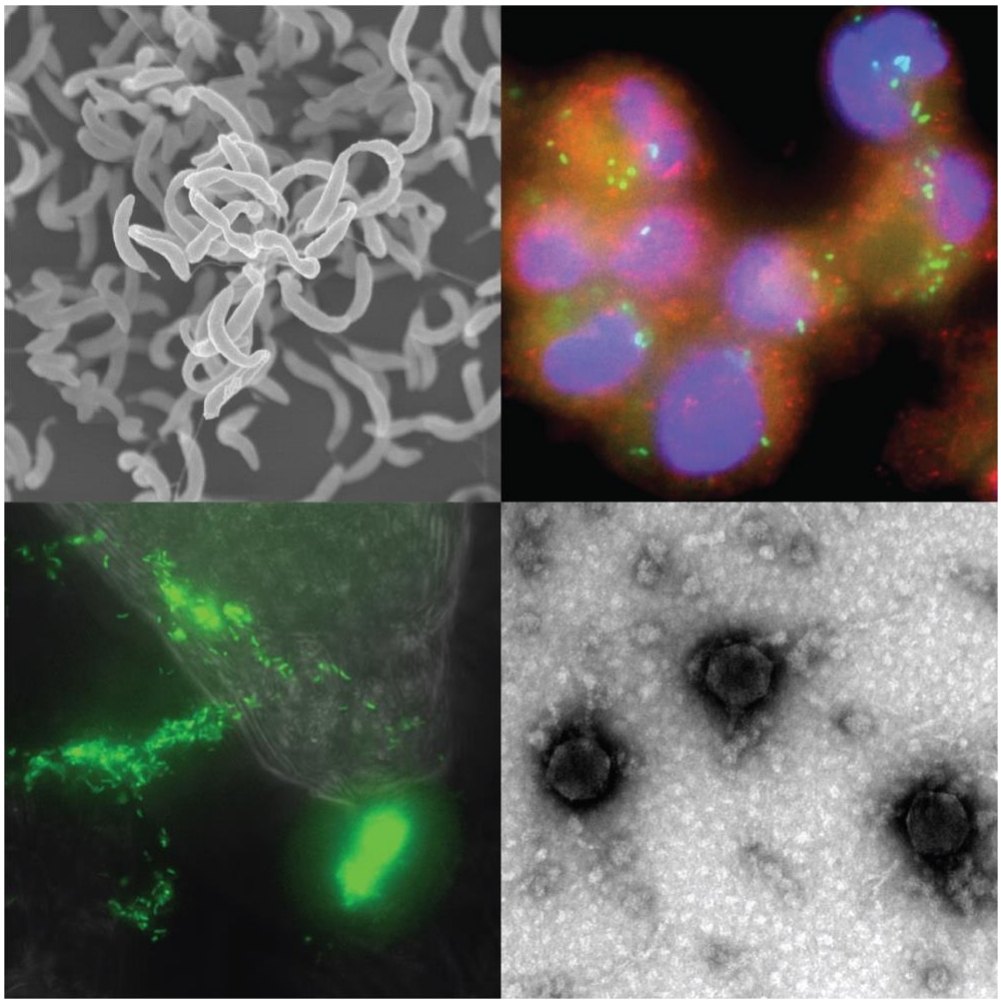
Michigan Branch
American Society for Microbiology
2026 Annual Meeting



March 28th, 2026

Hosted by:

FLINT COLLEGE OF INNOVATION & TECHNOLOGY

Contributions

Meeting Organizer:
Maeve McLaughlin, *University of Michigan–Flint*

Keynote Lecture:
Anne Thompson, *Portland State University*

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Maddy Quinlan, *Michigan State University* (Lower Left)
Maeve McLaughlin, *University of Michigan–Flint* (Lower Right)

Acknowledgements:
This meeting could not have happened without support from the College of Innovation and Technology. I also want to thank Laurel Ming for her expert help planning and organizing this meeting. Without her, this could not have happened.

Table of Contents

Schedule

Time	Event
8:00-9:00 AM	Registration and Continental Breakfast
9:00-9:10 AM	Introductions
9:10-9:25 AM	**Speaker:** Nico Fernandez, PhD (Peggy Cotter Awardee) **Institution:** University of Michigan-Dearborn **Title:** "Two Distinct Regulatory Systems Control Pulcherrimin Biosynthesis in *Bacillus subtilis*"
9:25-9:40 AM	**Speaker:** Brendyn St. Louis **Institution:** Wayne State University **Title:** "Cleavage of the Hippo kinases by the non-canonical inflammasome activates apoptosis in macrophages upon pathogenic *Legionella* infection"
9:40-9:55 AM	**Speaker:** Emily Capehart **Institution:** Michigan State University **Title:** "Characterizing the Antigenic Variation System of the Tick-Borne Relapsing Fever Agent *Borrelia hispanica*"
9:55-10:10 AM	**Speaker:** Paul Breen, PhD (Peggy Cotter Awardee) **Institution:** University of Detroit Mercy **Title:** "Pla-mediated Cleavage of the C-terminus of Factor H on *Y. pestis* Prevents Inactivation of C3b to iC3b"
10:10-10:25 AM	**Speaker:** Rylan Hissong **Institution:** University of Michigan-Ann Arbor **Title:** "IL-10 is Crucial for Protection of CNS from Inflammatory Damage during Cryptococcal Meningoencephalitis"
10:30-12:00 PM	Poster session
12:00-1:00 PM	Lunch
1:00-1:15 PM	**Speaker:** Yifan Wang (Peggy Cotter Awardee) **Institution:** University of Michigan Medical School **Title:** "Host-specific determinants of *Toxoplasma gondii* fitness revealed by genome-wide CRISPR screens"
1:15-1:30 PM	**Speaker:** Claire Albright **Institution:** University of Michigan-Ann Arbor **Title:** "Aerotolerant Capacity of the Lung Commensal *Prevotella melaninogenica*"
1:30-1:45 PM	**Speaker:** Maddy Quinlan **Institution:** Michigan State University **Title:** "Dissecting an Endophytic *Caulobacter-Bradyrhizobium* Partnership that Enhances Soybean Nodulation and Drought Resilience"
1:45-2:00 PM	**Speaker:** Joseph M. Krampen **Institution:** University of Michigan-Ann Arbor **Title:** "Rising Ocean Temperatures Reduce the Hawaiian Bobtail Squid's Egg Defense Mediated by Symbiont Metabolites"
2:00-3:00 PM	Networking
3:00-4:00 PM	**Keynote speaker:** Anne W. Thompson, PhD

Schedule

	Institution: Portland State University **Title:** "The invisible forest: Life and death of the Earth's most numerous photosynthetic cells"
4:00-4:30 PM	Presentation Awards and Closing Remarks
4:30-5:00 PM	Board meeting

Keynote

Anne Thompson, PhD

ASM Distinguished Lecturer

Portland State University

Title: The invisible forest: Life and death of the Earth's most numerous photosynthetic cells

Anne Thompson, Ph.D., is an assistant professor at Portland State University. Her lab, the "Bluewater Lab," combines approaches in SCUBA, sea-going oceanography, molecular biology, modeling and classic microbiology to understand how the massive populations of microorganisms in the sea make a living. Her work advances microbial oceanography in areas of microbial interactions with predators and symbiotic hosts, trace metal chemistry, microbial physiology and single-cell gene expression.

Thompson received her Ph.D. from the MIT-Woods Hole Oceanographic Institution Joint Program in biology oceanography, working with mentors Sallie (Penny) Chisholm, Ph.D., (MIT) and Mak Saito, Ph.D., (WHOI). She did post-doctoral training with Jonathan Zehr, Ph.D., (UC Santa Cruz), where she and colleagues discovered that a globally abundant nitrogen-fixing cyanobacterium is symbiotic with a single-celled alga, in what is now recognized as a nitrogen-fixing organelle. Single cell analysis and flow cytometry were central to that discovery, which led Thompson to become a senior scientist at the Institute for Systems Biology (Seattle, Wash.), working with Nitin Baliga, Ph.D., and the Advanced Cytometry Group at BD Biosciences. In this position, Thompson developed innovative techniques in flow cytometry for diverse microbial systems.

In 2016, Thompson joined the biology faculty at Portland State University in order to continue pursuit of fundamental research in microbial oceanography and to work with a population of students who are severely underrepresented in geosciences and microbiology, including first-generation college students and students from minority backgrounds.

Oral Presentation #1

Two Distinct Regulatory Systems Control Pulcherrimin Biosynthesis in *Bacillus subtilis*

Nico Fernandez

Regulation of transcription enables bacteria to respond to external stimuli with appropriate timing and magnitude. In the soil bacterium *Bacillus subtilis*, transcriptional regulation is central to developmental processes required for survival. Gene expression during the transition from exponential to stationary phase is controlled by transcription factors known as transition state regulators (TSRs). TSRs influence processes such as biofilm formation versus motility, genetic competence, and sporulation, but their broader effects on bacterial physiology remain incompletely understood. Here, we show that two TSRs, ScoC and AbrB, together with the MarR-family transcription factor PchR, negatively regulate production of the iron chelator pulcherrimin in *B. subtilis*. Genetic analysis indicates that all three factors are required to limit pulcherrimin production during exponential phase and affect both the rate and total amount produced. Consistent with this, expression of the pulcherrimin biosynthesis gene *yvmC* was controlled by ScoC, AbrB, and PchR and correlated with pulcherrimin levels in each background. Finally, our *in vitro* data suggest a weak direct role for ScoC in controlling pulcherrimin production alongside AbrB and PchR. Given its role in protection against reactive oxygen species, its layered regulation by two distinct regulatory systems highlights the importance of tightly controlling this metabolite in *B. subtilis* physiology.

Oral Presentation #2

Cleavage of the Hippo kinases by the non-canonical inflammasome activates apoptosis in macrophages upon pathogenic *Legionella* infection

Brendyn M. St. Louis, Sydney M. Quagliato, Yu-Ting Su, Pei-Chung Lee

Department of Biological Sciences, Wayne State University, Detroit, MI 48202

The Hippo kinases MST1 and MST2 are well-characterized initiators of the canonical Hippo signaling pathway that governs cell proliferation and differentiation. Interestingly, individuals with *MST1* mutations are immunocompromised and experience recurrent bacterial infection, suggesting an unexplored role in innate immunity. Recent advances reveal that MST1/2 are cleaved by caspase-1 following the detection of bacterial flagellin by the NLRC4 inflammasome to induce apoptotic cell death and restrict replication of the human pathogen *Legionella pneumophila*. Here, we discover that MST1/2 are required for apoptosis in macrophages infected with a *Legionella* strain lacking flagellin (*LpΔflaA*) bypassing host NLRC4 detection and caspase-1 activation. Specifically, maximal cell death was only observed when both MST1/2 were present, while MST1 or MST2 alone were sufficient to restore cell death. We show that caspase-11, a non-canonical inflammasome activated by bacterial lipopolysaccharides, is involved in this alternative death process. Reconstitution of mouse caspase-11 or the homologous human caspase-4/5 in a cell model activates the cleavage of MST1/2 and subsequent cell death. Consistently, knocking out *caspase-11* in macrophages reduces cleavage of MST1/2 and apoptosis in response to *LpΔflaA* and direct LPS stimulation. Unlike caspase-1 which directly cleaves MST1/2, caspase-11 requires additional host proteins to cleave MST1/2. Finally, the results show that post-translational modifications of caspase-11's canonical substrate, gasdermin D, have a limited role in *LpΔflaA*-induced cell death, supporting the importance of the cleavage of the Hippo kinases as central death regulators following the activation of different immune pathways against bacterial pathogens.

Oral Presentation #3

Characterizing the Antigenic Variation System of the Tick-Borne Relapsing Fever Agent *Borrelia hispanica*

Emily E. Capehart[1], Samantha Hatter[1], Dalen W. Agnew[1], John C. Blazier[2], Luke J. Tallon[3], Artem S. Rogovsky[1]

[1]Michigan State University, East Lansing, Michigan 48824; [2]Texas A&M University, College Station, Texas 77843; [3]University of Maryland, Baltimore, Maryland 20742

In the Iberian Peninsula, *Borrelia hispanica* is the primary cause of tick-borne relapsing fever (TBRF), a disease caused by several species in the genus *Borrelia*. The human disease is characterized by febrile relapses driven by the interplay between the bacterium's antigenic variation system and the host's adaptive immune system. To date, the pathogenesis of *B. hispanica* is poorly understood and highly understudied, in part due to the lack of an appropriate immunocompetent mouse model and limited knowledge of its genome. Thus, our work here aims to identify mouse model(s) and to fully define the genome of *B. hispanica*. The infectivity of *B. hispanica* was tested in immunodeficient (CbySmn.Cg-Prkdcscid/J (SCID); 3 males, 3 females), and immunocompetent C3H/HeJ (C3H; 3 males, 3 females) and Collaborative Cross 046 (CC046; 10 males, 8 females) mice. To detect spirochetemia, blood was periodically examined by dark-field microscopy and culture. To detect systemic dissemination, brains, bladders, hearts, spleens, livers, ear pinnae, and tibiotarsal joints harvested at the time of sacrifice were tested by culture. The *B. hispanica* genome was sequenced by long-read PacBio and Oxford NanoPore technologies. The results demonstrated a significantly impaired infectivity of *B. hispanica* in C3H mice, the most used model of other TBRF pathogens, whose spirochetemia was rare and all harvested tissues tested negative. In contrast, all SCID mice developed persistent spirochetemia and disseminated infection, however, spirochetemic relapses were not detected due to the lacking antibody response. Excitingly, all CC046 mice developed spirochetemia with multiple relapses, the hallmark of human relapsing fever. Moreover, the use of the CC046 model also revealed the neurotropic nature of *B. hispanica*. Our assembly of PacBio/NanoPore sequences defined the complete genome of *B. hispanica*, which consisted of a chromosome, 16 linear and 5 circular plasmids. CC046 line is a novel model for studying the pathogenesis of *B. hispanica* and, along with the now fully defined genome, provides new opportunities for investigating this highly understudied pathogen.

Oral Presentation #4

Pla-mediated Cleavage of the C-terminus of Factor H on *Y. pestis* Prevents Inactivation of C3b to iC3b

Paul Breen[1], Sarah C. Plecha[1], Michael J. Rowse[1], Eric S. Krukonis[1]

Division of Integrated Biomedical Sciences, University of Detroit Mercy School of Dentistry, Detroit, MI, USA.

Yersinia pestis, the pathogen responsible for plague, evades the complement Membrane Attack Complex (MAC), through utilization of its adhesion protein Ail. Ail recruits the host complement regulatory protein Factor H (FH) present in serum, to the pathogen surface. FH is then able to recruit the protein Factor I (FI) which, along with FH, is able to cleave complement protein C3b into inactive iC3b, preventing formation of the MAC. Ail recruits FH by binding to short consensus repeats (SCRs) 5-7 (there are 20 SCRs within FH) and upon recruitment to the bacterial surface, FH is degraded by the *Y. pestis* protease Pla, but FH still associates with the bacterial cell surface after degradation. We sought to define which terminus of FH is cleaved off of the protein once it becomes associated with the bacterial surface and if the remaining FH fragment can recruit FI. Using monoclonal antibodies directed towards either the N-terminus or C-terminus of FH, we showed the C-terminus of FH is cleaved off by Pla. In vitro, in the absence of *Y. pestis*, this cleaved form of FH can bring FI and C3b together to generate iC3b. However, when pre-cleaved FH bound to an Ail+ strain lacking Pla (Δ*pla*, to prevent further cleavage), FH could no longer facilitate iC3b generation. These data suggest loss of the C-terminal SCR19-20 C3b-binding site in FH renders Ail-bound FH unable to facilitate FI-mediated cleavage of C3b to iC3b. Thus, the ability of Ail to prevent MAC formation may be due to alternative modes of MAC inhibition, such as preventing C5b-C9 recruitment through Ail binding to vitronectin, or potentially by Ail directly inhibiting C9 polymerization in the membrane of *Y. pestis*. Future experiments will explore these possibilities.

Oral Presentation #5

IL-10 is Crucial for Protection of CNS from Inflammatory Damage during Cryptococcal Meningoencephalitis.

