaments. Flagellins are diverse yet conserved, and almost half of flagellated species contain more than one flagellin-encoding gene, leading to studies regarding the regulation of multiple flagellins. <i>Azospirillum brasilense</i>, a plant-growth-promoting rhizobacterium, expresses a single polar flagellum in liquid. The filament was thought to be composed of one flagellin, but we found that <i>A. brasilense</i> encodes three flagellins (FliC1A, FliC1B, and FliC2). This work focuses on examining the structure, function, and regulation of <i>A. brasilense</i>'s flagellar filament.</p> <p>Methods: We mutated genes encoding each of the three flagellins in <i>A. brasilense</i>, then used transmission electron microscopy (TEM), protein gel electrophoresis, and swim assays to assess phenotypic effects. We then mutated genes encoding proteins of a stress-related regulatory pathway and evaluated phenotypes using transmission electron microscopy, protein gel electrophoresis, transcriptional expression assays, and swim assays.</p> <p>Results: In ΔfliC1A, the filament fails to form, SDS-PAGE shows decreased protein, and swim assays show decreased swimming. ΔfliC1B forms a filament but has similar yet diminished phenotypes to ΔfliC1A in the SDS-PAGE and swim assays. FliC2 is not detectable in normal conditions, but in a mutant that over-activates a stress pathway (Δasf6), FliC2 is upregulated. Δasf6 has an altered filament and decreased swimming. We then mimicked the phenotype of Δasf6 with external oxidative stress.</p> <p>Discussion: This study supports the hypothesis that FliC1A is the major flagellin, FliC1B is a minor flagellin, and FliC2 is not detected in the filament in normal lab conditions. We then show evidence that FliC2 expression is induced by oxidative stress through the activation of the RpoE6-Asf6 pathway.</p>
Gbp25	<p>Incorporation of polyfluoroalkyl carboxylates into bacterial membrane lipids</p> <p>Broquell Wong, Gao Chen, Cindy Swift, Andrew Stai, Robert Hettich, Frank Loeffler University of Tennessee Knoxville</p> <p>Background: Per- and Polyfluoroalkyl substances (PFASs) are widespread and persistent environmental contaminants that have been associated with negative effects on human and environmental health. Due to their unique chemical properties, PFASs have been widely used in various application such as food packaging, firefighting foam, nonstick cookware, and other consumer and industrial products. A key aspect in regulating PFASs is understanding their interactions with microbiota to predict PFAS environmental fate and transformation. <i>Pseudomonas</i> sp. strain 273, a soil isolate, grows with terminally fluorinated alkanes, and the metabolic machinery of the bacterium directs fluorinated catabolites into fatty acid and phospholipid synthesis, resulting in the formation of monofluorinated glycerophospholipids, which are incorporated into the membrane (Xie et al., 2022).</p>
PDP1	<p>Dissecting the Impact of HIV-1 and Antiretroviral Therapy on Pancreatic β-Cell Function</p> <p>Shilpak Bele^{1,2}, Prem Prakash^{1,2} and Chandravanu Dash^{1,2} 1Center for AIDS Health Disparities Research, School of Medicine, Meharry Medical College, Nashville, Tennessee 37208 2Department of Microbiology, Immunology and Physiology, School of Medicine, Meharry Medical College, Nashville, Tennessee 37208</p> <p>As antiretroviral therapy (ART) extends life expectancy in people with HIV (PWH), chronic metabolic complications such as Type 2 Diabetes (T2D) have become increasingly prevalent. While systemic insulin resistance has been well-documented, the direct effects of the virus and/or ART on pancreatic β-cell physiology remain largely unexplored. Viral proteins, including Tat, Nef, and gp120, are implicated in inducing oxidative stress, mitochondrial injury, and ER stress in metabolic tissues. However, their β-cell-specific mechanisms and interactions with ART-related toxicity remains unknown. To span this knowledge gap, Rat INS-1 cells were exposed to either supernatant or co-culture of Jurkat cells infected with HIV-1 particles (200ng/ml) and clinically relevant concentrations of ART drugs (raltegravir, dolutegravir, bictegravir, efavirenz and cabotegravir; 1μM). β-cell viability and cytotoxicity were quantified by LDH assay. Expression of β-cell transcription factors (PDX1, MAFA, Nkx 6.1), glucose-sensing markers (Gck, Glut2), and metabolic and stress defense markers (SOD2, ATF4, NRF2, P65-NFkB) were