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High throughput mapping of the interaction of Streptokinase reveals residues critical for binding to plasminogen and potentially *Streptococcus* pathogenesis

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Several pathogens hijack the blood coagulation and fibrinolytic systems in humans, including the important human pathogen, Group A *Streptococcus* (GAS). GAS express streptokinase (SK), a critical virulence factor that non-enzymatically activates the host zymogen, plasminogen (PLG), to an active form, PLG_{SK}, resulting in the degradation of fibrin clots, which in turn facilitates bacterial dissemination. This is in contrast to the physiologic activation of PLG to the serine protease plasmin via a proteolytic mechanism. As a potent thrombolytic, recombinant SK has been used as a therapeutic to treat heart attacks and strokes. GAS SK is highly-specific for human PLG, having little effect on the PLG of other species; however the individual amino acid residues mediating this specificity are unknown. Sequence variation between SK from different GAS strains has been linked to differences in its binding affinity for PLG, as well as disease pathogenicity and severity, however, the contributions of individual amino acids to these differences are poorly understood. To address this gap in knowledge, we use deep mutational scanning (DMS) coupled with high-throughput phage display to map the effects of ~70% of all possible single amino acid substitutions within SK on its ability to bind human PLG. We first demonstrate that SK expressed as a fusion protein to the p3 coat protein of M13 filamentous phage retains its capacity to bind human PLG. The results of our DMS screen further identify regions in SK, both at and distal to the protein-protein interface, in which amino acid substitutions are likely to increase or decrease its affinity for PLG. This finding suggests a complex protein-protein interaction in which long-range protein dynamics influence the conformational activation of PLG to PLG_{SK}. Future studies will apply this DMS approach to explore SK sequence and structural requirements for PLG activation. Taken together, the results of these studies lay the foundation for linking SK variation between GAS strains to differences in virulence, as well as mapping the determinates of GAS SK's human specificity.

Vibrio cholerae modulates cyclic di-GMP in response to zinc and quorum sensing via a horizontally acquired genomic island

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Secondary messenger cyclic-di GMP is involved in many crucial biological processes including biofilm formation, motility, and secretion in *Vibrio cholerae*. The intracellular level of cyclic-di GMP is regulated by diguanylate cyclases (DGCs) that synthesize cyclic-di GMP from GTP and phosphodiesterases (PDE) that degrade cyclic-di GMP. Understanding the regulation of these enzymes and the environmental signals that control their activity is important to understand how and when *V. cholerae* switches between motile and sessile lifestyles in different environments. Ongoing 7th pandemic *V. cholerae* El Tor strains have acquired two genomic islands, VSP-1 and 2, that are hypothesized to be important for their pathogenicity and adaptability. However, the functions of several genes in these genomic islands are unknown. Recently it has been shown that genes *vc0512-vc0515* in the VSP-2 island are repressed by zinc via the Zur repressor. This region includes the predicted cyclic-di GMP PDE, *vc0515*. My research seeks to understand how acquisition of a horizontally acquired PDE that responds to metal availability enables *V. cholerae* adaptation. To accomplish this, we delineated the role of zinc in the regulation of VC0515. We have shown that VC0515 is an active PDE, and mutation of the active site ELL-AAA rendered the protein inactive. The *Δvc0515* mutant had higher cyclic di-GMP levels and reduced motility when compared to the wild-type owing to its phosphodiesterase activity. Higher levels of extracellular zinc increased intracellular concentrations of zinc thereby decreased expression of *vc0515* via Zur repressor which was exacerbated in *ΔznuABC* importer mutant. Additionally, we have shown that Zinc disrupts the phosphodiesterase activity of VC0515 whereas manganese activates it. In addition to Zur repressor, we have also shown that *vc0515* is repressed by the quorum sensing master regulator HapR via the upstream coding region of *vc0515.* Our results demonstrate that *V. cholerae* alters cyclic di-GMP levels and associated phenotypes in response to zinc and cell density through regulation of the VC0515 PDE, identifying zinc as an important cue that control *V. cholerae* biology.