Rylan Hissong*, Hailong Li*, Kristie Goughenour, Jane Barnett, Maia Lintner, Alexander Ballesteros, Heineken Queen, Anutosh Ganguly, Grace Y. Chen, Jintao Xu*, Michal A. Olszewski*

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**Contributed equally*

Cryptococcus neoformans meningoencephalitis (CM) is a highly lethal fungal disease. Th1/IFN- g are crucial for fungal clearance but are also profoundly damaging in the brain. In CM patients with inflammatory syndromes (c-IRIS/c-PIIRS), elevated T-cell responses and elevated IFN- g in cerebrospinal fluid were markers of poor prognosis and brain damage. The cytokine IL-10 is one of the major anti-inflammatory factors produced by immune cells, but its role in CM and PIIRS is unknown.

Using an established murine model of CM/PIIRS, we applied fungal burden analysis, flow cytometry, immunofluorescence microscopy, and single-cell RNA sequencing to assess the effects of IL10 deficiency on inflammation and pathology during CM. Additionally, a murine coma and behavioral scale (MCBS) was used to assess neurological health. IL-10-/- mice were compared to wild-type C57BL/6 counterparts.

Our investigation revealed that: 1) IL-10 is induced in the CM brain; 2) regulatory T cells are the major source of this cytokine in the brain. Compared to WT mice, IL-10-/- mice with CM exhibited: 1) drastically worsened weight loss and neurological status of the infected mice, inducing 100% mortality despite enhanced fungal clearance; 2) increased CD45+ leukocyte infiltration into the brain, with a notable expansion of T-cells; 3) enhanced activation and inflammatory cytokine (IFN-□ and TNF-α) production by CD4+ T cells in the CNS; and 4) accelerated recruitment of monocytes and increased iNOS upregulation in these cells as well as in the resident microglia. Pathological examination further revealed increased neuronal damage in IL-10-/- mice with CM with severe depletion of the synaptic protein Syt7 at the perimeter of cryptococcal lesions and intense staining of cleaved caspase-3 in neurons, a marker of neuronal apoptosis.

These results collectively indicate that IL-10 plays a critical role during CM as an essential immunoregulatory factor protecting the brain from inflammatory damage.

Oral Presentation #6

Host-specific determinants of *Toxoplasma gondii* fitness revealed by genome-wide CRISPR screens

Jiawen Fu[1 *], Priscilla O. Gyan[1,2 *], Yao Gu[3,4], Lamba Omar Sangaré[4,5], Jeroen P. J. Saeij[4,#], Yifan Wang[1,4 #]

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[5]Department of Biology, Texas A&M University, College Station, TX, USA

Toxoplasma gondii is an obligate intracellular parasite that is remarkable for its ability to infect nearly all warm-blooded animals and to invade any nucleated cell, establishing lifelong infections in humans and animals. This exceptional host range requires the parasite to continually adapt its strategies for nutrient acquisition, immune evasion, and survival across genetically diverse host species. During infection, macrophages act as a crucial first line of defense against *Toxoplasma*; however, the parasite can survive within these immune cells by overcoming macrophage antimicrobial mechanisms. By integrating cross-species genome-wide CRISPR screens using primary macrophages from laboratory mice and rats, our study reveals that genetic determinants of parasite fitness differ among hosts and immune activation states. We demonstrate that the secreted effectors GRA42 and GRA43 mediate evasion of interferon-gamma (IFNγ)–induced restriction specifically in rat macrophages. Functional validation shows that loss of either gene sensitizes *Toxoplasma* to IFNγ-mediated parasite inhibition and host cell death in rat, but not murine, macrophages. Additionally, by comparing with prior CRISPR screens in human cells, we identified a *Toxoplasma* amino acid transporter TgApiAT6-3 as critical for parasite fitness in rodent cells but dispensable in human cells, underscoring species-specific adaptation for amino acid acquisition. We found that Δ*TgApiAT6-3* parasites exhibit markedly reduced growth specifically in murine and rat cells but not in human cells. We further determined that supplementation with L-cystine, but not other essential amino acids, rescues the growth defect of Δ*TgApiAT6-3* parasites in murine cells, suggesting that TgApiAT6-3 may function as a bona fide cystine transporter. Together, these findings advance our understanding of how *Toxoplasma* adapts broad host microenvironment and highlight the value of comparative CRISPR screening for unraveling host-specific determinants of parasitic fitness. Further mechanistic studies are underway to more precisely define the molecular basis of these host-specific determinants.

Oral Presentation #7

Aerotolerant Capacity of the Lung Commensal *Prevotella melaninogenica*

Albright[1], Claire E; Anil[1], Gouri; Evans[1], Jacob; Ntamubano[1], Souzane; Kozik[1], Ariangela

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Prevotella melaninogenica is a highly prevalent commensal in the healthy human lower respiratory tract (LRT), accounting for ~10% of the microbial community and persisting in chronic respiratory disease. This ecological predominance presents a notable paradox: while the LRT is a highly oxygenated environment, *P. melaninogenica* has been repeatedly described as a strict obligate anaerobe, unable to survive above 0.05% O_2. This discrepancy underscores a significant gap in our understanding of the physiology and adaptive strategies that enable its competitive success in the human airway.

To address this discrepancy, we quantified *P. melaninogenica* growth, viability, and consumption across O_2 concentrations. Strikingly, *P. melaninogenica* exhibited robust growth at 2% and 5% O_2, with no significant reduction in maximum cell density or viability at 24 hours compared to 0% O_2. Consistent with active growth, these cultures consumed oxygen, indicating ongoing metabolic activity. While growth ceased at 8% O_2, a significant number of cells remained viable and capable of anaerobic rescue. Most notably, some cell survival was found after 24-hour exposure to 21% O_2 (ambient air), revealing an oxygen tolerance that far exceeds previous assumptions.

We next performed transcriptomic profiling to elucidate the genetic mechanisms underlying this aerotolerance. RNA sequencing in 0% vs. 2% O_2 revealed significant upregulation of genes encoding putative ROS scavenging enzymes, ETC components, and iron sequestration proteins important for detoxification. Furthermore, we found many putative DNA repair and protection genes to be upregulated, suggesting a coordinated system for maintaining genomic integrity under stress.

Together, these findings provide novel insight into *P. melaninogenica* physiology and raise new questions regarding microbe-microbe and host-microbe dynamics in the human respiratory tract.

Oral Presentation #8

Dissecting an Endophytic *Caulobacter-Bradyrhizobium* Partnership that Enhances Soybean Nodulation and Drought Resilience

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Although historically regarded as freshwater oligotrophs, studies over the past decade have identified *Caulobacter* species in diverse plant-associated microbial communities, including those of maize, hibiscus, watermelon, poplar, and wild *Arabidopsis*. Notably, a *Caulobacter* strain (RL271) was recently identified as one of five hub taxa isolated from the root endosphere of soybean plants cultivated in a no-till agricultural field at the Kellogg Biological Station in southern Michigan. Soybean seedlings inoculated with RL271, either alone or as part of a five-member hub microbial consortium, showed enhanced drought tolerance and a significant increase in root nodulation by the nitrogen-fixing symbiont *Bradyrhizobium diazoefficiens* (hereafter *Bradyrhizobium*) (Longley, 2022). Like several other plant-associated *Caulobacter* species, RL271 is a riboflavin auxotroph due to absence of the *ribD/E/AB/H* operon encoding riboflavin biosynthesis genes. Co-culture experiments on soil-extract agar demonstrated a syntrophic interaction in which *Bradyrhizobium* supports RL271 growth likely by supplying riboflavin, while RL271 enhances the thermotolerance and general stress resistance of *Bradyrhizobium*. Additionally, RL271 stimulates adhesin production in *Bradyrhizobium* which may promote its attachment to plant roots and support subsequent nodulation leading to enhanced plant resilience. RNA-seq analysis of RL271 in co-culture with *Bradyrhizobium* on soil-extract agar revealed significant induction of nitrate/nitrite assimilation and membrane transport pathways, suggesting that competition for nitrogen may structure this microbial interaction. Overall, our results provide evidence that *Caulobacter sp.* RL271 is a functionally significant member of the rhizosphere, capable of improving soybean drought resilience through metabolic cross-feeding and modulation of a nitrogen-fixing partner. Elucidating the molecular basis of this *Caulobacter-Bradyrhizobium* interaction has the potential to advance microbiome-based strategies for crop stress tolerance.

Longley R. 2022. Impact Of Agricultural Management And Microbial Inoculation On Soybean (*Glycine max)* And Its Associated Microbiome. Michigan State University, East Lansing, Michigan.

Oral Presentation #9

Rising Ocean Temperatures Reduce the Hawaiian Bobtail Squid's Egg Defense Mediated by Symbiont Metabolites

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As global climate continues warming, increasing ocean temperatures are expected to disrupt many aspects of marine life. The Hawaiian bobtail squid, *Euprymna scolopes*, is a model organism of bacterial symbiosis well known for its light organ association with the bioluminescent bacterium *Vibrio fischeri*. Female Hawaiian bobtail squid have a second organ, the accessory nidamental gland (ANG), that houses a symbiotic bacterial consortium shown to protect eggs during embryogenesis. We hypothesize that rising ocean temperatures will affect the metabolomic profile of the squid's symbiotic bacteria which could perturb egg protection. We utilized untargeted metabolomics to evaluate changes in metabolomes of squid bacterial symbionts and squid egg clutches incubated at the current ocean water temperature and two higher temperatures predicted for future Hawaiian waters. Symbiont and egg metabolomes significantly shifted due to temperature, including changes in the abundances of several key antimicrobial metabolites. Using imaging mass spectrometry of eggs, we found spatial metabolite changes upon changing temperatures. Finally, we measured antifungal bioactivity of symbionts and eggs to determine how temperature impacts egg protection. Findings from these studies will provide additional understanding of how host-symbiont relationships are affected by increasing ocean temperatures.

Poster Presentation #1

Defining the Mechanism of Action and Resistance of New *Mycobacterium abscessus* MmpL3 Inhibitors

Bassel M. Abdalla[1,2], Matt Giletto[2], Nazli Goksel Carpa[3], Angela K. Wilson[3], Edmund Ellsworth[2], & Robert B. Abramovitch[1*]

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[3]Department of Clinical Pathology, The University of Utah, Salt Lake City, UT

Non-tuberculous mycobacterial (NTM) infections comprise an underrecognized yet emergent source of infections. *Mycobacterium abscessus* (Mab) is an attractive model for NTMs due to its growing prevalence, multidrug resistance, and long course of therapy (1-2 years). This long course of therapy is associated with the emergence of further drug resistance, necessitating the discovery of new antimycobacterial agents. MmpL3, an inner membrane transporter of trehalose-monomycolate, is an extensively studied drug target because it is essential for all mycobacteria's survival. However, less is known about the outcomes of MmpL3 inhibition on mycobacterial pathogenesis, drug sensitivity/resistance profiles, and ultimately clinical prognosis. Using a variety of biochemical, pharmacological, microbiological, and genetic strategies in combination with computational modeling, we are studying the structure of MmpL3, rationally designing efficacious MmpL3 inhibitors, and characterizing the outcomes of the treatments in wild-type, mutant strains, and clinical isolates. Toward these goals, we screened 450 novel MmpL3 inhibitors against wild-type *M. abscessus* (ATCC19977), isolated 16 unique resistant *M. abscessus* mutants against four inhibitors and examined the cross-resistance profiles between the mutants and a subset of analogs. Additionally, we studied the frequency of resistance and induced collateral sensitivity of several inhibitors as well as putative fitness defects associated with *mmpl3* mutations. We also tested a subset of inhibitors against WT *M. abscessus* in Bone marrow-derived macrophages, against 30 different clinical isolates, and in SCID acute infection mouse models. We further studied several molecular and biochemical alterations induced by MmpL3 inhibition. The Screening process yielded several potent and efficacious analogs *in vitro*, inside macrophages (EC_{50} <1 uM) and *in vivo*, comparable to the standard-of-care treatments. Cross-resistance profiling of the mutants and clinical isolates revealed differential patterns of sensitivity/resistance, likely underlying differential ligand-protein interactions and reflecting differences in the MmpL3 structure. Several inhibitors exhibited low frequencies of resistance (10^{-8}-10^{-9}) and induced collateral sensitivity to standard-of-care drugs when used in combination. Fitness studies hinted at putative fitness defects associated with specific *mmpl3* mutations. MmpL3 inhibition resulted in distinctive sets of differentially regulated genes, which may impact bacterial virulence, drug sensitivity, and biofilm production capacity of the bacteria. Together, these findings give us more insights into the clinical value of MmpL3, bacterial responses associated with its inhibition, and how it influences the pathogenesis of NTMs.

Poster Presentation #2

Host and pathogen genetics jointly drive inflammation in Lyme neuroborreliosis

Neumann, Natanel[a]; Batool, Maliha[b]; Aceino, Alexander[b]; St. Jean, Samantha[b]; Kasantikul, Tanit[a]; Agnew, Dalen[a]; Chien, Rory C.[c]; Stoica, George[b]; Wiener, Dominique J.[b]; Huang, Mingze[b]; Crawford, Scott[b]; Lynch, Rachel M.[d]; Threadgill, David W.[e]; Rogovskyy, Artem S.[a]

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The collaborative cross (CC) is a panel of recombinant inbred mice generated through the breeding of eight founder strains via a funnel breeding scheme, enabling a wide range of reproducible genetic diversity. Despite extensive research into *Borrelia burgdorferi* sensu lato complex (*Bb*), Lyme neuroborreliosis (LNB) remains challenging to study due to the lack of a reliable animal model that can consistently mimic human disease pathogenesis. Aside from a recently described C3H meningitis model, non-human primates (NHP) remain the most successful model, consistently showing both central and peripheral nervous system involvement. However, ethical, economic, and logistical constraints limit their use. Although laboratory mice offer advantages over the NHP model, no commonly used laboratory inbred mouse strain has been shown to reliably permit *Bb* entry into the central or peripheral nervous system, develop significant inflammatory lesions in neural tissues, or manifest neurological symptoms. In the current study, we employed four CC lines and four *Bb* strains to identify reproducible host-pathogen interactions that result in LNB-relevant inflammatory lesions, testing how host genetics and *Bb* genotype shape disease outcomes. Inflammation burden was assessed utilizing an inflammation score following a 3-month borrelial infection study. The results showed that most infected mice developed inflammatory lesions in neural tissues, but with substantial variability in both lesion frequency and cumulative inflammatory burden across the tested CC lines and *Bb* strains. Furthermore, cumulative inflammation scores demonstrated a statistically significant host–pathogen interaction ($p<0.05$), indicating that LNB-relevant neuroinflammation is jointly shaped by both host genetic background and bacterial genotype. *Bb*-infected female mice exhibited approximately twice as many inflamed tissues ($p=0.006$) and 50% higher cumulative inflammation ($p=0.0042$) compared to *Bb*-infected males. Collectively, these findings establish our four CC lines as novel mouse models of LNB.

Poster Presentation #3

Norovirus non-structural proteins alter glycolysis for efficient virus replication

Hyejin Lee, Christiane Wobus

During viral infections, viruses survive and reproduce by rewiring several metabolic pathways of infected cells. Pathways involved in central carbon metabolism, such as glycolysis, are especially targeted since they supply viruses with energy and macromolecular precursors to sustain prolonged replication. However, the effect of viruses on host cell metabolism varies widely by viral species and host cell type, and the molecular mechanism behind these alterations remains largely unclear. This study seeks to uncover the mechanism NoVs utilizes to rewire glycolysis as we previously showed it plays an important role in promoting successful NoV replication. Through analysis of glycolytic metabolites and the expression of glycolytic enzymes, we have confirmed that both MNV and HNoV upregulate glycolysis during their replicative cycles. Inhibition of glycolysis through 2-deoxyglucose (2DG) treatment and glucose deprivation revealed both viruses require glycolysis for optimal replication in intestinal enteroid models. Additionally, early mechanistic analysis revealed that MNV infection increased the enzymatic activity of hexokinase (HK), a key rate limiting enzyme in the glycolytic pathway, and follow-up analysis further showed this increase in HK enzymatic activity is mediated by the murine norovirus (MNV) nonstructural protein (NS) NS6 and human norovirus (HNoV) NS1/2 protein. Current studies aim to determine the underlying mechanisms MNV NS6 and HNoV NS1/2 utilize to increase HK enzymatic activity. Collectively, these data demonstrate the importance of glycolysis for productive NoV infection and the ability of NoV proteins to alter the activity of a key metabolic enzyme.