analyzed via qPCR and Western blotting. HIV-infected Jurkat supernatant strongly compromised β-cell transcriptional machinery and glucose sensing. Raltegravir offered partial but not complete rescue, showing that the virus could contribute to β-cell dysfunction, but other immune-mediated factors may also be involved. We also observed that HIV-infected human T lymphocyte cells compromised β-cell identity and function, both via soluble factors (cytokines, chemokines, and extracellular vesicles) and direct interactions (viral proteins), but with stronger effects in co-culture. High p65 suggests that β-cells are undergoing an inflammatory or stress-induced signaling cascade, which can alter gene expression patterns linked to survival, apoptosis, and insulin secretion at the same time elevated NRF2 expression in pancreatic β-cells indicates activation of an antioxidant stress response to counteract oxidative damage. Collectively, the interaction between immune cells, HIV infection, and ART is critical in modulating β-cell physiology. Currently we are a) planning rescue experiments using metformin and the GLP-1 receptor agonist liraglutide and b) cytokine secretion profiles by multiplex immunoassays.</p>
PDP2	<p>Development and Validation of High-Specificity PCR Assays for the Detection of <i>Erwinia tracheiphila</i></p> <p>Mohammad Fazel Soltani Gishini, Ali Taheri, Korsi Dumenyo Tennessee State University</p> <p>Fresh produce can become contaminated with antimicrobial-resistant bacteria and resistance genes through agricultural water. This risk may be greater when growers rely on irrigation sources such as creeks, streams, ponds, and wells. This study investigated microbial community patterns and antimicrobial resistance gene (ARG) profiles in irrigation water, soil, and lettuce produced under these conditions. Lettuce cv. Coastal Star was established in field plots containing mulched and bare soil and irrigated with water from 4 sources: creek, stream, pond, and well. A total of 16 water samples were analyzed, including 4 samples each from creek, well, stream, and pond. In addition, 192 lettuce samples were collected, comprising 48 samples per water source. Microbial community was characterized by 16S rRNA gene sequencing, and antimicrobial resistance determinants were examined using shotgun metagenomic sequencing. Alpha diversity was assessed using Shannon and Chao1 indices, and taxonomic differences were further evaluated using LEfSe-based comparisons. Microbial communities differed among water, soil, and lettuce. Soil, water, and lettuce were distinguished by the relative abundance of Proteobacteria, Firmicutes, and Actinobacteria. Both Shannon diversity and Chao1 richness indicated that soil and lettuce, including lettuce grown on both mulched and bare soil had greater diversity and richness than water samples. Beta-diversity analysis also separated lettuce into 2 distinct clusters based on production on mulched versus bare soil. Multiple ARGs were detected across all matrices. Surface irrigation water carried genes such as aac(6)-I, aadA2, and several bla genes, whereas soil showed the broadest ARG profile. Lettuce irrigated with creek, stream, and pond water carried ARGs including aadA9, qacE, tet(W), tetA(P), blaSHV, blaTEM, mcr-10, mcr-3, and mcr-7. These findings show that different irrigation sources may contribute to the movement of antimicrobial resistance determinants into soil and lettuce production, highlighting the need for improved irrigation water monitoring and grower education on produce safety.</p>

PDP3	<p>Functional and proteomic characterization of Escherichia coli Nissle 1917–derived factors inhibiting Campylobacter jejuni</p> <p>Ahmed Habib1, Ilhem Messaoudi2, Yosra A. Helmy1*</p> <p>1Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, USA 2Department of Microbiology, Immunology, and Molecular Genetics, College of Medicine, University of Kentucky, Lexington, Kentucky, USA</p> <p>Background: Campylobacter jejuni is a leading cause of foodborne bacterial gastroenteritis worldwide and is primarily associated with poultry consumption. The increasing prevalence of antimicrobial resistance (AMR) in C. jejuni represents a significant public health concern, underscoring the urgent need for alternative, non-antibiotic strategies to reduce pathogen burden within the food chain. The probiotic Escherichia coli Nissle 1917 (EcN) has emerged as a promising non-antibiotic approach for controlling enteric infections; however, the molecular mechanisms underlying its antimicrobial activity remain poorly understood.