Intestinal inflammation promotes *Campylobacter jejuni* growth during infection

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Campylobacter jejuni is a leading global cause of bacterial gastroenteritis, typically presenting as self-limiting but occasionally severe bloody diarrhea, fever, and abdominal pain lasting two to five days. The mechanisms underlying *C. jejuni* growth within the gut lumen during infection remain poorly understood. In our study, we observed that *C. jejuni* infection of ferrets induces moderate to severe gastroenteritis and colitis signs within two to three days, closely resembling human infections. Infection promotes colonic crypt hyperplasia, leading to increased epithelial oxygen levels and altered gut metabolite profiles. We observed a elevated levels of $Ki67^{+}$ transient amplifying, undifferentiated cells in infected colonic tissues, likely leading to significantly elevated lactate levels in the colonic lumen during the acute phase of infection. Our findings suggest that the elevated L-lactate serves as a key carbon source for *C. jejuni* during inflammation, as a *C. jejuni* mutant lacking the L-lactate transporter (*lctP::kan*) exhibits impaired growth during acute infection. We performed transcriptomic analyses *in vitro* on *C. jejuni* cultivated in L-lactate or pyruvate, uncovering significant increase in transcripts encoding nitrate respiratory regulatory genes (*napABCD*) in L-lactate-grown cultures. Additionally, in vitro growth assays demonstrated that nitrate significantly enhances *C. jejuni* proliferation beyond the effect of L-lactate alone. To assess the role of nitrate respiration in *C. jejuni* fitness during infection, we generated a nitrate reductase (*napA*) mutant (*napA::kan*). The mutant displayed significantly reduced colonic colonization compared to the wild-type strain at day three post-infection in ferrets. Our findings suggest that L-lactate upregulates nitrate respiratory pathways, which facilitate *C. jejuni* growth during infection. These results highlight metabolic adaptations that contribute to *C. jejuni* pathogenicity and may inform therapeutic strategies targeting bacterial growth in the gut.

Breaking the barrier: Discovery and profiling of novel MmpL3 inhibitors against *Mycobacterium abscessus*

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Non-tuberculous mycobacterial (NTM) infections comprise an underrecognized, yet emergent source of infections. *Mycobacterium abscessus* 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 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 pharmacological, microbiological, and genetic strategies, 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 400 novel MmpL3 inhibitors against wildtype *M. abscessus* (ATCC19977), isolated 15 unique resistant *M. abscessus* mutants against four inhibitors, and examined the cross-resistance profiles between the mutants and a subset of 16 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 and against 30 different clinical isolates. We further studied several molecular and biochemical alterations induced by MmpL3 inhibition as well as novel potential therapeutic strategies combining MmpL3 inhibitors and standard-of-care treatments. The Screening process yielded several potent and efficacious analogs *in vitro* and inside macrophages (EC_{50} <1 uM), 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^{-7}-10^{-8}) 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 the biofilm production capacity of the bacteria among other aspects. Together, these findings give us more insights into the clinical value of MmpL3, bacterial responses associated with its inhibition, and how it alters the pathogenesis of NTMs and offers potential optimization to the current standard-of-care treatments for NTMs.