Poster Presentation #4

Phosphate- and lactate-dependent cytoplasmic acidification drive growth arrest of *Mycobacterium tuberculosis* on lactate at acidic pH

Adam P. Kibiloski, Shelby J. Dechow, Bassel J. Abdalla, Heather M. Murdoch, Robert B. Abramovitch

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Mycobacterium tuberculosis (Mtb) cultured in minimal medium at acidic pH arrests growth when provided specific single carbon sources, including glycerol, propionate, or lactate, a phenomenon we refer to as acid growth arrest. We hypothesized that growth arrest on lactate is a regulated physiology that could be suppressed by mutations. We plated a transposon mutant library on minimal medium plates buffered to acidic pH with lactate as a sole carbon source and successfully isolated suppressor mutants. Four of the suppressor mutants had transposon insertions in *phoT,* which encodes for ATPase component of a phosphate ABC transporter. Mtb is also capable of growing in minimal media at acidic pH supplemented with lactate when phosphate is depleted, supporting the conclusion that Mtb growth arrest on lactate was dependent on phosphate. We hypothesized that lactate causes cytoplasmic acidification and measured cytoplasmic pH over time in a range of phosphate and lactate concentrations. We found that lactate and phosphate together contribute to acidification of the cytoplasm. The *phoT::Tn* mutant has a more neutral cytoplasm, likely by preventing phosphate-associated protons from entering the cell. Transcriptional profiling showed the *phoT::Tn* mutant upregulates the *senX3*/*regX3* two-component regulatory system regulon. Using a Δ*regX3* mutant, we demonstrate growth on lactate at low phosphate requires RegX3 Analysis of WT transcriptional profiles also indicates that components of the electron transport chain and phosphate uptake genes are strongly upregulated in lactate at acidic pH. These data support that phosphate and lactate cause disruptions to pH homeostasis and membrane energetics in Mtb. We propose a model where 1) the combined impact of acidic pH, lactate and phosphate cause cytoplasmic acidification and decreased proton motive force that is associated with arrested growth; and 2) low phosphate or a mutated phosphate transporter causes upregulation of *senX3-regX3* which may promote growth by inducing ESX-5 and PPE/PE-based import mechanisms, altering the mycomembrane or nutrient uptake in a manner that promotes growth at acidic pH.

Poster Presentation #5

Identification of novel motility regulators in an atypical *Brucella* sp.

Jeannette Farnham, Aretha Fiebig, Rinosh Mani, Sean Crosson

The presence of flagellar genes in the genus *Brucella* remains an unresolved mystery in the field. Classical Brucella species retain a full set of flagellar genes, but they have lost motility through multiple mutation events. Some of the remaining flagellar genes have been identified as virulence factors, although the exact mechanisms governing their effect on infection are poorly understood. Atypical Brucella species, a relatively recent discovery, are motile with a completely functional complement of flagellar genes. We study atypical Brucella isolates cultured from diseased and deceased frogs from local Michigan zoos. We have established an atypical *Brucella* species as a genetic model for studying motility in a genus long classified as non-motile. Using a randomly-barcoded transposon library, we identified two potent regulators of motility: *clpA* (encoding a protease subunit) and BIVDL1_002263 (hereafter 2263), a previously uncharacterized gene conserved in *Brucella* species and in related a-proteobacteria. Targeted deletion of *clpA* or 2263 produced a hypermotile, multiflagellated phenotype compared with wild-type cells, while overexpression of 2263 produced a hypomotile phenotype. We propose that 2263 encodes a ClpA protease adaptor that modulates motility via regulated proteolysis of flagellar regulators or components. Our development of an atypical *Brucella* model system has the potential to provide insight into the regulatory mechanisms governing motility and flagellar expression in *Brucella* species and to clarify how flagellar components may contribute to host interactions.

Poster Presentation #6

A *Brucella abortus* Δ*wbkA* mutant highlights differential attenuation between strains with engineered and naturally rough LPS

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Brucella spp. are Gram-negative, intracellular pathogens that cause brucellosis across a diverse host range. The zoonotic *Brucella abortus* can induce chronic human infection and spontaneous bovine abortion, posing significant health risks and economic disruptions. Lipopolysaccharide (LPS) is a key outer membrane determinant of *Brucella* virulence, but how LPS structure influences *Brucella*-host interactions is not fully known. We are focused on a central paradox in *Brucella* infection biology: loss of the LPS O-polysaccharide (O-antigen; "roughness") is often associated with enhanced innate immune recognition and attenuation, yet naturally rough species, such as *B. ovis* and *B. canis*, remain competent intracellular pathogens in their preferred hosts and *ex vivo* models (1). Consistent with previous work on rough mutants of naturally smooth B. melitensis and *B. suis* (2), we saw that a rough *B. abortus* mutant lacking the O-antigen glycosyltransferase *wbkA* (Δ*wbkA*) elicited strong inflammatory cytokine responses and showed significantly reduced fitness at 48 h post-infection in an ex vivo model relative to wild-type *B. abortus*. *In vivo*, the Δ*wbkA* mutant also showed significant attenuation in bovine lymph nodes at 3 weeks post-infection through both mucosal and intravenous routes of entry. In contrast, infection with the naturally rough ovine pathogen *B. ovis* induced inflammatory cytokine release ex vivo without comparable attenuation. Although both rough *Brucella* strains triggered inflammatory cytokine production, they produced different intracellular infection outcomes, indicating that inflammatory signaling activation alone does not predict intracellular control. Ongoing comparative infections with *B. abortus* and *B. ovis* in bovine and murine cell models are aimed at identifying host and bacterial determinants of restriction and testing how LPS context, rather than presence or absence of O-antigen, dictates *Brucella* intracellular survival. Ultimately, uncovering mechanistic pathways with our bacterial and cell model systems could highlight novel therapeutic targets in both the host and bacterium.

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2. Rittig MG, Kaufmann A, Robins A, Shaw B, Sprenger H, Gemsa D, Foulongne V, Rouot B, Dornand J. 2003. Smooth and rough lipopolysaccharide phenotypes of *Brucella* induce different intracellular trafficking and cytokine/chemokine release in human monocytes. *J Leukoc Biol* 74:1045–1055.

Poster Presentation #7

The N-terminal Domain of SusC is Necessary for Starch Transport in *Bacteroides thetaiotaomicron*

Aarav R. Raju, Michael Cadigan, Nicole M. Koropatkin

Bacteria in the human gut impact host health by breaking down complex carbohydrates such as starch. *Bacteroides* comprises gram-negative, anaerobic prokaryotes and is one of the most abundant genera of gut bacteria. The *Bacteroides* transport starch from the environment into the periplasm and then the cell using the Starch Utilization System (Sus), which has long served as a model of polysaccharide utilization. In this system, several outer membrane lipoproteins work together to bind and cleave long chains of starch into smaller pieces for import. The TonB complex of the inner cell membrane generates energy for this transport, and SusC, a TonB-dependent transporter (TBDT), moves these starch fragments into the periplasm. Existing models in E. coli suggest that the SusC and TonB interaction is mediated through the TonB box peptide at the N-terminus of SusC. However, predictive structural models using Alphafold suggest an uncharacterized N-terminal extension (NTE) domain of SusC may mediate further interactions between SusC and TonB through parallel beta strand pairing. We hypothesize that in *Bacteroides thetaiotaomicron* (Bt) starch import is primarily mediated by this NTE's interaction with TonB. A Bt SusC NTE knockout strain showed no growth in potato starch but normal growth in glucose, whereas wild-type Bt grew in both sugars. Upregulating the Sus using maltose before growth on starch produced a partial rescue but still resulted in a significant growth delay, suggesting SusC remained functional but impaired, a likely lethal growth defect in a natural environment. This data suggests that the N-terminal region of SusC is essential for Bt growth in starch. We hope to further understand how interactions between SusC and TonB are mediated using in vitro structural techniques like X-ray crystallography and in vivo techniques like isothermal calorimetry to determine the strength of the proposed SusC-TonB interaction. This data demonstrates the necessity of the SusC N-terminal extension for starch uptake in Bt and may extend to other polysaccharide-targeting TBDTs within Bt and across *Bacteroides*.

Poster Presentation #8

Wastewater Monitoring of Norovirus GI and GII in Michigan

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The COVID-19 wastewater surveillance (WS) program in Michigan facilitated the development of complementary public health approaches to monitor viral infections in communities. Leveraging existing capacity enables the framework to expand surveillance to other viral infections of public health concern, particularly in areas with limited capacity for clinical surveillance. The Centers for Disease Control reports that Norovirus causes 58% of foodborne illness and is linked to 2,500 outbreaks yearly in the US. Norovirus clinical data are limited, and this study focuses on utilizing WS for monitoring Norovirus prevalence in Michigan communities.

Composite influent wastewater samples (24-hour, n=335) were collected from eleven different wastewater treatment plants around Michigan from October 2023 to February 2026. Virus concentration was carried out using PEG precipitation and centrifugation to recover concentrated viral pellets and RNA extraction (Flood et al, 2021). Targets for Norovirus GI and GII markers were quantified with the GT-ddPCR Wastewater Test kits (GT Molecular, USA), per instructions using 5 µL of RNA template per reaction well. Droplet digital PCR was performed using BioRad's 1-Step RT-ddPCR Advanced kit with a QX200/600 ddPCR system (Bio-Rad, USA).

Positivity rates for Norovirus GI were 95.83% (322/336) and Norovirus GII was 100% (336/336). The average concentration of the Norovirus GI marker was 6.31×10^4 gene copies/100mL and the Norovirus GII marker was 3.23×10^5 gene copies (GC)/100mL. Norovirus GI was more prevalent in the months of December, January, and June, with a yearly average concentration range of 1.63×10^3-1.01×10^5 GC/100mL, and Norovirus GII was more prevalent in March and April, with a range of 4.95×10^4-1.24×10^6 GC/100mL.

WS can be used as a cost-effective and non-invasive approach to monitoring pathogens such as Norovirus. It can be used to detect infection hotspots and track trends over entire population areas and can fill gaps that clinical testing may leave. WS can provide rapid information to enable public health officials to take timely and targeted mitigation measures to prevent disease spread.

Poster Presentation #9

Human cytomegalovirus particles released from stem-loop binding protein depleted cells transdominantly inhibit viral infection

Kylee Morrison (Western Michigan University) and Robert F Kalejta (University of Wisconsin-Madison)

Virally infected (producer) cells release infectious progeny virions as well as an array of non-infectious particles, vesicles, soluble proteins, and other small molecules called a secretome. Subsequent infections in recipient cells are initiated by the released virions, but the recipient cells are also exposed to producer cell secretomes. Stem-loop binding protein (SLBP) is a cellular protein required in producer cells to make fully infectious human cytomegalovirus (HCMV) stocks that consist of both virions and their associated secretomes. Here, we show that HCMV virus stocks produced in SLBP-depleted producer cells show deficits and delays in early viral gene expression, and profound defects in viral genome replication and late protein accumulation. These infection defects in recipient cells are not elicited by producer cell secretomes; rather, they are driven by the virus particles themselves. Importantly, we demonstrate that HCMV particles produced in SLBP-depleted cells trans-dominantly inhibit co-infecting wild-type HCMV by reducing and delaying viral genome replication and late protein accumulation. Our work here identifies SLBP as a cellular protein required to prevent the generation of trans-dominantly inhibitory HCMV particles.

Importance:
Virion assembly is a complex process mediated by both cellular and viral factors. Proper assembly generates particles that can initiate new infections. There are multiple examples of cellular defenses that modulate virion infectivity for antiviral effect, as well as cellular proteins required for the generation of infectious viral particles. Here, we report a novel example of a cellular protein required for the full infectivity of released virus by preventing the generation of trans-dominantly inhibitory viral particles. Our work expands the interrelatedness of two vibrant areas of research (virus particle assembly and cellular defenses) and offers an opportunity to further define this previously unappreciated example of a virus commandeering a highly conserved cellular protein to support infection in ways distinct from its canonical cellular function.

Poster Presentation #10

Elucidating the molecular grammar of a retrotransposon prion-like domain in budding yeast

Alexa MacKersie, Justin Romero, Awesome Abraham, Sean Beckwith*

Biology Department, Hope College, Holland, MI

Retrotransposons and retroviruses shape genome evolution and can negatively impact genome function. The Ty1 retrotransposon of the budding yeast *Saccharomyces cerevisiae* provides an excellent model for studying fundamental mechanisms of retroelement propagation. Retrotransposition of Ty1 requires a prion-like domain (PrLD) within the Gag protein that contains similar amino acid composition to known yeast prions. Here, we probe the sequence constraints governing Ty1 PrLD function. We find that a truncated PrLD as small as 36 amino acids supports robust retromobility and a PrLD twice as long as wildtype supports partial retromobility. Whereas glutamine and asparagine are critical for the function of several yeast prions, these residues are dispensable for Ty1 PrLD function. However, prolines are required for retromobility. Furthermore, the Ty1 PrLD does not tolerate charged residues. Future work will continue characterizing the sequence parameters that dictate the rules of the Ty1 PrLD's "molecular grammar".

Poster Presentation #11

Real-time, live-cell dynamics of a retrotransposon membraneless organelle in budding yeast

Awesome Abraham, Sean Beckwith*

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Retrotransposons and retroviruses shape genome evolution and can negatively impact genome function. The Ty1 retrotransposon of the budding yeast Saccharomyces cerevisiae provides an excellent model system for studying fundamental mechanisms of retroelement propagation. Ty1 RNA is reverse transcribed into cDNA within virus-like particles (VLPs) and is then inserted into a new genomic location, increasing the total copy number of Ty1 within the host genome. The cytosolic foci (termed retrosomes) that nucleate VLP assembly are not well understood. Here, we use live-cell fluorescent microscopy of GFP-tagged Ty1 Gag protein to study retrosome structure and real-time dynamics. This approach will be used to test for liquid-liquid phase separating properties of Ty1 retrosomes and behavior in response to environmental and cellular stress.

This work was supported by the Herbert H. and Grace A. Dow Scholars Award.

Poster Presentation #12

Sequence constraints on retrotransposition of Ty1 in the budding yeast *Saccharomyces cerevisiae*

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Retrotransposons and retroviruses are key elements in genome evolution. The Ty1 retrotransposon of the budding yeast *Saccharomyces cerevisiae* provides a useful model for the study of fundamental mechanisms of retroelement life cycles. Ty1 retrotransposition requires an intrinsically disordered prion-like domain (PrLD) within the Gag protein that contains amino acid sequence composition similar to other known yeast prions. We are investigating the sequence constraints including length and charge through a series of mutant PrLDs by tagging Ty1 with a *his3-AI* genetic marker, which measures retrotransposition. Our work underscores the importance of sequence composition in Ty1 PrLD function and highlights a gap in our understanding of the molecular grammar of intrinsically disordered domains.

Poster Presentation #13

Ji Luth, Jonathan Finkel

The human mouth is awash with bacteria, fungi, and viruses that typically live at equilibrium to maintain a healthy environment. An imbalance of these microorganisms in their environment is responsible for some common diseases in the human mouth, including cavities (caries) and periodontal disease. *Candida albicans* and *Streptococcus mutans* are among the microorganisms that have long been associated with dental caries. One of the major mechanisms that microorganisms use to cause caries is multi-organismal biofilms. The adhesion between *C. albicans* and the bacteria already present in the oral cavity provides an ideal breeding ground for *C. albicans* growth into biofilms. Biofilms are defined as microbial communities that bind to a substrate and are encased by an extracellular matrix. This research aims to identify cell wall genes required for *C. albicans* and *S. mutans* biofilm formation. Identification of these gene products will allow for new drug targets that decrease the cospecies adherence frequencies and host caries formation.