</p> <p>Methods: Functional assays and comparative proteomic analysis were used to characterize antimicrobial factors derived from EcN. Cell-free supernatant (CFS) was fractionated to identify active components, and antimicrobial activity was evaluated using agar well diffusion, biofilm formation, adhesion, invasion, and intracellular survival assays. In parallel, comparative proteomic profiling was performed to identify proteins enriched in EcN relative to non-probiotic E. coli K-12 control strains.</p> <p>Results: Fractionation of EcN-CFS identified a high-molecular-weight fraction (>100 kDa) with strong inhibitory activity against C. jejuni in agar well diffusion assays, whereas equivalent fractions from non-probiotic E. coli K-12 strains showed no detectable activity, indicating that the effect is specific to EcN. The active EcN-CFS fraction also inhibited biofilm formation and disrupted established biofilms. In addition, pretreatment of polarized human intestinal epithelial (HT-29) cells with EcN-CFS significantly reduced C. jejuni adhesion and invasion, resulting in no detectable intracellular bacteria. Comparative proteomic analysis revealed distinct clustering of EcN and control strains, indicating strain-specific protein expression profiles. Differential abundance analysis identified proteins significantly enriched in EcN, highlighting candidate factors potentially associated with antimicrobial activity.</p> <p>Conclusions: These findings demonstrate that EcN secretes bioactive molecules capable of suppressing C. jejuni and provide mechanistic support for probiotic-derived factors as candidates for antibiotic-sparing strategies to reduce pathogen colonization and limit AMR dissemination at the animal–human interface.</p>
PDP4	<p>Role of TbTim17 in mitochondrial stress response in Trypanosoma brucei</p> <p>Hira Karim, Raylin Webster, Minu Chaudhuri</p> <p>Department of Microbiology, Immunology, and Physiology, School of Medicine, Meharry Medical College, Nashville TN 37208</p> <p>Mitochondrial protein translocases Tim17 and Tim23 play important roles in stress-induced signaling pathways via activating the transcription factors (ATFs) to maintain organellar homeostasis in eukaryotes. Trypanosoma brucei, the infectious agent for African trypanosomiasis, is a unicellular parasite that diverged very early during evolution. T. brucei lacks ATFs but possesses TbTim17, a major and essential component of the TIM complex in mitochondria. However, it has not been investigated whether TbTim17 plays a role in the mitochondrial stress response. Here, we show that depletion of TbTim17 increased T. brucei tolerance to paraquat, increasing the EC50 by 3- to 4-fold compared with the wild type. Subsequent analysis revealed that increased levels of mitochondrial reactive oxygen species (ROS) resulting from TbTim17 knockdown upregulate mitochondrial superoxide dismutase (SODA) and preadapt cells to resist paraquat-induced oxidative stress. This is supported by the fact that Pre-treatment of TbTim17 knockdown cells with N-acetyl cysteine decreased the EC50 of paraquat. Paraquat treatment of the wild-type parasite didn't induce mitochondrial Hsp70 levels but increased the levels of trypanosome alternative oxidase (TAO) and SODA, as well as TbTim17 in mitochondria and Bip, the Hsp70 homolog, in the ER. Furthermore, TbTim17 knockdown did not inhibit protein translation; however, it caused a moderate reduction of SL RNA and a 2-fold increase in the TSNAP42 transcript levels, suggesting that stresses induced by mitochondrial protein import activate the splice leader silencing response. Together, these results show that TbTim17 plays a role in mitochondrial stress; however, it acts differently than in other eukaryotes.</p>