Identification of contingency loci in phage

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Phage face stringent challenges to survive in and transmit between bacterial hosts due to a myriad of bacterial phage defense systems. Phage adapt to their host through acquisition of specific counter defense mechanisms that inhibit host defenses or through selection of mutant phage that are insensitive to these defenses, the latter of which is not yet fully understood. My research suggests that contingency loci, homopolymeric nucleotide repeats that have an elevated mutation rate that drives genetic variation, are abundant in phage. Although contingency loci have been extensively studied in all branches of life, they have yet to be described in phage. My discovery of phage contingency loci arose from screening a *Vibrio cholerae* genomic library in *Escherichia coli* for novel phage defense systems. From this screen, I discovered two genes, vc1767 and vc1766, that protect against T-even coliphage infection and are homologous to gmrSD, a Type IV restriction system. Thus, we renamed these genes TgvAB (Type I-embedded gmrSD-like system of VPI-2). I identified T2 mutants resistant to TgvAB had frameshift mutations in agt. Agt encodes an alpha glycosyl-transferase, that adds a glucose to the 5-hydroxy-methyl-cytosine (5hmC) of T- even phage DNA, suggesting TgvAB targets glucosylated phage genomes. All mutations identified in agt were insertions or deletions in thymine repeat sequences, which I hypothesize are contingency loci. Using another Type IV restriction system, I showed that reversion to wildtype agt occurs at the identical contingency locus at a much higher rate versus single-nucleotide polymorphisms (SNPs). Liquid infection experiments reveal that T2 mutants resistant to TgvAB arise within one culture cycle, demonstrating high mutation rates of agt. Additionally, through sequencing we have identified hundreds of SSRs in genomes of many phage, and analysis of these repeats shows that they exhibit high mutation rates. Our results suggest that replication of many phages does not produce a homogeneous population but rather a highly heterogenous population with frequent insertions or deletions in contingency loci allowing for phenotypic plasticity to adapt to divergent bacterial hosts or environments. More broadly, my results are the first description of contingency loci in phage, demonstrating how phage can harness mutation rates to adapt to bacterial host defense.

The E3 Ubiquitin Ligase of ICP0 Blocks the Hippo Kinase Cleavage Triggered by Inflammasome Activation in HSV-1 infection

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MST1 and MST2 (mammalian sterile 20-like kinases 1 and 2) are the core components of the Hippo pathway that govern critical life events like cell proliferation, organ formation, carcinogenesis, and immune development. A recent study revealed a non-canonical Hippo signaling pathway, where caspase 1 driven proteolytic cleavage of MST1/2 triggers apoptosis to restrict bacterial infection. Although recent studies suggested the role of MST1/MST2 in antiviral immunity, their role in HSV-1 infection remains unexplored. In this study, we report that (i) low multiplicity of infection (MOI) of wild-type HSV-1 (F) causes a mild accumulation of the cleaved MST1/2 N terminal fragments as well as cleaved PARP1 an apoptotic hallmark, similar to intracellular bacterial infection (ii) overexpression of the cleaved N-terminal MST1 fragment reduces viral protein expression at low MOI but not at high MOI, suggesting that HSV-1 infection events overcome the inhibitory effects of MST1 cleavage at high MOI (iii) while the MST1/2 cleavage is seemingly associated to multiple cell death pathways, viral protein ICP0 is responsible for counteracting the caspase 1 controlled inflammatory cell death. The E3 ubiquitin ligase activity of ICP0 is key to blocking the inflammasome activation through MST1/2 cleavage, whereas the cleavage reaction of MST1/2 is uniquely absent in U2OS cells, the permissive cell line for the ICP0 null virus. Taken together, inflammatory responses associated with the Hippo kinase cleavage promote a multifaceted anti-HSV defense, which is partly countered by the ICP0 E3 ubiquitin ligase activity.

Standardizing an antimicrobial susceptibility testing protocol for *Mycoplasma bovis*

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Mycoplasma bovis is a highly contagious bacterial pathogen affecting cattle, leading to substantial production losses and disruption in the cattle industry. Infections of *M. bovis* may result in respiratory disease, mastitis, and/or arthritis. There are many barriers to treatment including inherent resistance to beta-lactam antibiotics and the emergence of resistance to multiple classes of antibiotics. Lack of commercially available vaccines and the contagious nature of this bacterium makes it challenging to prevent the spread of infection in a herd. Antimicrobial susceptibility testing (AST) against *M. bovis* is not offered by many diagnostic laboratories due to the fastidious nature of the organism and the absence of a standardized AST procedure. With the aim of developing a standardized procedure for AST we have validated a label-free bacterial enumeration method using an impedance flow cytometer for rapid and accurate counting of *M. bovis* cells, a pre-requisite for AST. We also validated a broth micro-dilution method of AST using the ATCC strain of *M. bovis* with a custom designed 96 well antibiotic plate. Currently this newly validated protocol is being used to plot the MIC distribution of clinical historic and current *M. bovis* isolates; the data generated will be used to determine epidemiological cut-off values for clinically relevant antibiotics for *M. bovis.* This study will help validate a protocol for *M. bovis* AST which can be adopted by other laboratories and support informed decision-making regarding the use of antibiotics for *M. bovis* infections.