Poster Presentation #14

Type 1 Conventional Dendritic Cells Drive Protective Th1 Immunity During Cryptococcal Infection

L. Borhani[1], R. Hissong[1], S. Hopson[1], M. Lintner[1], J. Barnett[1], J. Xu1,[2], K. Goughenour[1,2], M. Olszewski[2]

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Cryptococcus neoformans is a pathogenic yeast that poses a serious health threat to immunocompromised individuals. Infection of *Cryptococcus neoformans* is responsible for approximately 200,000 deaths every year. It has been previously established that defense against cryptococcal infection relies on Th1 activated immunity and production of interferon-gamma (IFN-γ). However, it remains unclear how this activation process occurs. This research focuses on the role of type 1 conventional dendritic cells (cDC1). We examined the immune response to cryptococcal infection in Batf3$^{-/-}$ mice, which lack the cDC1 subset. Our results demonstrate that cDC1s are largely responsible for recruitment and activation of immune cells in the lungs and brain. Absence of the cDC1 population resulted in higher fungal burdens and lower levels of IFN-γ across the brain, lungs, and spleen. Additionally, Batf3$^{-/-}$ mice expressed significantly lower levels of interleukin 12. This result supports our hypothesis that cDC1 secretes IL-12, but further experimentation is needed to determine the exact function and importance of this cytokine. We conclude that the cDC1 subset is an essential part of the activated immune response. It is indirectly responsible for the proper activation of T cells and the production of specific cytokines. This pathway could potentially be utilized to develop treatments for cryptococcal infection.

Poster Presentation #15

The Elusive Role of iNOS in Antifungal Defenses: Regulating CNS Immunity During Cryptococcal Infection

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Cryptococcus neoformans (*C. neo*) is an environmental fungal pathogen that results in severe infections in immunocompromised individuals. *C. neo* infection has a high mortality due to the limited number of therapeutic options, as many antifungals are toxic or ineffective. Inducible nitric oxide synthase (iNOS/NOS2) is a hallmark of classically activated myeloid cells with known antimicrobial functions, but its role in regulating CNS immunity during cryptococcal meningoencephalitis (CM) remains unclear. In this study, we investigated the contribution of iNOS to fungal control, neuroinflammation, and CNS pathology using a murine model of cryptococcal infection. Wild-type (WT) and Nos2-knockout (Nos2-/-) mice were infected intravenously with *C. neo* strain H99 and monitored for survival and neurological disease. Despite comparable fungal burdens in the brain and peripheral organs, Nos2-/- mice exhibited significantly accelerated mortality relative to WT controls. iNOS deficiency was associated with increased blood–brain barrier permeability and exacerbated CNS pathology. Characterization and analysis of brain leukocytes revealed increased neutrophil recruitment in Nos2-/- mice, a population known to contribute to inflammatory tissue damage. Consistent with this, neutrophil depletion in iNOS-deficient mice significantly improved survival. Together, these findings demonstrate that iNOS is dispensable for fungal clearance during CM but plays a critical immunoregulatory role in limiting excessive neuroinflammation and protecting against immune-mediated CNS damage.

Poster Presentation #16

Surfactant Proteins A and D Differentially Regulate Early Pulmonary Cryptococcus neoformans Infection Following TPS1 Disruption

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The lack of effective antifungal therapies and increasing resistance to drugs demonstrate the need for new antifungal targets. Trehalose-6-phosphate synthase (TPS1) is a key enzyme required for full virulence of *Cryptococcus neoformans*, one of the most common causes of serious fungal infections worldwide. In a pulmonary infection model, TPS1-deficient (*tps1Δ*) *C. neoformans* strains are rapidly cleared from the lung, a process accompanied by early inflammatory activation and increased recruitment of innate immune cells. We found that during *tps1Δ* infection, there was an increase in the murine expression levels of surfactant proteins A and D (SP-A and SP-D), which are major resident immune factors in the alveolar fluid. Based on this finding, we investigated whether these surfactant proteins contribute to the elimination of *tps1Δ Cryptococcus neoformans* from the lungs. Incubation of *C. neoformans* with purified SP-A or recombinant SP-D resulted in greater inhibition of *tps1Δ* compared to wild-type strains, indicating increased susceptibility of the mutant to surfactant protein activity. To assess *in vivo* relevance, infection outcomes were compared in mice lacking these surfactant proteins. In SP-D KO mice *tps1Δ C. neoformans*, were better cleared on 1dpi compared to WT mice. This effect was lost by 2dpi. In contrast, SP-A KO mice failed to control *tps1Δ C. neoformans* as well as WT fungi, with significantly higher fungal burdens on 1dpi. Therefore, we conclude that while the loss of *tps1* may render cryptococcus susceptible to both SP-A and D *in vitro*, the surfactant proteins are playing diverging roles *in vivo*.

Poster Presentation #17

Shigella c-di-GMP phosphodiesterases regulate virulence and biofilm formation

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Shigella causes bacillary dysentery, known as Shigellosis. Shigellosis is characterized by fever, abdominal cramps and diarrhoea. Shigellosis affects an estimated 80-165 million persons yearly. Currently, there are no vaccine for shigellosis. Antibiotics are effective; however, Shigella's advancing antibiotic resistances makes treatment challenging. Shigella's success is owed to its low infectious dose and ability to invade epithelial cells.

Shigella uses the second messenger c-di-GMP to regulate various bacterial phenotypes including virulence and biofilm. C-di-GMP homeostasis within a bacterial cell is maintained by two classes of enzymes: diguanylate cyclases (DGC) which synthesize c-di-GMP and specific phosphodiesterases (PDE) which hydrolyse c-di-GMP.

Shigella encodes 4 putative DGCs, and 6 putative PDEs. These enzymes contain sensory domains which interacts with environmental cues, and in turn dictates their activities. Deletion of Shigella DGC's results in decreased invasion, plaque size, biofilm, and increased resistance to acid shock. However, we do not know how c-di-GMP specific PDE's regulate these phenotypes.

The objective of my research is to determine how PDEs regulate some of Shigella's phenotypes. I created Shigella knockouts of the 6 PDEs to characterize their impact on Shigella's behaviour. I have found that Shigella's PDE knockout strains formed larger plaques, had greater invasion frequency in Henle-407 cells, increased biofilm formation and decreased resistance to acid shock. I have also noted that some PDEs contribute in controlling the global c-di-GMP pool while other PDEs are possibly operating at a local signalling level.

Shigella PDE mutants behave diametrically opposite to DGC mutants. Here we demonstrate how varying c-di-GMP levels in bacterial signalling can impact phenotypic expression. This study will provide a greater understanding of Shigella's ability to overcome environmental hurdles through regulating their c-di-GMP levels, which in-turn enables it to cause its grave disease.

Poster Presentation #18

Alyssa Jarvis, Jonathan Finkel

Bacteriophages encode diverse proteins capable of manipulating or disrupting bacterial processes, including toxic gene products that may serve as a new antibacterial agent. The mycobacteriophage Fred313, isolated at the University of Detroit Mercy, contains several genes previously identified as toxic to Mycobacterium smegmatis, yet the structural basis for this toxicity remains unclear. In this study, the Fred313 gene library was refined through verifying gene-specific primers using PCR amplification and gel electrophoresis, confirming correct base-pair lengths for the majority of genes that were tested. Three previously identified toxic genes—19, 51, and 54—were examined further using toxicity dot assay using anhydrotetracycline induction varying in concentration levels. All three genes demonstrated lethality in *M. smegmatis*, confirming their toxic phenotypes. To investigate the mechanism of toxicity, AlphaFold structural predictions were generated for genes 19, 51, and 54. Gene 19 formed a β-rich stopper fold similar to known protein SPP1 gp16. Gene 51 displayed a large, multi-domain enzyme that was consistent with nucleotide-modifying reductase, that disrupts essential metabolic pathways. Gene 54 consisted of α-helical folds relating to interaction based toxins in TA systems. Together, the structural and phenotypic evidence presented explores the functional characteristics of Fred313 and the potential therapeutic advances of phage-derived toxins as an antibacterial.

Poster Presentation #19

Pseudotime reconstruction of viral infection dynamics from single-cell high-content screening

Dina G. Tekle[1], Craig J. Dobry[2], Jonathan Z. Sexton[2], & Elliott D. SoRelle[1,3]

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Viral infection in any cell population is inherently asynchronous and results in heterogenous biological outcomes. These properties pose a challenge to studying infection dynamics that require the use of single-cell methods and, typically, time-resolved measurements. Here, we introduce a method utilizing single-cell high-content screening and morphological pseudotime trajectory calculation to resolve infection dynamics from fixed cells. We demonstrate this technique's proof-of-principle by examining nuclear chromatin remodeling and DNA damage responses (DDR) during lytic reactivation of Epstein-Barr virus (EBV), a prevalent gammaherpesvirus that contributes to several cancers and autoimmunity. Lytic infection can be triggered by diverse physiologic and pharmacologic stimuli, yet the specificity and breadth of host-virus responses they elicit are unclear. We analyzed eight B cell lymphoma models and seven lytic induction methods using high-content microscopy. Cells were assayed for immediate-early and late EBV lytic proteins, DNA replication, and double-stranded break DDR essential for lytic infection. Single-cell segmentation, measurement, and dimensional reduction yielded a phenotype atlas of ~1x10^6 cells. Lytic stimuli generated spatiotemporally distinct host-virus responses including genotoxin-induced abortive reactivation. Confirming prior studies in hybrid cell lines, reactivation in B cells produced kinetically distinct replication compartments and cellular chromatin reorganization, which we resolved along pseudotemporal phenotype trajectories. During lytic infection, DDR markers γH2AX and 53BP1 were notably depleted in replication compartments; γH2AX outside of compartments was pan-nuclear, whereas 53BP1 was virtually undetectable in lytic cells. This approach reveals previously unknown dynamics of host-directed DDR dependent on the stage of lytic infection. Thus, our approach enabled new insights into EBV replication and DDR kinetics at the single-cell level without live-cell imaging. In addition to providing a platform for high-throughput screening of viral gene functions and antiviral drug mechanisms, we expect this method will be readily adaptable to study dynamic host-virus interactions across diverse viral families.

Virus Type: Herpesviruses
Virus Research Area: Novel Methodology & Technology

Poster Presentation #20

Tracking Antibiotic Resistance Genes, Antimicrobial Resistance and Multidrug Resistance in the Huron River.

Evan Siemienkiewicz, Maitreyee Mukherjee

Antibiotic resistance arises when bacteria acquire and disseminate genetic determinants that enable survival under antimicrobial selective pressure, frequently through horizontal gene transfer of antibiotic resistance genes (ARGs) among diverse taxa. Despite increasing global concern, the environmental prevalence and distribution of antimicrobial-resistant (AMR) and multidrug-resistant (MDR) bacteria remain insufficiently characterized. The Huron River in southeastern Michigan is a critical freshwater resource subject to substantial anthropogenic influence, raising concerns regarding the environmental persistence and propagation of AMR and MDR bacteria and their associated ARGs. In this ongoing investigation, AMR and MDR phenotypes, along with corresponding ARG profiles, were evaluated over a six-month period (May–October 2025). Surface water samples were collected monthly from ten sites across the river, and *Escherichia coli* and *Enterococcus* spp. were isolated and subjected to antimicrobial susceptibility testing against eight antibiotics using the Kirby–Bauer disk diffusion assay. Quantitative polymerase chain reaction (qPCR) was employed to quantify the presence and relative abundance of targeted ARGs. *Escherichia coli* isolates exhibited resistance to tetracycline and erythromycin and frequently demonstrated multidrug resistance, most commonly involving co-resistance to tetracycline and erythromycin in combination with additional antimicrobial classes. *Enterococcus* isolates showed resistance to ciprofloxacin, with elevated occurrence during June and August, and exhibited lower rates of multidrug resistance compared to *E. coli*. Molecular analyses revealed consistently high abundance of the *int1* (class 1 integron integrase), *tetW* (tetracycline resistance), *sul1* (sulfonamide resistance), and *ermF* (macrolide resistance) genes across all sampling months. Continued sampling is planned through May 2026 to strengthen spatiotemporal resolution.

Poster Presentation #21

Characterizing an LRP homolog's role in the regulation of antibiotic sensitivity

Katheren Barger[1], Maeve McLaughlin[1]

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Bacteria's ability to survive relies on modulating gene expression in response to changes in their environment. These changes in gene expression are often mediated by transcription factors (TF) that can respond to environmental signals (e.g. antimicrobial agents; nutrient availability). To understand how bacteria survive in stress conditions, it is crucial to study how TF sense and respond to environmental signals. *Caulobacter* species are gram-negative Alphaproteobacteria that play important roles in many plant-associated and aquatic communities. This along with their genetic tractability, makes *Caulobacter* an excellent model system for studying how transcriptional regulation impacts survival in different environments. Leucine-responsive regulatory protein (LRP)-family TFs are a common family of TFs in bacteria that alter gene expression in response to binding a ligand (usually an amino acids). Data suggests that disruption of the LRP-family TF *CCNA_03281* in *C. crescentus* has a strong impact on fitness during growth with different amino acids or upon exposure to cell envelope stressors. Here, we investigate *CCNA_03281*'s role in regulating sensitivity of *C. crescentus* to the antibiotic vancomycin. Using ChIP-seq, we identified putative gene targets for CCNA_03281, including genes involved amino acid metabolism and several TonB-dependent transporters (TBDT). In particular, one putative gene target is ChvT, a TBDT that has been previously shown to impact vancomycin sensitivity in *C.* crescentus. Taken together, this suggests that CCNA_03281 may impacts antibiotic sensitivity by regulating expression of nutrient transporters. This system provides an excellent opportunity to study the interplay between transcriptional regulation, cellular metabolism, and antibiotic sensitivity.

Poster Presentation #22

Characterization of a Transcription Factor to Determine Its Role in Adhesin Development in *Caulobacter crescentus*

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The application of techniques to bacterial exploration that start at gene expression for adaptations and survival could lead to greater breakthroughs in medicine and human mechanisms. Bacteria need to coordinate gene expression with both their metabolic state and cell-cycle progression to survive and adapt to their environment. In the studied organism, *Caulobacter crescentus,* the *pilA* gene encodes the major pilin protein required for adhesion and affects susceptibility to bacteriophage at this stage. Its expression is strictly regulated during the cell cycle. The means by which metabolic signals influence this regulation is not fully understood, however. In this study, we characterized CCNA_02225 (SahR), an ArsR-Family transcription factor that responds to the methylation-cycle metabolite S-adenosylhomocysteine (SAH). Data from another lab has shown that SahR acts as a metabolite-responsive regulator of *pilA* expression rather than a strict repressor. Our preliminary ChIP-Seq data also suggest that SahR may regulate additional genes across the *C. crescentus* genome. Overall, our results support a model where SahR links the methylation cycle to cell-cycle regulation to regulate *pilA* expression, helping ensure pili are produced at the correct stage and supporting the overall cell fitness in *Caulobacter crescentus*.

Poster Presentation #23

Examination of global binding profile of CCNA_02595 in *Caulobacter crescentus*

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Transcription factors are essential regulators of cell-cycle progression and stress adaptation. In *Caulobacter crescentus*, the General Stress Response (GSR) is overseen by a top-down regulatory network in which the alternative sigma factor activates downstream effectors, including the non-coding RNA GsrN, to coordinate survival under oxidative and envelope stress. However, the full complement of transcriptional regulators operating within this network remains incompletely characterized. In this study, we investigate CCNA_02595, a currently hypothetical protein lacking a published annotation. The computer program AlphaFold does structural predictions based on the amino acid sequence. When inputting our gene of interest's sequence, it revealed a TFIIB-like fold featuring an N-terminal Cys4-Type zinc ribbon domain and C-terminal Helical regions consistent with protein-protein interactions, suggesting a role in transcriptional regulation or co-regulator binding. *CCNA_02595* is in the same operon as an HtpX-like protease, is upregulated during iron limitation, and co-elutes with the sRNA *gsrN*. To examine its binding repertoire, we tagged CCNA_02595 with a C-terminal 3x-FLAG tag, expressed it from a cumate-inducible promoter, and performed ChIP-seq. Here, we examine our preliminary global binding data and putative targets of CCNA_02595.

Poster Presentation #24

Characterization of the *Arthrobacter* phage Chesters genome

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Bacteriophages or phages (i.e. viruses that infect bacteria) are the most abundant organisms on earth with an estimated 10^{31} viral particles. Due to their ability to kill bacteria, phages have been used for medical purposes as therapeutics and for agricultural purposes as biocontrol agents. These applications have seen increased interest in recent years as the rates of antimicrobial resistance increase. Phage use structural proteins to attach to a host bacterium, ejecting their genetic material into the host, replicate within the cytoplasm, and often lysing the host in order to release progeny phage into the environment. Here, we studied the genome of *Arthrobacter* phage Chesters which was found in a soil sample collected in Bay City, MI. Arthrobacter phage Chesters is a FC cluster phage with a 195,574 bp genome with ~17,000 bp terminal repeats and a 54.1% GC content. Automated annotation predicted that Arthrobacter phage Chesters encoded 322 genes and 34 tRNA. We have annotated the first 90 genes in Chester's genome, predicting their start site and potential functions using various bioinformatic tools. About 20% of the genes annotated had a viable function. Roughly 80% of the genes that were found to be present in the genome were purely hypothetical. Understanding the functions of different phages helps show the diversity of all the phages and assists in understanding what phages can do.