PDP5	<p>Using High-Throughput Phenotyping to Explore the Genetic Basis of Biological Nitrification Inhibition in Populus</p> <p>Jessica LaFond1, Dana Carper1, Stephen Zambrzycki1, Mengjun Shu1, Brandon Sloan2, John Field2, and John F. Cahill1</p> <p>1.Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN</p> <p>Background: Plants can regulate the soil microbiome by exuding chemicals that inhibit nitrification. Biological nitrification inhibition (BNI) is emerging as an avenue for increasing nitrogen use efficiency and reducing loss of nitrogen to the atmosphere and waterways. This work evaluates variation in nitrification, measured as gross nitrification rate (GNR), across Populus genotypes. The objective is to determine whether variation in GNR among Populus genotypes has a genetic basis and to identify associated candidate genes.</p> <p>Methods: Soil cores (~top 10-15 cm) were collected from two Department of Energy Populus common gardens (Tennessee and Oregon) in summer and spring, respectively. The TN site included 15 genotypes in replicate and 6 background samples (away from trees) while ~320 genotypes were sampled at the OR site. GNR was measured using the established 15N isotope dilution method followed by nitrate derivatization and analyzed via immediate drop-on demand technology coupled with an open port sampling interface (I.DOT/OPSI) for rapid high-throughput quantification.</p> <p>Results: Preliminary results indicate substantial variation in GNRs across genotypes. At the TN site, average GNRs of all genotypes were below the background rate of 5.29 ± 0.5 mg-N kg⁻¹ d⁻¹, with the lowest genotype, ILL-101, having a 49% reduction in GNR. In a linear mixed model, genotype explained ~17% of GNR variation, suggesting a meaningful genetic contribution. In the larger OR campaign, GWAS linked genotype-specific sequence variation with GNR. Preliminary findings identified single nucleotide polymorphisms (SNPs) exceeding a significance threshold of $-\log_{10}(p\text{-value}) > 5.0$, mapping to ~40 genes potentially associated with GNR. These candidate genes include those putatively involved in root modulation, RNA and DNA binding, defense response, and vesicle trafficking.</p> <p>Conclusions: Populus genotypes showed significant variation in gross nitrification rates. Initial GWAS results indicate a potential genetic basis for nitrification in Populus and highlight candidate genes for further investigation of related plant-microbe interactions.</p>

PDP6	<p>Deep Metagenomic Sequencing Reveals Threshold for Detecting Metagenomic-Assembled Genomes of Low-Content Salmonella Species</p> <p>Jia Wang, , Henk C. den Bakker, Daniel W. Bryan, Thomas G. Denes, Department of Food Science, The University of Tennessee, Knoxville, Tennessee 37996, USA Center for Food Safety, Department of Food Science and Technology, College of Agriculture and Environmental Sciences, University of Georgia, Griffin, Georgia 30223, USA</p> <p>Wastewater metagenomics is increasingly used for foodborne pathogen surveillance, yet reconstructing genomes of low-abundance pathogens such as <i>Salmonella enterica</i> remains difficult due to complex microbial communities and interference from closely related taxa. In this study, we systematically evaluated short-read and hybrid metagenomic assembly strategies for recovering <i>Salmonella</i> metagenome-assembled genomes (MAGs) from raw and enriched wastewater microbiomes. Taxonomic profiling revealed comparable microbial community structures between Illumina and Nanopore datasets. However, direct assembly and binning approaches using whole metagenomes, taxonomically classified reads, or reads mapped to a reference genome failed to produce medium-quality <i>Salmonella</i> MAGs (completeness < 50%, contamination > 10%). Using in silico spiking experiments, 3 medium-quality <i>Salmonella</i> MAGs were generated and at least 20× genome coverage was required to reconstruct medium-quality MAGs from enriched wastewater metagenomes. Reducing background microbiome complexity to one-tenth of the dataset lowered this threshold to 10× coverage, and 7 medium-quality <i>Salmonella</i> MAGs were reconstructed. Core virulence genes including 28 <i>Salmonella</i> pathogenicity island and 12 invasion genes were consistently detected across reconstructed MAGs, demonstrating that conserved pathogenicity markers can be captured even in complex environmental samples. Hybrid assembly did not consistently improve MAG reconstruction or virulence gene recovery. These findings establish practical coverage benchmarks and highlight key methodological challenges for metagenomic reconstruction of low-abundance foodborne pathogens in wastewater surveillance.</p>