***Sneathia*: An Emerging Pathogen in the Female Reproductive Tract**

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Sneathia is a fastidious bacterium of the vaginal microbiome. Through molecular investigations, it has been associated with bacterial vaginosis, increased risk of sexually transmitted diseases, and spontaneous preterm birth, which is the leading cause of neonatal morbidity and mortality worldwide. Despite its association with women's reproductive health, there has been only one representative isolated in pure culture from vaginal fluid. Our novel isolation strategies, including refined transport medium, specific uptake of media dyes and fluorescence, and confirmation of presence via *Sneathia* specific primers, have allowed us to overcome prior barriers to cultivation from mixed microbial communities of vaginal fluid. We recently cultivated 19 *Sneathia* isolates from vaginal fluid. Genomic data of these isolates have enabled us to explore the metabolic and virulence capabilities of *Sneathia*. One of the two *Sneathia* species, *S. sanguinegens,* is less prevalent in vaginal fluid and is more difficult to culture, in part because it requires whole blood for growth. Our assays have revealed that hemoglobin and albumin, as well as hemoglobin and riboflavin supplemented together, result in *S. sanguinegens* growth that is almost comparable to growth in a whole blood medium. Additionally, through pangenomic analyses of all publicly available *S. vaginalis* and *S. sanguinegens* genomes, including our novel *Sneathia* isolates, we have identified heme as a critical nutrient. The pangenomic data suggests the challenge lies in *S. sanguinegens* heme uptake through a TonB-dependent receptor that is absent from *S. vaginalis* genomes. Overall, this research is foundational in elucidating the previously unknown metabolic and virulence mechanisms involved with the pathogenicity of *Sneathia*. Targeting these mechanisms promise to mitigate *Sneathia*'s pathogenic impact on women's reproductive health.

PhoPR remodels *Mycobacterium tuberculosis* metabolism to restrict growth on propionate at acidic pH

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Mycobacterium tuberculosis (Mtb) is an ongoing public health problem in many communities including the indigenous community. Mtb is a successful pathogen, in part because it can adapt to environmental cues encountered during infection, including the acidic pH of the macrophage. In response to this acidic environment Mtb modulates its metabolism. Mtb uses a two-component system, PhoPR, that is stimulated by acidic pH and causes differential expression of metabolic genes. Mtb arrests its growth on specific single carbon sources at pH 5.7, such as propionate. However, Mtb grows well on these carbon sources at pH 7.0. We hypothesized that arrested growth is genetically controlled and that mutants could be selected that gain the ability to grow on these carbon sources at acidic pH. To test this hypothesis, we selected for transposon mutants that grew on propionate, as a sole carbon source, at pH 5.7. All selected transposon mutants were identified with mutations in *phoR* or *phoP*. We propose a model where at acidic pH, PhoPR diverts carbon away from central carbon metabolism for lipid anabolism and slows growth at acidic pH. When PhoPR is inactivated, propionate is instead metabolized by the methyl citrate cycle to supply carbon to the TCA cycle resulting in enhanced growth. This model is experimentally supported by manipulating different pathways such as the methylcitrate cycle and the methyl malonyl-CoA pathway. For example, when the methylcitrate cycle is blocked, Mtb loses the ability to grow on propionate at acidic pH. Slow growth in the PhoPR mutant is restored, via methyl malonyl-CoA pathway, by supplementing the media with vitamin B12. Together, these findings support that arrested growth on propionate at acidic pH is caused by metabolic remodeling that is dependent on PhoPR.