Poster Presentation #25

Genomic comparison of Arthrobacter phage Pauu and Nobs

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Bacteriophages are the most abundant organisms on the planet, with ~10^{31} estimated viral particles across the earth. Due to their ability to kill bacteria, phages have been used as therapeutics for infections and as biocontrol agents for agriculture. The vast majority of phage, however, remain uncharacterized. Efforts were focused last year to identify phages with the SEA-PHAGES program to isolate bacteriophages from environmental samples. Soil samples were collected around a decomposing tree and from a flower garden. Subsequently, we isolated phage from enriched cultures and purified them over several rounds. Arthrobacter phages Pauu and Nobs (Pauu: 42.84949° N, 83.45899° W; Nobs: 42.8164995, -83.7848909) were identified using the host bacterium *Arthrobacter globiformis* NRRL B-24025. The Arthrobacter phage Pauu and Nobs genomes were sequenced and comparison indicated that they were 94% identity and belong to the FE cluster of phages. Interestingly, while Pauu displayed a Myoviridae morphology, Nobs displayed a Podoviridae morphology. While the genomes were highly similar, the plaque morphologies of Pauu and Nobs were distinct, suggesting that the genetic differences were impacting infection dynamics. To compare the differences between the two genomes, we used Glimmer and GeneMark, along with Starterator to identify the start codons of predicted genes within Pauu and Nobs. To identify potential functions of the predicted gene products, we used HHpred, CDD, Alphafold, Phamerator, and DeepTMHMM. This information will allow us to identify the genetic difference within these phages.

Poster Presentation #26

Characterization of the *Arthrobacter* phage Jeremy4pt0 genome

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Bacteriophages (phages) are viruses that infect bacteria and are the most abundant group of organisms on Earth with an estimated 10^{31} viral particles. Phages play important roles in shaping microbial evolution, population dynamics, mediate genetic exchange, and nutrient flow through the environment. Additionally, phage can be used as therapeutics and agricultural biocontrol agents due to their ability to kill bacteria. To better understand these processes, it's important to understand the genetic composition and diversity of phage genomes. Interestingly, a considerably large portion of phage genome encode for genes with no known function, leading to the presence of "viral dark matter". Many ongoing research programs are currently underway in hopes of bringing this "dark matter" into the light.

Here we report the discovery of a novel *Arthrobacter* phage, Jeremy4pt0, the 24th member of the cluster AW phage. *Arthrobacter* phage, Jeremy4pt0 was isolated from a soil sample from Flint, Michigan in 2025 against the host *Arthrobacter globiformis B-2979*. The phage is sequenced using Illumina by the Pittsburgh Bacteriophage Institute, revealing a double-stranded DNA (dsDNA) genome of 54,197 base pairs with a 51.8% GC content. 86 genes have been identified in this genome, whose functions are attributed in this study. Jeremy4pt0 is highly similar to the other members of the AW cluster, which on average have genomes of 54,511 bp, 51.7% GC content, and 86.9 genes, and are generally lytic. Jeremy4pt0's genome consists of X% genes with official functions, while the remaining have been called hypothetical proteins or membrane proteins. Based on structural predictions and sequence similarity, we identified that gene product 6 (gp6), which is annotated as a "hypothetical protein" for other AW cluster phage, likely encodes the head-to-tail adaptor.

Poster Presentation #27

Characterizing *CCNA_03379*'s role as a transcriptional regulator in *Caulobacter crescentus*

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Bacteria often encounter environment stressors that can impact their survival abilities, including physical and chemical stressor. In order to survive, bacteria need ways to successfully sense and respond to these new. Transcriptional regulators are essential in this process, as they can control the expression of different genes that are involved in survival pathways. Using *Caulobacter crescentus* as our model organism, we were able to look specifically at how one predicted XRE-family transcription factor is binding in this gram-negative Alphaproteobacterium. This family of Xenobiotic Response Element transcription factors have been previously reported to repress gene expression in response to different stress conditions. Previously, there have been findings on these factors in *C. crescentus*. However, with the variations within this XRE family, many predicted transcription factors remain unknown. We were specifically interested in characterizing the genome-wide binding patterns of the previously uncharacterized XRE-family transcription factor, CCNA_03379. Through ChIP-seq, we were able to investigate genomic binding activity and structure identification. ChIP-seq binding peaks showed multiple binding sites across the *C. crescentus* genome. Yet, had minimal binding overlap when compared to various other XRE regulators in our model organism. This is indicative of different genomic impact and regulatory functions in comparison to other XRE genes. Our data suggested that gene CCNA_03379 controls stress response mechanisms, oxidoreductase activity, and antibiotic resistance. The observations made in this research allowed us to further understand XRE network connections within *C. crescentus*.

Poster Presentation #28

Annotation and function of the newly discovered *Arthrobacter* phage Pauu

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Bacteriophages are the most abundant biological entities on Earth with ~10^{31} predicted phage particles, outnumbering their bacterial hosts by approximately 10:1. Phage inhabit a wide range of environments. Their capacity to kill bacteria led to phage being used as therapeutics for bacterial infections prior to the widespread usage of antibiotics. The rise of antibiotic resistance in recent years has renewed interest in phage-based therapies as alternative and complementary treatments. Because phages are both highly abundant and often species-specific, many remain undiscovered, and newly identified phage genomes are frequently unannotated. In this study, the genome of a newly discovered bacteriophage, *Arthrobacter* phage Pauu, was annotated using an array of bioinformatic tools to predict both gene location and potential gene function. *Arthrobacter* phage Pauu is a cluster FE phage with a 15612 bp genome that contains 15 bp 3' sticky overhangs and has 67.3% GC content. Gene prediction programs such as Glimmer and GeneMark were used to detect likely coding regions and confirm gene boundaries within the genome. Comparative analysis with Phamerator, HHPred, CDD, and the official protein database helped group genes based on sequence similarity to known phage genes, providing insight into possible functions. Additionally, AlphaFold was used to predict protein structures, supporting functional predictions and improving confidence in the overall genome annotation. Through these analyses, we identified 24 predicted genes and 0 tRNA. Our analysis suggested that the genome encodes 8 hypothetical proteins, 3 HTH-containing proteins, 3 transmembrane domain-containing proteins, and 10 structural/lysis-related proteins.

Poster Presentation #29

Analysis of global binding of the XRE-family transcription factor *CCNA_03612*

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To survive, bacteria need to sense and respond to fluctuating environments. Transcription factors play a central role in this process by detecting chemical, metabolic, or stress-related signals and altering gene expression in ways that drive changes in cell physiology. The model Alphaproteobacteria *Caulobacter crescentus* is an excellent system to study how transcriptional regulators impact environmental acclimation. Here, we examined the previously uncharacterized XRE-family TF CCNA_03612 and the LRP-family TF CCNA_03281. Publicly available fitness data suggest that disruption of CCNA_03612 or CCNA_03281 increases cellular fitness in numerous stress conditions, indicating that these TFs may normally contribute to regulatory pathways that influence cellular survival under stress. Given their similar fitness profiles, we asked whether CCNA_03612 and CCNA_03281 control related transcriptional targets. Using ChIP-seq, we defined the global binding profiles of both regulators. We detected 821 binding sites for CCNA_03612 and 91 binding sites for CCNA_03281. Despite their parallel stress phenotypes, the two TFs shared only one overlapping binding site, the promoter region of CCNA_03612. This finding suggests that CCNA_03612 is a direct target of CCNA_03281 and that CCNA_03612 likely autoregulates its own expression. To place CCNA_03612 within a broader regulatory context, we compared its binding profile to that of CdxA, a previously characterized XRE-family TF. CCNA_03612 and CdxA showed co-localized binding at genes required for pH homeostasis (carbonic anhydrase) and motility (CheYIII), indicating shared regulatory logic. Overall, this work expands our understanding of transcription factor targets in *C. crescentus* and identifies CCNA_03612 as a key regulator in the bacterium's response to environmental stress.

Poster Presentation #30

Developing a Random Barcoded Bacteriophage Transposon Library

Ashlynn Linet, Chris Waters

This project aims to develop a strategy for generating bacteriophage transposon mutant libraries, similar to the genetic screening technique Tn-Seq used in bacteria, for the identification and characterization of essential bacteriophage genes. Three major hurdles exist to the development of such a tool: 1) identification of a suitable bacterial host, 2) identification of bacteriophage-specific selectable markers, and 3) isolation and propagation of transposon-containing bacteriophage.

Thymidylate synthase (ThyA) is an essential bacterial enzyme in nucleotide biosynthesis that catalyses the conversion of dUMP to dTMP. Despite requiring deoxynucleotides for genome replication, many bacteriophages lack their own *thyA* and are likely dependent on their host to synthesize dTMP. Therefore, we constructed an *Escherichia coli* thymidine auxotroph (Δ*thyA*) and an arabinose-inducible transposase and transposon encoding *thyA* as a selectable marker (TN-*thyA*) on a plasmid (pTN-thyA). We hypothesized that only bacteriophages harboring a TN-*thyA* will be capable of infecting and replicating in the Δ*thyA* host.

Bacteriophages were propagated on *E. coli* DH10B hosts containing pTN-*thyA* while inducing transposase expression to facilitate transposition into the bacteriophage genome. The resulting bacteriophage progeny were selected on Δ*thyA* and successful transposition events were confirmed by arbitrary PCR and sequencing. Using this approach, we have constructed transposon mutant libraries for multiple, phylogenetically diverse bacteriophages. Notably, these libraries are barcoded to improve downstream sequencing and identification. These results support the development of a broadly applicable method for constructing bacteriophage transposon mutant libraries.

Poster Presentation #31

BR-Bodies Promote Envelope Stress Survival and Intracellular Fitness in *Brucella*

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Brucella spp. are intracellular bacterial pathogens and the etiological agents of the zoonotic disease brucellosis. Their ability to survive and replicate within host cells, an environment that contains numerous antimicrobial stressors, requires acute control of gene expression. A key post-transcriptional regulator is RNase E (Rne), a highly conserved ribonuclease that contains a C-terminal intrinsically disordered region (IDR). Rne with an intact IDR can undergo phase separation to form biomolecular condensates known as BR-bodies. Here, we investigate how BR-bodies contribute to *Brucella ovis* stress survival and to infection of mammalian macrophages. To test whether Rne forms BR-bodies in *B. ovis*, we imaged strains expressing fluorescently labeled full-length Rne (*rne*-msfGFP) or an IDR deletion variant (*rne*ΔIDR-msfGFP) by phase-contrast and fluorescence microscopy. To assess whether BR-bodies contribute to *Brucella* fitness during stress treatment, we compared wild type (WT) to a BR-body null (*rne*ΔIDR), a strain in which full length Rne was restored at the native locus (*rne*$^{+}$), and chimeric Rne expressing the IDR domain of *Caulobacter crescentus* (*rne*^{CcIDR}). To model physiologically relevant stress *in vitro*, the strains were exposed to H2O2 or spotted on TSAB +/- polymyxin B and carbenicillin. Finally, we assessed intracellular fitness via a gentamycin protection assay using THP-1 cells and enumerating CFUs at 2-, 24- and 48-hours post infection. Full length Rne formed dynamic intracellular foci, whereas a *rne*ΔIDR mutant did not, indicating that the IDR is required for BR-body formation. The *rne*ΔIDR was more sensitive to all *in vitro* stress conditions and had severely diminished intracellular fitness compared to wild type, and restoration of full length *rne* rescued the fitness defects. The chimeric *rne*CcIDR strain had variable sensitivity to stress, fully rescuing growth on H2O2 but not on polymyxin B or carbenicillin, and partially rescued the infection defect. Together, these results demonstrate that BR-bodies contribute to *Brucella* survival under multiple stress conditions and promote fitness in the intracellular niche.

Poster Presentation #32

Importance of IL-10 in Protection of the Central Nervous System from Inflammatory Damage during *Cryptococcus* Meningoencephalitis

Rylan Hissong, Jasmine Chang, Hailong Li, Kristie Goughenour, Maia Litener, Shelby Hopson, Lauren Borhani, Jintao Xu, and Michal Olszewski

Cryptococcus neoformans meningoencephalitis (CM) is a lethal fungal disease with a strong inflammatory component. While the inflammation is driven by the effector T-cells producing INF-g and TNF-a inflammatory cytokines, there are also anti-inflammatory factors produced by regulatory T cells, which serve as the "brakes" of the immune system. One of such anti-inflammatory factors that Tregs can make is IL-10 cytokine, however its role in cryptococcal meningitis is unknown. We hypothesize that IL-10 restricts inflammation during CM and a murine model of CM is used to test this hypothesis. IL-10-/- and their wild type (WT) counterparts C57BL6 mice, were infected intravenously to induce CM. The outcomes were compared between the groups including weight loss and other symptoms, brain fungal burden, immune cell responses (flow cytometry) in the dispersed brains, and immunofluorescent microscopy of brain tissue.

As expected, Tregs in IL10-/- were unable to make IL-10 in contrast with IL-10-abundant Tregs in the WT mice. Comparison of IL-10-/- to WT mice revealed exacerbated weight loss and 100% mortality in the knockout mice, along with a significant increase in immune cell recruitment to the brain, and a significant increase of inflammatory signaling proteins (IFN-γ and TNF-α) of the effector T cells. We observed enhanced brain pathology, including increased inflammatory infiltrates and neuronal damage and death. Results show that IL-10 plays a vital role in regulating the immune response during CM, protecting the brain from inflammatory damage. This outcome phenocopied our previous findings with mice depleted of Tregs, suggesting that IL-10 is the major anti-inflammatory mechanism employed by Tregs.

Moving forward, we aim to confirm that regulatory T-cells anti-inflammatory function relies on IL-10 production. We plan to transfer IL-10 competent T-cells to the IL-10-/- mice with CM, with expected improvement of their disease outcomes if our hypothesis is correct.

Poster Presentation #33

Investigating Pathogenic Mechanisms of Enterotoxigenic *Bacteroides Fragilis* in Colorectal Cancer

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As the second leading cause of cancer-related deaths with growing incidence among the younger populations in the USA, colorectal cancer (CRC) is a mounting public health concern. Increasing evidence suggests that the gut microbiome plays a significant role in the development of CRC. *Bacteroides fragilis* is a part of the normal human gut microbiota and is usually considered to be a commensal organism. However, in some cases of CRC, enterotoxigenic *B. fragilis* (ETBF) have been shown to be significantly increased. These ETBF strains release *B. fragilis* toxin (BFT), a metalloprotease that breaks down gut epithelial lining proteins. Previous studies have used genetic engineering techniques to knock out the *bft* gene responsible for the toxin production in *B. fragilis*. The aim of this study is to compare the metabolomic profiles of ETBF, the knockout mutant, and wildtype non-enterotoxigenic *B. fragilis* (NTBF) strains to investigate other possible pathogenic mechanisms used by ETBF to progress CRC development. Untargeted metabolomics was used to analyze the metabolomic profiles of these strains and bioassays against methicillin-susceptible *Staphylococcus aureus* (MSSA) was used to study the pathogenicity of each strain. Preliminary results from the metabolomic study revealed significant differences between each strain, suggesting that knocking out the *bft* gene did not completely revert the ETBF metabolomic profile to that of NTBF. Furthermore, the knockout mutant showed the greatest inhibition against MSSA growth. These findings indicate that besides the BFT toxin, the ETBF strain possesses other mechanisms of pathogenesis that could aid it in colonizing the gut microbiome. This highlights the importance of adaptations in *B. fragilis'* metabolomic profile that may impact the development of CRC.

Poster Presentation #34

Temperature-Dependent Motility and Intestinal Colonization of *E. marmotae* using the Zebrafish Model

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Escherichia marmotae is a recently recognized member of the *Escherichia* genus that was historically misidentified as *Escherichia coli* due to overlapping biochemical profiles and limitations of standard diagnostic assays. Advances in mass spectrometry-based biomarker detection and TaqMan PCR now enable reliable differentiation of *E. marmotae* from other *Escherichia* species. *E. marmotae* has been implicated in human disease, including sepsis and urinary tract infections, yet its true prevalence and pathogenic potential in clinical infections remain unclear.