FP1	<p>High quality reference genome sets that represent the genomic diversity of epidemiologically important <i>Salmonella enterica</i> serovars in Tennessee</p> <p>Lauren K. Hudson, Daniel W. Bryan, Thomas G. Denes University of Tennessee, Knoxville</p> <p>Background: <i>Salmonella enterica</i> serovars Newport, Javiana, and Braenderup are among the top ten most isolated clinical serovars in Tennessee. Many bioinformatic analyses depend on high-quality, complete reference genomes. However, publicly available assemblies for these serovars vary widely in quality. The goal of this project was to generate high-quality assemblies of representative strains of each serovar to serve as a public resource for academic research, epidemiologic applications, and related work.</p> <p>Methods: Identifiers were obtained for Javiana (n = 144), Newport (n = 192), and Braenderup (n = 28) isolates sequenced as part of routine surveillance by the Tennessee Department of Health. When available, existing assemblies were downloaded from NCBI; otherwise, Illumina reads were retrieved from the Sequence Read Archive (SRA) and assembled using SPAdes. For each serovar, assemblies and reference isolates (from previous phylogenetic studies) were analyzed using kSNP to delineate phylogenetic clades and subclades. One representative isolate per subclade was selected for Nanopore sequencing. Long-read assemblies were generated with Flye, polished using both long reads and Illumina reads from NCBI, and annotated with PGAP.</p> <p>Results: From the three phylogenies, sixty-seven total representative strains were selected (Javiana [n = 33], Newport [n = 24], and Braenderup [n = 10]). The long-read assemblies averaged two contigs, 4.7 Mb N50, and 1 L50. The quality was substantially better than Illumina-only assemblies or assemblies available on NCBI (average 42 contigs, 0.4 Mb N50, and 6.5 L50). Annotated assemblies and metadata will be publicly available on NCBI.</p> <p>Conclusions: This work provides high-quality, complete genomes for three epidemiologically important <i>Salmonella</i> serovars in Tennessee, representing the genetic diversity observed across these populations. These annotated genomes will be available for public health and comparative genomics applications. They can improve resolution and accuracy for pathogen tracking and outbreak investigations and serve as strong references for comparative and evolutionary studies.</p>
FP2	<p>Irrigation Water as a Reservoir of Antimicrobial Resistance in Lettuce Production on Small-Scale Farms</p> <p>Agnes Kilonzo-Nthenge Tennessee State University</p> <p>Alternative irrigation water sources used on small-scale farms may facilitate preharvest dissemination of antimicrobial resistance in fresh produce systems. This study characterized bacterial community structure and antimicrobial resistance genes (ARGs) in irrigation water, soil, and lettuce (<i>Lactuca sativa</i> var. Coastal Star) produced under field conditions. One-month-old seedlings were transplanted into mulched and bare soil plots and irrigated with creek, stream, pond, or well water. Water samples (n = 16) and lettuce samples (n = 192) were analyzed by 16S rRNA gene sequencing and shotgun metagenomic sequencing.</p> <p>Distinct microbial assemblages were observed across water, soil, and lettuce niches, with additional separation between lettuce grown on mulched and bare soil. Community structure was driven primarily by Proteobacteria, Firmicutes, and Actinobacteria. Alpha-diversity metrics showed lower richness and diversity in irrigation water than in soil and lettuce, while beta-diversity analyses confirmed habitat-specific clustering.</p> <p>Resistome profiling detected ARGs across all sample types. Surface irrigation water carried <i>aac(6)-I</i>, <i>aadA2</i>, <i>bla2a</i>, <i>blaACT</i>, <i>blaFIA</i>, <i>blaFRI</i>, <i>blaOXA</i>, and <i>blaPER</i>. Soil harbored the broadest ARG repertoire, including <i>qacA</i>, <i>qacE</i>, <i>sat2</i>, <i>str</i>, <i>sul1</i>, <i>sul2</i>, <i>tet(30)</i>, <i>tet(Q)</i>, <i>tet(S)</i>, <i>tet(T)</i>, <i>tetB(P)</i>, <i>van</i>, <i>vanS-O</i>, <i>vanS-Pt</i>, <i>vat(E)</i>, <i>vga(A)</i>, <i>aph(3')-IIIa</i>, <i>vanXYC</i>, <i>tet(33)</i>, <i>erm(33)</i>, and <i>blaAIM</i>. Lettuce irrigated with surface water carried <i>aadA9</i>, <i>qacE</i>, <i>tet(W)</i>, <i>tetA(P)</i>, <i>nimC</i>, <i>vanTc</i>, <i>blaSHV</i>, <i>blaTEM</i>, <i>cfxA</i>, <i>cfxA6</i>, <i>cmIB1</i>, <i>floR2</i>, and <i>mcr-10/mcr-3/mcr-7</i>. These findings position irrigation water not merely as a contamination source, but as a biologically active reservoir shaping the field resistome and amplifying the risk of ARG transmission to fresh produce.</p>