The Role of a HRxxN Histidine Kinase in *Brucella ovis* General and Detergent Stress Responses

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All organisms require the ability to sense and respond to environmental changes/stress. One method of stress response in bacteria is the general stress response (GSR) where many potential stressors will activate a large regulon of genes, mounting a broad response to the initial stressor(s). In Alphaproteobacteria, the GSR is activated via two component systems, which are composed of histidine kinases and response regulators. One family of histidine kinases, the HRxxN kinases, are of interest due to atypical oligomeric states and protein interactions. Studies have implicated HRxxN kinases as alphaproteobacterial GSR activators and repressors; however, many HRxxN kinases remain uncharacterized with unknown roles in stress response pathways.

Brucella ovis, an alphaproteobacteria and facultative intracellular sheep pathogen, contains 3 HRxxN kinases (BOV_1602, BOV_1607, LovhK). This small number makes *B. ovis* a model organism for characterizing the functional roles of HRxxN kinases. There has been evidence of LovhK positively regulating the GSR in *Brucella spp*. through distinct molecular mechanisms, but it is not known if other HRxxN kinases directly regulate the GSR system. In a recent study, the deletion of *BOV_1602* conferred dramatic resistance to detergent stress. Preliminary data showed that *ΔBOV_1602* detergent resistance requires an intact GSR system and the BepCFG efflux pump system. Bacterial-two hybrid assays provided evidence for protein-protein interactions between BOV_1602 and LovhK, implying that BOV_1602 may be regulating the GSR by interactions with LovhK. Size exclusion chromatography revealed multiple oligomeric states BOV_1602 could adopt. Finally, genetic and RNAseq data implicated BOV_1602 as both a positive and negative regulator of the GSR. This research will not only clarify stress adaptation in *Brucella spp.* but will be informative about how the broader alphaproteobacteria use HRxxN kinases for stress responses.

Understanding the differential substrate recognition by the E3 ubiquitin ligase activity of HSV-1 immediate early protein ICP0

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Upon infection, herpes simplex virus 1 (HSV-1) immediately contends with the promyelocytic leukemia nuclear bodies (PML-NBs) that converge with the incoming viral DNA to repress gene expression. Infected cell protein 0 (ICP0), an E3 ubiquitin ligase, is deployed to degrade PML, the architectural organizer of PML-NBs, to disperse the NB components and derepress the viral genome. Interestingly, we found that the ICP0 adopts different biochemical mechanisms to target the PML isoforms, based on their sequence specificity, SUMOylation status and subcellular localization. We previously reported that ICP0 degrades all SUMOylated PML via its SUMO-interacting motif (SIM) but targets the unSUMOylated PML I through an undefined N terminal motif. We showed that failure in the cytoplasmic SUMO-independent degradation of PML I causes PML-NBs to reform in late infection and impairs viral yield. Here, we sought to characterize the mechanisms of this differentiation. We report that (i) the ICP0 N-terminal sequences recognizing the unSUMOylated PML I coincide with the binding site of RNF8, a cellular E3 critical for DNA damage responses; (ii) VprBP, the adaptor of CUL4 E3 complex, competes for the same binding site; and (iii) the isoforms of another core component of PML-NBs, Sp100, are also differentiated. While ICP0 targets Sp100 isoforms A, C, and HMG via the SUMO SIM interaction, it recognizes Sp100 B in a SUMO-independent manner. However, the mechanism of SUMO-independent targeting of Sp100 B by ICP0 E3 is distinct from that of unSUMOylated PML I. Taken together, ICP0 has a complex ability to distinguish its E3 substrates. This differentiating capability is even more important considering PML-NBs are dynamic hubs, having more than 150 proteins transiting the foci upon environmental cues. With reports indicating PML NBs pro-viral activities, HSV-1 may exploit the dynamic interaction between ICP0 and PML-NB to harness useful factors while targeting the inhibitory core.