While *E. marmotae* can adhere to and invade epithelial cells, the molecular mechanisms by which it contributes to infection are not well defined. Preliminary RNA-seq and proteomic analyses revealed temperature-dependent regulation of potential virulence traits, with upregulation of motility- and adhesion-associated genes at 28 °C and increased expression of the outer membrane protease gene *ompT* at 37 °C. We hypothesized that *E. marmotae* employs specific colonization mechanisms that promote adherence to eukaryotic cells.

To test this hypothesis, we used an adult zebrafish infection model to examine intestinal adherence and invasion, and to evaluate how deletion of genes involved in temperature-dependent motility affects colonization. We demonstrate that *E. marmotae* adheres to and invades the Zebrafish gut at 24 and 48 hours post-infection. Furthermore, mutant strains targeting motility-associated genes (*fliA*, *motA*, *fliC*) and *ompT* exhibited significantly reduced gut colonization compared to the wild-type.

This model provides valuable insights into the pathogenicity of *E. marmotae*, highlighting the role of temperature-regulated genes in modulating its ability to colonize and evade host defenses.

Poster Presentation #35

GGDEF Domain Missense Mutations Influence Cyclic-di-GMP Signaling in *Vibrio Cholerae*

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Vibrio cholerae is a gram-negative bacterium that lives in aquatic environments and causes diarrheal disease. Cyclic di-GMP – an important secondary messenger located in *V. cholerae* – controls the switch between the microorganism's motile and stationary states, with high intracellular c-di-GMP levels driving biofilm formation. Diguanylate cyclase (DGC) enzymes encode GGDEF domains and synthesize c-di-GMP while phosphodiesterase (PDE) enzymes encode EAL domains and degrade c-di-GMP. Isolation and sequencing of a hyper-biofilm forming mutant of *V. cholerae* strain *N16961* revealed it had an amino acid substitution of asparagine (N) instead of aspartic acid (D) in the GGDEF domain of *vc0653* – a GGDEF-EAL dual domain containing protein. We hypothesized that this mutation was responsible for the mutant's elevated c-di-GMP levels and higher biofilm production.

To test this hypothesis, we replicated this point mutation in two other GGDEF domain containing proteins – *vc1370* and *vc1353* – but these GGNEF mutant proteins formed lower levels of c-di-GMP, which did not support our hypothesis. However, c-di-GMP levels increased when this mutation was made in *vc2750*, which is a GGDEF-EAL dual domain containing protein. Therefore, we now hypothesize that the GGNEF mutation increases inhibition of the EAL domain, leading to greater c-di-GMP levels.

My future analysis of *vc0653* will test this hypothesis as I will homologously recombine three constructed mutant *vc0653* proteins - *vc0653* GGDEF ΔEAL, *vc0653* GGNEF EAL, and *vc0653* GGNEF ΔEAL – into the genome of *V. cholerae* strains *N16961* and *C6706* and perform biofilm assays on the mutated and wildtype strains to elucidate c-di-GMP levels.

Also, bioinformatics analysis of all DGCs across bacterial phyla showed that 16% were GGNEF variants and 23% were GGQEF variants, suggesting that the GGDEF domain is amenable to different amino acids, which may impact DGC enzymatic function. To pursue this idea, we will analyze the activity of GGAEF, GGQEF, and GGVEF mutations in different *V. cholerae* proteins including *vc0653* to compare c-di-GMP levels and biofilm formation.

Our research will provide insight into the flexibility of the DGC GGDEF domain and how changes in the amino acid structure of the GGDEF domain modulate c-di-GMP levels and control important physiological processes in bacteria such as motility and biofilm formation.

Poster Presentation #36

Analysis of *Enterococcus* Contamination Levels in the Huron River, a Major Recreational Water Source in Washtenaw County, Michigan

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Background: The Huron River represents an important freshwater resource in Southeast Michigan, providing ecological, recreational, and societal benefits to surrounding communities including Ann Arbor and Ypsilanti. *Enterococcus* spp. is commonly occurring; typically nonpathogenic bacteria associated with human and animal fecal material and are widely utilized as microbial indicators of fecal contamination in aquatic environments. The detection and quantification of *Enterococcus* in surface waters therefore serves as a proxy for potential exposure to fecal-derived pathogens that may pose risks to human health. Given the extensive recreational use of the river, maintaining microbial water quality is of considerable public health importance. The objective of this study was to investigate the spatial and temporal variability of *Enterococcus* concentrations within the river system to better characterize patterns of fecal contamination and provide data that may inform future water quality monitoring and management strategies.

Methods: Water samples were collected across 10 sites along the Huron River each month between May-November 2025. Samples were immediately analyzed following EPA method 1600 for *Enterococcus* to enumerate colony forming units (CFU) per 100ml of water.

Results: Elevated concentrations of *Enterococcus* were detected throughout the Huron River, with numerous samples exceeding the recreational water quality criteria established by the United States Environmental Protection Agency, indicating substantial fecal contamination. Spatial analysis revealed that sampling sites within Ypsilanti exhibited comparatively higher bacterial concentrations than other locations along the river. Temporal patterns indicated that contamination levels were generally higher during the summer months, although an anomalous increase was also observed in October.

Future Directions: We plan to continue with collection and data analysis through winter and spring through 2026. We also intend to analyze these samples for microbial source tracking (MST) markers to identify and characterize the potential sources of fecal contamination within the Huron River watershed.

Poster Presentation #37

Assessment of Culturable Microbial Loads via R2A Heterotrophic Plate Counts in the Huron River

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The Huron River is a major waterway flowing through Ann Arbor and Ypsilanti, Michigan, and upports extensive recreational use. The river experiences substantial temperature variation due to depth changes and seasonal shifts, and it has faced ongoing water quality concerns. Heterotrophic plate counts (HPC) are an important indicator of the overall microbial load in recreational waters and provide insight into changes in microbial community abundance associated with environmental conditions or contamination events. Monitoring HPC helps assess general water quality and can signal conditions that may favor the presence or growth of opportunistic or pathogenic microorganisms. This project aimed to monitor spatial and temporal patterns in microbial abundance to better understand overall water quality conditions. Water samples were collected monthly from May to November 2025 across 10 sites along the Huron River. Heterotrophic plate counts (HPC) on R2A agar were performed within four hours of sampling. Serial dilutions were plated via spread plating, and colony-forming units (CFU) per 100 mL of water were enumerated. All sampling sites contained substantial levels of viable, culturable heterotrophic bacteria. Microbial counts were consistently higher during the summer months peaking in July and declining with decreasing temperatures and reduced recreational activity in the fall and early winter. Sampling and HPC analysis will continue throughout 2026. Collected data will be used to investigate potential contamination sources using microbial source tracking approaches.

Poster Presentation #38

The role of several *Candida albicans* genes in multidrug-resistant *Candida auris*

Makenna May, Ian Cleary

Candida auris is an emerging fungal pathogen first isolated from the ear canal of a patient in Japan in 2009. Since then, *C. auris* has spread to all 6 inhabited continents. It is unique in its ability to survive on surfaces for an extended period of time and withstand certain disinfectants, allowing for enhanced transmission in healthcare settings. *C. auris*, as a pathogenic yeast, has the ability to form biofilms, a meshwork of interconnected cells that can accumulate on surfaces such as intravenous catheters leading to systemic infection. Due to the pathogen's relative novelty, little is known about the genetic basis for its biofilm formation, particularly because it does not behave similarly to other *Candida* species. In our study, we looked at several genes that have been shown to affect biofilm formation in the better-understood *Candida albicans* (*19.2446*, *HAL22*, *PHO13*, *19.4444*, and *19.1405*), and one that is a *C. auris* ortholog to *19.4444* (*BNJ08_004228*) and assessed their effects on biofilm formation and the morphology of *C. auris,* as well as their impact on antifungal susceptibility. Our results suggest that the overexpression of *HAL22* leads to a decrease in biofilm formation and a difference in cellular morphology. Additionally, the overexpression of *19.4444* led to an increase in biofilm formation. Upon testing antifungal resistance, we found that the strains exhibited a wide range of minimum inhibitory concentrations, suggesting that the overexpression of such genes has an impact on the organism's ability to withstand amphotericin B, caspofungin, and fluconazole.

Poster Presentation #39

Characterizing an Uncultured Pathogen: *Sneathia* in the Female Reproductive Tract

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Sneathia is a fastidious, anaerobic bacterium of the vaginal microbiome that is strongly associated with bacterial vaginosis, increased risk of sexually transmitted diseases, and spontaneous preterm birth, the leading cause of neonatal morbidity and mortality worldwide. Despite its clear impact on women's reproductive health, *Sneathia* is reputably uncultured, with only one *Sneathia* representative isolated in pure culture from vaginal fluid. We hypothesized that our novel isolation strategies, including refined transport medium, specific uptake of media dyes and fluorescence, and confirmation via *Sneathia* specific primers would result in successful isolation of multiple *Sneathia* representatives from vaginal fluid. We found that this methodology has allowed us to overcome prior barriers of cultivation, resulting in 21 *Sneathia vaginalis* isolates and 3 *Sneathia sanguinegens* isolates. While this isolation methodology has proven highly successful, *S. sanguinegens* is disproportionately isolated far less than *S. vaginalis* – a phenomenon we sought to resolve. We hypothesized that a component of whole blood is essential for *S. sanguinegens* growth and it is not provided sufficiently in our protocol. Our assays revealed that hemoglobin results in *S. sanguinegens* growth that exceeds growth in a whole blood medium, suggesting that hemoglobin promotes *S. sanguinegens* growth and that other properties present in whole blood inhibit growth. This both highlights the physiological differences between the two *Sneathia* species and aids in refining the isolation methodology to increase *S. sanguinegens* isolation for its use in future *in vitro* and *in vivo* experimentation. Now that we have obtained these novel *Sneathia* isolates, future directions will include the use of comparative genomics to identify new virulence factors and further establish *Sneathia*'s pathogenic role in women's reproductive healthy.

Poster Presentation #40

Metabolic Constraints on Pathogen Virulence: Characterizing Isoleucine Catabolism in *Shigella*

Benecia Angelica, Benjamin Koestler

Shigella is a highly infectious human pathogen that causes severe diarrheal disease worldwide. To survive and adapt to different microenvironments within the human body, *Shigella* has adapted its metabolism to cope with specific stresses. For example, *Shigella* uses amino acids produced by the human body to survive acid stress. *Shigella* induces a branched-chain amino acid (BCAA) starvation in the human body, indicating that isoleucine (ILE), valine, and leucine are consumed by *Shigella* when the bacteria are growing within a host cell. In this study, we explored Shigella the ILE catabolism pathway, by knocking out 4 genes in the *S. flexneri* ILE catabolic pathway (*ilvE, fadA, fadD,* and *acnB*) and examining their metabolism and virulence. We found that the disruption of some of these genes impairs *Shigella'*s metabolism and reduces its ability to cause infection. The findings highlight important connections between genes involved in amino acid catabolism and *Shigella* virulence.

Poster Presentation #41

Quorum Sensing Regulates *Vibrio cholerae* Growth in a Selective Environment Mimicking Infection

Drew Johnson, Christopher Waters

During its life cycle, the bacterial pathogen *Vibrio cholerae* must adapt and survive to many different environments including the small intestine. In order to examine how *V. cholerae* survives in selective environments, I serially passaged *V. cholerae* on the Vibrio-selective medium Thiosulfate Citrate Bile-Salts Sucrose (TCBS) agar. This bile salt containing medium induces stress in *V. cholerae* and partially mimics *in vivo* growth conditions. After 10 serial passages, 6 *V. cholerae* isolates were examined by whole genome sequencing. All isolates had evolved mutations in the transcription factor *luxO* (*luxO-L104R*), a central regulator in *V. cholerae* quorum sensing (QS). QS allows bacteria to regulate gene expression in response to cell density, controlling behaviors like biofilm formation and virulence in the human pathogen *Vibrio cholerae*. To study the physiological impact of the *luxO-L104R* mutation, I regenerated this mutation in a WT *V. cholerae* strain. Using a bioluminescence reporter, I found that the *luxO-L104R* mutation locked the cells into a low-cell density QS state. Moreover, this mutant exhibited improved fitness on TCBS relative to the WT strain, demonstrating a link between QS and *V. cholerae* fitness in this selective condition. To understand how QS impacts TCBS fitness, I performed a transposon mutagenesis screen in a *luxO-L104R* background, selecting for mutants that return to a WT-like phenotype and mapping these transposon insertions on the *V. cholerae* genome. My initial results suggest that catabolite repression may be involved in the QS-driven fitness. My results demonstrate QS impacts growth on harsh environments, suggesting how detection and isolation using selective media may be influenced by bacterial communication systems.

Poster Presentation #42

Monitoring *E. coli* Levels to Assess Recreational Water Safety in the Huron River

Depowski,Mary; Lehn,Molly; Cherian,Luke; Siemienkiewicz,Evan; Gao, Zi Jie; Mukherjee,Maitreyee

The Huron River serves as a recreational water source in Southeastern Michigan, spanning 900 square miles. Maintaining its safety is a public health priority, as the river supports diverse recreational activities including swimming, kayaking, and fishing. This study focuses on assessing water quality by monitoring the concentration of *E. coli*, which serves as the primary fecal indicator bacteria (FIB) for determining the presence of potential pathogens in water sources. According to the US Environmental Protection Agency, the recreational water quality standard is set to 126 CFU (colony forming units) of *E. coli* allowed per 100mL of water. Exceeding this threshold may indicate contamination levels that may pose significant health risks to the public.

To evaluate the extent of this contamination, we investigated the prevalence and distribution of *E. coli* across ten sites along the river. Water samples were collected monthly, May to November 2025. Each sample was processed within a four-hour window following collection and analyzed using EPA methods. Following incubation, colonies were enumerated to determine the CFU per 100mL of water. The results revealed substantial *E. coli* contamination across all ten sites.

Data shows a significant temporal trend, with contamination peaking in the summer months and decreasing during colder months. These findings suggest that recreational users may be at an increased risk during peak summer activity. The research provides a critical baseline for understanding the FIB makeup of the Huron River.

Poster Presentation #43

Integrated Metagenomics and Metabolomics to Uncover Novel Colorectal Cancer Drivers and Therapeutic Targets

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Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide, with rising incidence in younger populations and poor prognosis for metastatic cases. Research over the past two decades has implicated the gut microbiome in CRC initiation and progression, although clinically effective, microbiome-targeted therapies remain elusive. In our study, we performed a deep integrated analysis using paired metagenomic and metabolomic data from a clinical cohort (n=90) spanning three stages: healthy controls (n=30), pre-cancerous adenoma (n=30), and confirmed CRC (n=30) patients. The objective of this study was to functionally define the microbial and metabolic drivers of CRC and identify novel biomarkers and therapeutic agents. Preliminary results revealed significant, stage-specific differences in both microbial community structure and metabolomic profiles. Fecal 16S rRNA community profiles showed CRC-associated enrichment of species such as *Fusobacterium nucleatum* and a greater abundance of putatively beneficial taxa (*Bacteroides*, *Clostridium*) in healthy individuals. Additionally, untargeted metabolomics also revealed distinct, stage-specific chemical signatures, particularly for early-stage adenoma, offering a significant opportunity for diagnostic biomarker development. Using a multi-omics integration approach, we identified five bacterial strains that tightly correlate with specific metabolites and disease stages. Antiproliferation testing of the crude extracts from one of the bacterial hits showed strong inhibition of CRC cell lines, with bioactivity significantly induced when co-cultured with its biofilm's co-inhabitant, suggesting inducible biosynthetic machinery in tumor biofilms. Together, this integrated, data-driven approach will guide targeted microbial culturing and bioactivity screening efforts to isolate and validate novel small molecules that may buffer against opportunistic pathogens, offering insights into microbiome-host interactions and paving the way for new CRC prevention, diagnostic, and therapeutic strategies.