FP3	<p>Determining the rate-limiting processes for cell division in Escherichia coli.</p> <p>Jaana Männik 1, Prathitha Kar 2 3, Chathuddasie Amarasinghe 1, Ariel Amir 4, Jaan Männik 1 1Department of Physics and Astronomy, University of Tennessee, Knoxville, TN, 37996, USA. 2John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, 02134, USA. 3Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, 02134, USA. 4Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel.</p> <p>Background: Constriction onset marks the transition from a mature Z-ring to an active septal cell wall synthesis machine in Escherichia coli. Although recruitment of FtsN has been proposed to trigger this transition, the rate-limiting processes that determine constriction onset in vivo remain unclear. We asked which divisome components set the timing of constriction onset during moderately fast growth.</p> <p>Methods: We quantitatively upregulated FtsZ, FtsN, and FtsA in live E. coli and measured constriction timing by high-throughput single-cell imaging in microfluidic devices combined with cell-cycle modeling. Protein levels were calibrated on-chip using three strains: the strain of interest expressing the unlabeled upregulated protein, a Reporter strain expressing a fluorescent protein from the same locus under the same promoter and Shine-Dalgarno sequence, and a Reference strain constitutively expressing the protein of interest fused to the same fluorescent protein from its native locus. Signals from the Reporter and Reference strains were used to estimate native-equivalent protein concentrations, and the method was validated by Western blotting. To interpret the effects of increased FtsZ levels, we used an FtsZ polymerization model to predict changes in protofilament number and organization.</p> <p>Results: FtsN and FtsA were not rate-limiting for constriction onset under physiological conditions. FtsN accelerated constriction only at high overexpression levels, whereas excess FtsA inhibited division. In contrast, increased FtsZ advanced constriction onset, identifying FtsZ abundance as a rate-limiting factor for division timing in E. coli. Modeling suggested that higher FtsZ levels primarily increase protofilament number, with only minor effects on filament length, consistent with enhanced lateral interactions and bundling.</p> <p>Conclusions: Our data support a model in which the cell-cycle-dependent increase in FtsZ abundance promotes earlier constriction onset at faster growth rates. In this model, increasing FtsZ levels favor formation of bundled protofilaments, which remodel FtsA into an antiparallel filament form competent for FtsN recruitment. FtsN recruitment would then activate the core divisome complex and trigger constriction onset.</p>
FP4	<p>From transcriptome to treatment: Genomics-informed fungicide discovery for woody ornamental pathogens</p> <p>Erik A. Powers, Robert Pawlak, Marcin Nowicki University of Tennessee, Knoxville</p> <p>Woody ornamental pathogens are often managed empirically despite growing availability of pathogen genomic resources. We combined RNA-seq target discovery with in vitro fungicide screening to prioritize treatments against <i>Discula destructiva</i> (DD; dogwood anthracnose) and <i>Juglanconis japonica</i> (JJ; branch dieback). Ranked DD DEGs were enriched for secreted and apoplastic enzymes involved in host penetration and cell wall degradation, including cutinases, pectate lyases, glycosyl hydrolases, glucanase transferases, and redox-associated proteins. Efficacy of 17 emergent candidate fungicides was evaluated using net growth, percent inhibition, and area under the growth curve. Tebuconazole and BannerMaxxII were the overall strongest treatments. For DD, tebuconazole reached 98.5% mean inhibition at 10 ppm with a MEC80 of 1 ppm, whereas BannerMaxxII reached 95.8% inhibition at 10 ppm and 97.7% at 50 ppm. For JJ, BannerMaxxII achieved 96.0% inhibition at 0.01 ppm and 99.0% at 10 ppm and tebuconazole reached 92.4% inhibition at 1 ppm. Dipotassium phosphite, neem oil, and Serenade also showed promise, especially against JJ, whereas copper formulations required comparably higher concentrations. The RNA-seq can identify biologically relevant pathogen vulnerabilities and accelerate rational fungicide selection. Our genomics-to-management workflow provides a practical framework for improving chemical control and guiding future validation across pathogen isolates.</p>