FT-IR Spectroscopy for the Identification of Binding Sites for Cr absorption in Bacterial Strains

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Chromium (Cr) contamination presents significant environmental and health hazards, necessitating a thorough understanding of its interaction with bacterial strains for effective remediation approaches. In this investigation, Fourier-transform infrared (FT-IR) spectroscopy was used to examine the binding sites responsible for Cr absorption in four bacterial strains (R3, R19, L2, and L30). The primary objective was to identify the functional groups involved in the binding process, thereby offering valuable insights into the mechanisms of Cr uptake. The bacterial cultures were exposed to 10 mg/L of Cr and FT-IR spectroscopy was utilized to record their spectra. The acquired spectrum underwent meticulous data analysis, encompassing peak identification and analysis. The FT-IR spectra of the bacterial strains exhibited changes in the intensity and position of specific absorption bands following Cr exposure. By means of peak identification and reference spectrum comparison, functional groups such as 1,3-disubstituted and 1,2-disubstituted aromatic Compound, alkenes ($C_2H_2R_2$) alkynes (C≡C), hydroxyls (R-CHO, R-OH), amines (C-C), and N- and P-containing functional groups (P-NH_2, P=N, P-NH), were identified as plausible binding sites for Cr absorption across all bacteria strains. This study collectively shows the effectiveness of FT-IR spectroscopy in the binding sites accountable for Cr absorption in bacterial strains. The integration of peak identification shows an analysis of the underlying mechanisms proceeding with Cr uptake. The identified functional groups hold valuable presenting information for further exploration in the development of efficient bioremediation strategies tailored for Cr-contaminated environments.

Phage Interactions with defense gene VCA0483

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Phage therapy has recently gained attention as an alternative to antibiotics due to the emergence of antimicrobial resistance. Phage are viruses that specifically infect bacterial cells and cause cell death/lysis. However, bacteria can inhibit phage infection by utilizing various molecular defense systems. Using a *Vibrio cholerae* genomic library in *Escherichia coli*, we identified a unique cosmid that protects against T2, T4 and T6 infection. Transposon mutagenesis revealed that *vca0483* is required for protection against T-even phage. I confirmed *vca0483* was sufficient for T-even protection by performing PFU counts with a series of 10 phages. To identify whether *vca0483* is protective against varying T-even phage, I screened related phages from the BASEL collection that were T-even like and identified BAS39 phage that was resistant to *vca0483* protection. An in-depth genomic analysis of BAS39 showed a unique hypothetical protein that wasn't encoded in the rest of the T-even and the T-even like phages in the collection. I am currently working on identifying whether this unique hypothetical protein can inhibit *vca0483* defense. This study will increase our understanding of how phage defense systems work, while highlighting important mechanisms by which phage can overcome defense systems to improve phage therapeutics.

Predicting transcriptional network evolution across Alphaproteobacteria

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Bacteria often use networks of transcription factors (TFs) to regulate gene expression and thereby mediate cellular decision making. Intriguingly, conserved TFs in related species can have significantly different regulatory networks due to rewiring of the genes that the TF targets, which can result in distinct phenotypic outputs. Examining how transcriptional networks evolve across species to influence bacterial fitness will help us to understand how microbes adapt to different environmental niches. We previously characterized an XRE-family TFs in *Caulobacter crescentus* known as CdxA, which was shown to bind hundreds of sites in the genome and regulated both biofilm formation and phage infection. Interestingly, homologs of *cdxA* were identified across most Alphaproteobacteria based on amino acid similarity and genome neighborhood analysis, suggesting that this transcription are ancestral in this class of bacteria. Genetic analysis indicates that *cdxA* homologs in other Alphaproteobacteria have distinct phenotypic outputs, suggesting that the transcriptional network has been rewired. To better understand how the CdxA transcriptional network has been rewired, we used the CdxA DNA binding motif from *C. crescentus* to bioinformatically predict where the CdxA homologs bind in ecologically and clinically relevant Alphaproteobacteria, including *Brucella ovis*, *Agrobacterium tumefaciens*, *Sinorhizobium meliloti*, Azorhizobium *caulinodans*, and *Caulobacter segnis RL271*. Specifically, we analyzed sequences upstream of annotated genes in each species for predicted CdxA binding sites using the FIMO motif scanning tool from the MEME suite and looked for binding sites in genes with known impacts on virulence, biofilm formation, and symbiosis.