Poster Presentation #44

Pragati Singh, Benjamin Koestler

Shigella is an intracellular pathogen that evolved from Escherichia coli and causes shigellosis, a severe diarrheal disease that leads to about 600,000 deaths each year, mostly in children under five. Although antibiotics are available, growing antimicrobial resistance and the absence of an effective vaccine have led the World Health Organisation to list *Shigella* as a priority pathogen. As Shigella evolved from E. coli, it lost many genes and developed pseudogenes, including mutations in important parts of the autoinducer-2 (AI-2) quorum-sensing pathway. AI-2 is made by LuxS from S-adenosylmethionine through the intermediate 4,5-dihydroxy- 2,3-pentanedione and helps bacteria communicate and coordinate their behavior. In *Shigella*, several genes needed for AI-2 uptake and processing (*lsrK, lsrFG,* and *lsrB*) are labeled as pseudogenes, which raises questions about how well this signaling system works. Early data show that even with these mutations, *Shigella* can still respond to AI-2. Interestingly, a ΔlsrK mutant forms more plaques in Henle-407 epithelial cell monolayers than the wild type, suggesting LsrK may have an unexpected role in controlling virulence.

This study aims to characterize the functionality of AI-2 pathway pseudogenes and determine their impact on intracellular survival and host-pathogen interactions. AI-2 production and uptake will be quantified in wild-type and mutant strains (ΔluxS, ΔlsrK, ΔlsrR, and ΔlsrK/ΔlsrR) using LC–MS, and transcriptomic differences between wild-type and mutant strains will be assessed via RNA sequencing. Additionally, to define how the host responds to AI-2 signaling during infection, cytokine production from Henle-407 epithelial cells infected with wild-type and mutant strains will be quantified. For exogenous AI-2 exposure, cytokine levels will be measured using ELISA to determine how externally supplied AI-2 influences epithelial inflammatory responses. To assess host responses to intracellular AI-2 accumulation during infection, multiplex cytokine profiling will be performed using a Bio-Plex assay, allowing simultaneous quantification of multiple inflammatory mediators. These experiments will determine whether AI-2—both extracellular and intracellular—modulates host inflammatory signaling and whether *Shigella* exploits AI-2 to promote immune evasion and intracellular survival.

By defining the functional relevance of AI-2 signaling in *Shigella*, this work will clarify how pseudogenes may retain residual or compensatory activity and uncover novel mechanisms of intracellular adaptation and immune evasion. Targeting AI-2–mediated communication may provide new strategies to attenuate virulence and enhance antibiotic efficacy against this globally significant pathogen.

Poster Presentation #45

Metabolomic investigation of the human microbiome as a source of inhibitory small molecules against *Klebsiella* pathogenesis

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Klebsiella spp. are opportunistic pathogens well known for their rapid and robust development of antimicrobial resistance. Consequently, the production of strains resistant to carbapenems, a class of last-resort antibiotics, and multiple drug resistant (MDR) strains generate a need for novel broadly effective therapeutics with new mechanisms of action. Because successful *Klebsiella* infection typically occurs during dysbiosis, the myriad microbial interactions and chemical communications governing a healthy human gut microbiome are likely significant mediators of *Klebsiella* levels and pathogenicity in the gastrointestinal tract. As such, the gut microbiome serves as a promising avenue of exploration in *Klebsiella* treatment research. To investigate this therapeutic potential, anaerobic competition and induction experiments were conducted and subsequently analyzed for bacterial viability in conjunction with mass spectrometry-based metabolomics. A clinically isolated strain of *Klebsiella oxytoca* was shown to substantially reduce growth of carbapenem-resistant *Klebsiella pneumoniae* in these biological assays. Moreover, these bioactive samples display distinct metabolomic profiles, indicating potential antimicrobial metabolites of interest. Future assays will determine which metabolites possess inhibitory activity, while further competition assays conducted with human fecal samples will investigate the diverse dynamics and metabolomic output of human gut microbiome interactions. Ultimately, this work will help to further elucidate the mechanisms that mediate a true healthy microbiome and provide a potential solution for antimicrobial resistance in *Klebsiella*.

Poster Presentation #46

Maria Alasbahi, KayLee Robinson, Brooklyn J. Dean, Madison Retz, Madison Hasse, and Emily D. Lavering

Antibiotic resistance is an increasing problem worldwide, indicating a need for new antimicrobial agents. Previous studies have shown that cannabidiol (CBD) exhibits antimicrobial activity. Here, analogs of the cannabinoid family were evaluated for potential antimicrobial activity against a range of gram-positive bacteria, including *S. aureus, S. pyogenes, S. agalactiae, S. epidermidis, E. faecalis, and B. subtilis*. These bacteria were selected for their clinical relevance, ranging from cavities to life-threatening conditions. We determined the minimum inhibitory concentrations (MICs) of our synthetic analogs. Numerous analogs exhibited strong antimicrobial activity, with some having MICs comparable to or lower than that of CBD. We also investigated the mechanism of inhibition of these compounds and preliminary results of our membrane permeability and membrane polarization assays indicate that these analogs disrupt the integrity of the bacterial cell membrane. Significant antimicrobial activity observed in several analogs indicates that building a library of molecules with subtle structural variations enables identification of structure-activity relationship and enhancement of antimicrobial activity. Future work will focus on better understanding the mechanism of action of these analogs.

Poster Presentation #47

Inhibiting methyltransferase-independent EZH2 activity induces cytotoxicity and inflammatory chemokine expression in EBV-positive B cell lymphoma

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Epstein-Barr virus (EBV) is a prevalent herpesvirus associated with ~2% of annually diagnosed cancers. Roughly half of EBV-associated cancers are lymphoid malignancies including endemic Burkitt Lymphoma (eBL), post-transplant lymphoproliferative disorders (PTLD), and Diffuse Large B Cell Lymphoma (DLBCL) in immunosuppressed individuals. Although EBV presence is routinely assayed for B cell lymphoma histopathology, current clinical guidelines are unaffected by tumor EBV status. EBV-tailored therapies are needed since multiple virus-positive lymphoma subtypes are particularly aggressive and carry worse clinical outcomes versus virus-negative counterparts. Host dependency factors in EBV-positive tumors constitute potentially favorable targets for such therapies. Prior studies indicate that one such dependency is the cellular histone methyltransferase EZH2, which interacts with EBV latency proteins EBNA3A and EBNA3C to epigenetically sustain proliferation via targeted H3K27me3 silencing of genes that direct terminal B cell differentiation into plasma cells. We investigated the effects of EZH2 inhibition across EBV-positive BL (Mutu, Akata+), DLBLC (IBL1), LCLs, and an EBV-negative control BL (Akata-) using cytotoxicity assays, RNA-seq, ELISA, flow cytometry, and high-content immunofluorescence microscopy. Surprisingly, S-adenosyl-methionine competitive inhibitors (EPZ-6348, GSK343) of EZH2 histone methyltransferase activity show low cytotoxicity in LCLs, DLBCL, and BL models (IC50> 25 μM in each line). By contrast, EZH2 pharmacologic degradation with a proteolysis-targeting chimera (PROTAC; MS177) yielded ~100-fold higher cytotoxicity across cell lines. RNA-seq analysis revealed potent inflammatory chemokine expression (C-C motif family) in MS177-treated cells, particularly LCLs and IBL1, and these transcriptional responses were validated at the protein level by ELISA. Comparison of isogenic EBV-positive and EBV-negative Akata further revealed virus-dependent type I and type II interferon responses. Western blot analysis confirmed differential expression of the EBV latency protein LMP-1 across lymphoma models, and flow cytometry revealed treatment-associated changes in B cell surface markers, consistent with altered phenotypes following EZH2 degradation. Interestingly, RNA-seq analysis of EBV transcripts and quantitative high-throughput microscopy of EBV Zta and viral DNA synthesis indicated MS177 minimally induces the viral lytic cycle. These findings suggest an important role for non-canonical EZH2 functions in maintaining latent EBV-mediated lymphoproliferation and support therapeutic potential of EZH2 degradation via direct cytotoxicity and inflammatory responses with minimal viral reactivation.

Virus Type: Herpesviruses
Virus Research Area: Chronic, Latent, & Persistent Infections

Poster Presentation #48

Evaluating a Mechanism Responsible for Break-Induced Replication at Hotspots on Yeast Chromosome IV

Kailey Krueger, Brady Malone, Shannon Rochon, Hanen Khasawneh, Eman Otaifah, Sydney Winfree, Anas Mohamad, Ethan Oats, Anne Casper

Genomic instability in human cancer cells can result from break-induced replication (BIR). In *S. cerevisiae*, a model organism, we analyzed BIR at Fragile Site 2 (FS2) on chromosome III, a region with inverted Ty1 transposons. Our lab previously found that BIR template selection favors two hotspots on chromosome IV; each hotspot contains inverted Ty1 elements that can form a hairpin. We tested whether hairpins drive template favoritism by deleting one Ty1 element at each hotspot to reduce hairpin formation, then analyzing whether those hotspots are still favored as BIR templates.

Poster Presentation #49

Interaction Between *Vibrio cholerae* and Intestinal Bile Acids

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Vibrio cholerae, the etiological agent of cholera, causes disease while colonizing the small intestine. Bile, a fluid made by the liver and secreted into the small intestine, induces antimicrobial pressure on *V. cholerae* during colonization. Bile acids (BA) are a main component of bile known to have inhibitory effects against bacteria. Previously, we showed that the bile component in Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, a selective medium for *Vibrios*, causes growth inhibition of a *V. cholerae* DNA-repair mutant lacking exonuclease VII (*exoVII*). Suppressor mutations in DNA gyrase restored growth of the *exoVII* mutant on TCBS, indicating that bile inhibits DNA gyrase like fluoroquinolone antibiotics. As these studies were done with ox bile, a complex mixture of BAs, it is not known which BA(s) cause DNA gyrase dysfunction in *V. cholerae* and how *V. cholerae* (and the gut microbiome) have adapted to deal with BA-mediated DNA gyrase inhibition in the small intestine. Using LC–MS/MS, we show that TCBS agar and ox bile contain high relative abundances of cholic acid, glycodeoxycholic acid, and glycoursodeoxycholic acid, followed by lower amounts of other BA derivatives. We found that 5.0 mg/ml of ox bile significantly reduces the growth of wild-type *V. cholerae* whereas the *exoVII* mutant is more sensitive to ox bile. Some gut bacteria can detoxify and modify BAs to lower their inhibitory effects, at least in part due to the action of Bile Salt Hydrolase (BSH), which conjugates and deconjugates amino acids to/from BAs. We have identified that the *bsh* gene in *V. cholerae* encodes a putative BSH and hypothesized it mediated BA resistance. However, a *bsh* transposon insertion mutant exhibited similar sensitivity to BAs as the WT strain. This result suggests that either BSH is not expressed in these conditions or it does not contribute to *V. cholerae* tolerance to bile, possibilities we are exploring. Additionally, *V. cholerae* strains exposed to 1 mM of different individual BAs revealed high sensitivity to deoxycholic acid (DCA), chenodeoxycholic acid, and cholic acid, with DCA most closely mimicking ox bile. My future directions will identify which BAs inhibit DNA gyrase and investigate the contribution of BSH to this phenomenon, increasing our understanding of how bacteria survive bile acid stress.

Poster Presentation #50

Identification of a new punitive phage defence system from *Vibrio cholerae*

Ariana E. Straus, Jasper B. Gomez, and Chris M. Waters

Since the discovery of antibiotics, antibiotic resistance has been rapidly evolving, leading to one of the greatest public health threats. Phage therapy, or the use of bacterial-specific viruses to treat infectious disease, has been studied as an alternative to antibiotics for centuries and has renewed interest as an effective treatment due to their ability to infect and lyse specific bacterial species. Bacteria, however, have evolved various phage defense systems to protect themselves from phage infection. To develop phage therapies, we must understand the mechanisms of phage defense systems. To identify new phage defense systems, we are using *Vibrio cholerae* as a model as it has evolved several phage defense systems due to its constant predation by phages in its environment. A previous screen of a *V. cholerae* cosmid library within *Escherichia coli* found a 25kb fragment that provides protection against T2 coliphage. Using a transposon mutant library, we identified that the gene *vca0483* was both necessary and sufficient to protect against T2, T4 and T6 phage infection. Expression of *vca0483* both from its native promoter and an inducible promoter in *E. coli* was sufficient for protection against T2, T4, and T6. Overexpression of *vca0483* inhibits cell growth, suggesting the protein it produces is toxic. A colony forming survival count shows overexpression of *vca0483* inhibits cell growth without decreasing the viable cell count. Alpha fold predicts the protein *vca0483* encodes as a transmembrane protein. My model for how *vca0483* functions is that *vca0483* is expressed during phage infection as a transmembrane protein that induces cell stasis, and inhibits the phage replication cycle. I will test this model by performing a live/dead stain experiment to determine cell viability during phage infection and by making a mNeonGreen C-terminus fusion to *vca0483* for visual protein localization. Overall, understanding the mechanisms bacteria have evolved against phage infections is paramount to developing more effective phage therapies.

Poster Presentation #51

Construction of ICP0 Mutant Viruses to study the role of Proteins from DNA repair pathway in substrate specific recognition of PML I by HSV-1 ICP0

Gaurik S. Sidhu, Eleazar Reward, Dr. Behdokht Jan Fada, Dr. Haidong Gu

HSV-1 is infamous for wide-spread recurring infection, enabled in large part by the viral E3 ubiquitin ligase activity of ICP0. Regulated by subcellular location, phosphorylation status, and the various ICP0 functional domains, the RING-type E3 ubiquitin ligase of ICP0 ubiquitinates host proteins to degrade them for effective viral infection. PML-NB is a dynamic nuclear structure organized by PML that executes key anti-viral defense in early HSV-1 infection. ICP0 mediated PML degradation leads to dispersal of PML-NB, releasing host cell restrictions on HSV-1 replication. We found that SUMO-SIM interaction is required to ubiquitinate most PML isoforms but not PML isoform I. SIM-independency in PML-I recognition results from a secondary targeting mediated by residues 1-83 of ICP0, preventing the reformation of PML-NB. Phosphorylation of T67 in ICP0 N-terminus has been shown to facilitate the recognition of a DNA repair regulator, RNF8, by the ICP0 E3 ubiquitin ligase. Here we report that ICP0 containing unphosphorylated T67 interacts with VprBp, another critical component in DNA damage responses. We seek to determine if RNF8 and VprBP compete for T67 to regulate the PML-I substrate recognition. Four ICP0 mutants were generated to facilitate this study. Mutants ICP0-T67A and ICP0-T67E mimic a constantly unphosphorylated and phosphorylated T67, respectively. Double mutations in both T67 and SIM of ICP0 enable us to investigate the PML I isoform specific substrate recognition without interference from SUMO-SIM interactions. These four mutants combined will elucidate ICP0's ability to tweak apart similar host proteins and target multiple biological pathways simultaneously during early lytic infection.

Poster Presentation #52

Phenotypic Response of Aquatic Microorganisms to Seasonal Changes

Allen Cureton, Trista Vick-Majors

Most lakes at moderate latitudes become mixed with regard to temperature twice per year. In the fall, this mixing occurs when the lake water column reaches 4 °C. Seasonal change likely induces a cascade of physiological changes in aquatic microbial communities; however, the consequences of this are not well understood. The goal of this research was to assess changes in natural microbial communities across a range of temperatures in the context of mixing. We expected that as temperatures decreased and the water column mixed, microbial communities would change in cell morphology and abundance. To test this, we collected surface water samples from Lake Mendota, a dimictic lake in Wisconsin, and assessed the responses of a bacterial isolate (IKW-07) as a pilot experiment. Surface water samples were collected from Lake Mendota in November 2025 prior to complete fall mixing. DNA samples and samples for cell abundance and morphology were collected and stored to capture initial community composition. Water samples were then incubated at 4 °C, 9.8 °C, and 20 °C and samples sacrificed at 24, 72, and 96 hours. Microbial cell counts were then conduced via epifluoresence microscopy and images analyzed using ImageJ to determine cell abundance, morphology, and sizes as equivalent spherical diameter. The results when it came to cell abundance, many of the IKW-07 samples were stagnant in abundance. Lake Mendota samples were more consistent, displaying a decrease in cell abundance and an increase in morphology over time. Additionally the morphological data showed an increase in rod-shaped bacteria in 20 °C culture after 96 hours . This could suggest that a community shift occurred within the 20 °C culture over time causing rod-shaped microbes to become more advantageous. In the future, Lake Mendota's DNA samples will be extracted and sqeunced to identify the microbial community within each sample. Our results suggest that lake turnover leads to phenotypic changes which likely co-occur with adaptitve activities in the changing environment.

Poster Presentation #53

Antimicrobial Resistance Monitoring Using Community Wastewater

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Antimicrobial resistant infection is a growing public health threat that is responsible for an average of $693 billion of hospital costs across the world (Naylor et al., 2025). Antimicrobial resistance genes (ARGs) and the bacteria that carry them can be spread via community wastewater sources including hospital and wastewater treatment plant (WWTP) effluent which is discharged back to the environment. Wastewater monitoring is an effective method for tracking many pathogens and is well established in Michigan. Integrating ARG monitoring into already established monitoring framework can be a cost effective and low barrier way to track progression of resistance spread. Using wastewater to understand prevalence of ARGs in a population could influence antibiotic use in healthcare and types of treatments used in WWTPs. An urban WWTP in Michigan was monitored for 13 ARGs, including mostly beta-lactamases due to prevalence of beta-lactam antibiotic use. Out of 13 ARGs monitored, 12 were significantly reduced from raw WWTP influent to final WWTP effluent. However, *blaIMP* was not significantly reduced by the treatment process, suggesting specialized treatments may need to be used to target that ARG. KPC and *tetW* which confer resistance to carbapenem and tetracycline, respectively, were found in the highest concentrations. This could lead to community specific recommendations for antibiotic use to help mitigate spread of antibiotic resistance.

Poster Presentation #54

Uncovering Fitness Effects Associated with Carriage of the Predominant Carbapenemase-encoding Plasmid in Michigan

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Carbapenems are a class of last-line antibiotics used to treat bacterial infections that are resistant to most other antibiotics. Unfortunately, carbapenem resistance is spreading, leading to the emergence of carbapenem-resistant *Enterobacterales* (CRE) that are nearly impossible to treat. A major driver of carbapenem resistance are *Klebsiella pneumoniae* carbapenemase (KPC) enzymes that cleave and inactivate carbapenems. KPC enzymes are especially concerning because they are carried on conjugative plasmids, allowing carbapenem resistance to spread into diverse bacterial strains and species. However, little is known about the molecular epidemiology of these KPC plasmids and why certain plasmids are more successful in spreading than others. Whole-genome sequencing of every KPC-positive CRE isolate submitted to the Michigan Bureau of Laboratories over a ten year period reveals AB978 as the predominant plasmid in the state. We hypothesize that its predominance is because AB978 has a low initial fitness cost in new hosts, and this fitness cost can be ameliorated by mutations over time. To test this hypothesis, we are using a CRISPR-based curing plasmid to remove AB978 from isolates, and comparing the growth of the plasmid-cured strains to their parental, plasmid-carrying strains. To measure in-host adaptation to AB978 over time, we selected early and late occurring isolates within clonal clusters, and we are assessing the fitness cost of AB978 in related strains collected over time. To date, we have found strains in which AB978 has a significant negative fitness effect. In both clusters where we have successfully cured the early and late isolates, we see a significant change in the fitness effects imparted by AB978, indicating that the host cells are adapting to AB978. We are currently working on curing more clinical isolates from our selected clusters and validating experimental results by complementation with the AB978 plasmid. Moving forward, we will identify genetic variants associated with KPC plasmid persistence, which may allow for more accurate risk assessments of new KPC strains as they emerge.

Poster Presentation #55

Angela Prete, Christopher Waters

Vibrio cholerae naturally resides in aquatic environments, but it makes drastic changes upon infection to colonize the human gut and cause disease. One of the major signaling molecules that facilitates this environmental adaptation is the bacterial second messenger bis (3'-5') cyclic dimeric guanosine monophosphate (c-di-GMP), which regulates several cellular processes such as biofilm formation, motility, virulence, and quorum sensing. C-di-GMP is synthesized by diguanylate cyclases (DGCs) and degraded by phosphodiesterases (PDEs), all of which regulate c-di-GMP levels within the cell in response to environmental signals. The c-di-GMP signaling network in *V. cholerae* is highly complex with dozens of DGCs and PDES contributing to c-di-GMP levels. Moreover, each of these enzymes encodes a signaling domain that is hypothesized to recognize and respond to specific environmental cues. However, what specific DGCs and PDEs, the environmental signals that they recognize, and in what conditions they control c-di-GMP is poorly understood. For example, only ~1/4 of the DGCs of *V. cholerae* appear to be active in standard laboratory conditions. In addition to DGCs and PDEs, other global regulatory pathways, like quorum sensing, regulate c-di-GMP levels. To understand this complexity, a systems level analysis of this signaling pathway is needed. To answer both of this question using *V. cholerae* as a model system, I will generate a randomly barcoded transposon mutant library of ~400,000 unique mutants using the process of RB-TnSeq. I will insert into this this library a dual tandem riboswitch-based fluorescent c-di-GMP biosensor, combining RB-TnSeq and fluorescence activated cell sorting (FACS) to isolate Tn mutants that both increase and decrease intracellular c-di-GMP. This will be performed in many different environments, generating a signaling network map across multiple conditions leading to the identification of environmental-specific DGCs and PDEs as well as new regulatory pathways that control c-di-GMP signaling. In addition, my novel experimental framework combining RB-TnSeq and FACS could be applied to understanding c-di-GMP in many bacteria.

Poster Presentation #56

IFN-γ–Mediated Restriction of *Toxoplasma gondii* in Rat Macrophages

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The protozoan parasite *Toxoplasma gondii* (*T. gondii*) is able to infect any nucleated cell in any warm-blooded animal, yet infection outcomes vary widely across hosts. In humans, acute infection is typically subclinical and progresses to a dormant chronic stage, whereas acute infection in laboratory mice can be lethal. Host resistance to *T. gondii* is widely attributed to IFN-γ/Th1 immune signaling, but the downstream IFN-γ-induced effector mechanisms that restrict parasite replication in humans and other naturally resistant hosts remain poorly defined. Investigating these mechanisms may uncover novel immune effectors involved in parasite control and aid the development of therapeutic strategies.

In this study, we propose the laboratory rat as a naturally restrictive host for toxoplasmosis. We observed that rats resist acute infection following high-dose *T. gondii* injection and subsequently establish a dormant chronic infection resembling human disease. Because macrophages are a primary infected cell type during in vivo infection, we examined parasite replication in primary macrophages isolated from rats and mice. We found that naive rat macrophages are less permissive to parasite replication than mouse macrophages, and this difference persists during IFN-γ stimulation. To investigate mechanisms underlying this divergence, we performed transcriptomic analysis of rat and mouse macrophages activated by IFN-γ to identify rat IFN-γ-activated effectors that may restrict parasite replication. Our analysis identified induction of orthologs of well-defined IFN-γ-stimulated genes, including genes encoding guanylate-binding proteins, as well as several candidate genes uniquely induced in rat macrophages, suggesting potential mechanisms underlying species-specific parasite control. Current work aims to validate the function of candidate genes through genetic knockdown and parasite replication assays.

Poster Presentation #57

Characterization of a novel phage defense system *Quartet*

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Antimicrobial-resistant (AMR) bacteria pose a major threat to public health, causing more than 1.1 million deaths annually. Bacteriophage therapy offers a promising alternative to antibiotics; however, a deeper understanding of bacteriophage defense systems is needed to avoid and overcome the development of phage resistance in pathogenic bacteria. To this end, we are characterizing a novel phage defense system we have named Quartet (QRT) encoded by a bloodstream clinical isolate of Citrobacter werkmanii . QRT is encoded by a four gene operon (qrtABCD), and while there are no known functions for these genes, we have identified over 800 bacterial genomes, including many pathogens, which contain at least one QRT protein homolog. Structural predictions generated using Alphafold suggest QRT proteins form a multimeric inner membrane complex resembling a pore. We hypothesize that QRT destabilizes the bacterial membrane in response to phage infection, triggering cell death thus preventing phage replication and restricts further infection within the bacterial population. Exogenous expression of qrtABCD protects Escherichia coli against many diverse phages, including greater than1,000-fold resistance to infection by phage BAS31. Thus far, we have observed that single gene knockouts of any QRT allele resensitize E. coli to BAS31 infection, suggesting that all four genes are required for QRT's antiviral activity and are currently validating these results by complementation. In addition, we have isolated BAS31 mutants capable of evading QRT antiviral activity that will be used to identify phage factors involved in QRT activation. Finally, we have identified a closely related qrtABCD operon in Citrobacter pasteurii, allowing for comparative studies to describe the conservation of QRT activity between these Citrobacter pathogens. Our characterization of this widely distributed phage defense system will inform the engineering of therapeutic phage to evade QRT and improve the application of bacteriophage therapies against AMR infections.

Poster Presentation #58

Ali Jomaa, Jeffrey Ram

Escherichia marmotae is a recently identified member of the Escherichia genus that was first isolated from fecal contamination in environmental waters. Although it shares nearly 100% phenotypic similarity with *Escherichia coli* in standard biochemical tests, its genomic similarity is only about 90%, indicating important genetic differences that may influence its physiology and pathogenic potential. Increasing attention has been given to *E. marmotae* due to its association with human infections, including sepsis and spondylodiscitis. One aspect of bacterial physiology is biofilm formation, a process that contributes to environmental persistence and virulence. Previous work in our laboratory demonstrated that biofilm formation in *E. marmotae* is temperature-dependent, forming biofilms more readily at 28 °C than at 37 °C. This suggests that environmental conditions may influence its ability to colonize surfaces. In this study, we investigated the role of specific genes in biofilm development by generating targeted gene knockouts. Mutant strains lacking motility-related genes, as well as strains lacking a biofilm-associated gene, were constructed using the Lambda Red recombination system with plasmid-based templates. Biofilm production was quantified using a crystal violet staining assay and measured spectrophotometrically at 590 nm. By comparing biofilm formation among wild-type and mutant strains, this study aims to determine how motility genes and biofilm-related genes contribute to surface attachment and biofilm development in *E. marmotae*. Understanding the genes that influence biofilm formation may provide insight into how this emerging species survives in environmental reservoirs and contributes to human infection.

Poster Presentation #59

The role of zinc and manganese on phosphodiesterases in *Vibrio cholerae*

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Cyclic di-GMP is a signaling molecule that regulates biofilm formation and motility and contributes to bacterial infection in *Vibrio cholerae*. Cyclic di-GMP is regulated by diguanylate cyclases (DGCs), which synthesize cyclic di-GMP, and phosphodiesterases (PDEs), which degrade it. We have previously found that zinc binds to PDE ZpdA to repress EAL activity while manganese activates ZpdA. We are investigating the role of zinc binding to two closely related PDEs, *vc0137* and *vc1851,* and their effect on cyclic di-GMP regulation. Deletion of *vc0137* or *vc1851* increased biofilm formation compared to WT, consistent with high levels of *cyclic di-GMP*. A manganese exporter mutant, Δ*vc0022,* showed decreased biofilm formation corresponding to low levels of cyclic di-GMP. These results highlight the role of zinc and manganese in regulating cyclic di-GMP levels in *V. cholerae*.

Poster Presentation #60

Opposing HRxxN Histidine Kinase Inputs Tune the *Brucella* General Stress Response

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To promote survival across diverse stress conditions, alphaproteobacteria employ a general stress response (GSR) system that controls large transcriptional programs via a conserved partner-switching mechanism. A phosphorylated response regulator, PhyR, binds the anti-sigma factor, NepR, thereby releasing an ECF sigma factor to activate GSR transcription. The output and dynamics of this pathway vary substantially across genera; therefore, one proposed explanation is variability in the regulation of PhyR phosphorylation by atypical HRxxN (HWE/HisKA_2) histidine kinases. *Brucella* spp. are intracellular pathogens that encode three HRxxN kinases (LovhK, PhyK, and BOV_1607) and utilize the GSR system to withstand host-associated envelope and physicochemical stresses encountered during infection. Currently, LovhK has been described as a GSR activator while the others have unknown roles. Thus, we have developed *B. ovis* as a model to test how distinct kinase inputs converge on PhyR and influence stress response phenotypes. Combining unbiased genetic selection, RNA sequencing, protein-protein interaction assays, and biochemical reconstitution, we show that these kinases form an antagonistic network that sets GSR output through opposing effects on PhyR phosphorylation. Deletion of *phyK* confers strong detergent resistance, and this phenotype requires an intact set of GSR activators including PhyR, RpoE1, and LovhK. Thus, PhyK normally limits GSR-dependent stress protection. Transcriptomic analyses support a model in which PhyK represses, whereas BOV_1607 promotes, GSR-dependent transcription. Mechanistically, PhyK associates with LovhK and lacks detectable autokinase activity, but serves to dephosphorylate PhyR. In contrast, BOV_1607 exhibits weak phosphotransfer to PhyR consistent with a role as a GSR activator. Together, our data supports a model in which a multi-histidine kinase network controls phosphorylation of a single protein, PhyR, to regulate *Brucella* GSR output.

Poster Presentation #61

Isolation and Annotation of Mycobacterium Phage Khan1

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Bacteriophages are highly diverse viruses that play important roles in microbial ecology and evolution, yet many remain uncharacterized. Here, we report the isolation and genomic characterization of *Mycobacterium* phage Khan1, a lytic siphovirus discovered in 2024 from an enriched soil sample collected in Detroit, Michigan. The phage was isolated using *Mycobacterium smegmatis* mc^2155 as a host through the SEA-PHAGES program at the University of Detroit Mercy. The Khan1 genome is 68,556 bp in length, circularly permuted, and has a GC content of 66.5%, consistent with other mycobacteriophages. Bioinformatic analysis assigned Khan1 to Cluster B, Subcluster B1, and identified 101 predicted protein-coding genes, with no tRNA or tmRNA genes detected. Morphological and plaque analyses indicate that Khan1 belongs to the family Siphoviridae, characterized by a long, non-contractile tail. The absence of lysogeny-associated genes and its classification as a lytic phage suggest potential applications in bacterial control. In this poster we describe the annotation of the Khan1 genome, expanding our understanding of phage diversity.

Poster Presentation #62

Annotation of the Arthrobacteriophage *Chicken* genome

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Arthrobacteriophage *Chicken* is a bacteriophage, which is a virus that targets bacteria, using the host's cells to replicate themselves and destroy the host cells. It was isolated from slightly dry, sandy soil collected from Los Angeles, California. Based on genomic similarity, *Chicken* has been classified to belong to the cluster AS1 and was isolated using the host *Arthrobacter globiformis B-2979*. This tells us that it has a shared evolutionary relationship and conserved genomic features among related phages. It has a temperate life cycle, where it can alternate between both the lytic and lysogenic cycles. This suggests the presence of regulatory genes involved in lysogeny, like integrases and repressors, which can be determined during annotation. The genome's length is a total of 39,511 base pairs (bp). Its genome is now being annotated by students and faculty of the University of Detroit Mercy by employing various gene annotation tools, including PECAAN, PhagesDB, NCBI, DeepTMHMM, and HHPred Databases. These tools will allow us to compare proteins and analyze their structure in the open reading frames. Although currently we are in a preliminary process of annotating the genome of *Chicken*, we look forward to determining the functions of all 64 possible genes and finalizing our project. This work will contribute to the growing database of arthrobacteriophages and help to provide valuable information such as genome organization and evolutionary relationships.

Poster Presentation #63

Genetic Analysis of *Candida albicans* and *Streptococcus mutans* Co-Biofilm Formation in Oral Disease

Hadeel Bazzi

Fungal infections, particularly those caused by *Candida albicans*, represent a growing clinical concern due to limited antifungal treatments and their association with systemic diseases. In the oral cavity, fungal infections are increasingly relevant with the rise of artificial dental devices and biofilm-associated infections. Traditional in vitro models, such as catheter-based assays, fail to accurately represent oral environments, showing the need for more relevant studies. This project investigates the genetic factors involved in biofilm formation between *Candida albicans* and *Streptococcus mutans*, a key contributor to dental caries. Specifically, we examine the role of *C. albicans* cell wall and kinase-related genes in co-biofilm development. Using a mutant library, individual gene disruptions are screened to determine their impact on biofilm formation and stability. Weekly assays evaluate multiple mutants, followed by quantitative analysis of biofilm growth and structure. Identifying genes essential for co-biofilm formation may reveal novel therapeutic targets for preventing fungal-bacterial interactions associated with oral disease. This research aims to improve our understanding of fungal contributions to dental pathology and support the development of more effective antifungal strategies.