Student version

Transcription factors (TFs) are able to impact gene expression through the direct binding of DNA. These interactions are modeled by transcription regulatory networks (TRNs). The xenobiotic response element (XRE) family of TFs has previously been characterized in *C. crescentus*. Notably, these TFs influence adhesion and biofilm formation in this species. Additionally, these TFs are highly conserved across numerous species in the *Alphaproteobacteria* class. The presence of homologs, or features evolved from a common ancestor, indicates that this family of TFs is important for regulation and may point to key similarities between species. Understanding the intricacies of TRNs allows for analysis of microbial decision making and the ability to assess microbial pathogenicity and adaptation. To examine the potential CdxA homolog binding sites across different *Alphaproteobacteria* species, we utilized the CdxA binding site motif found from *Caulobacter*: INSERT MOTIF

SEQUENCE? Using FIMO Motif Scanning tool on MEME Suite software, the CdxA binding motif was inserted with the DNA sequences from the Alphaproteobacteria species of interest: *Brucella ovis*, *Sinorhizobium meloti*, *Azorhizobium caulinodans,* and *Agrobacterium tumefacians*. These species are of interest due to virulence mechanisms (*B. ovis*) or ecological functions (*S. meloti, A. caulinodans, A. Tumefacians*). From the motif scanning, binding sites within a probability of 1E-4 were examined. These binding sites were examined in correspondence to the genes that they regulate. Considering the genes regulated by these binding sites, an emphasis was placed on their roles and how that may enhance or regulate their functions. Understanding the role of CdxA gene regulation in these organisms may provide further insight into their mechanistic functions and ability to interact with each other. If consistent binding sites are found throughout the species of interest, other species may be investigated to identify the same binding sites. Identifying CdxA regulated genes across different species of *Alphaproteobacteria* can lead to further research involving protein alignments of XRE transcription factors to compare their structural similarities and how that may impact their function to promote transcription of genes in *Alphaproteobacteria* species.

Profiling of Fecal-Derived Microbial Species in the Rouge River Using DNA qPCR Arrays

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Michigan's Rouge River has been highly polluted for years, notably with human fecal matter, affecting both the river's health and human activities. This study uses qPCR techniques to identify the most prevalent bacteria in various locations along the lower watershed, including Canton, Inkster, Wayne, Dearborn, and Detroit. Field data and water samples were collected and analyzed in the lab. After filtering the water samples, microbial gDNA was extracted and subjected to qPCR to target fecal-derived microbes. The most prevalent bacterial strains detected in nearly all samples were *Aeromonas* species and *Bacteroides* vulgatus. However, several species were entirely absent across all sampled locations. These absent species included *Campylobacter upsaliensis*, *Desulfovibrio vulgaris*, *Legionella pneumophila*, *Ruminococcus obeum*, *Salmonella enterica*, *Streptococcus suis*, and *Vibrio vulnificus*. The detection of *Aeromonas* species and *Bacteroides vulgatus* in almost all samples indicates a common and widespread contamination of these bacteria in the environment being studied. The prevalence of *Aeromonas* species is particularly significant as they are often linked to gastrointestinal issues, especially diarrhea. This suggests that the environmental samples might be influenced by fecal contamination, pointing towards potential public health concerns. Understanding the distribution and prevalence of these bacterial species can inform public health strategies and environmental management practices. This research highlights areas where contamination control measures might be necessary and helps identify potential risks associated with the presence of pathogenic bacteria in the environment.

Understanding Bacterial-Metal Interactions Analyses of ATR-FTIR Spectroscopy Data

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Fourier Transform Infrared (FT-IR) Spectroscopy was used to observe changes in the chemical structure of bacterial cells exposed to varying concentrations of cadmium (Cd). Strains R3, R19, L2, and L30 were grown on media with 0, 10, 20, and 50 mg/L of Cd. FTIR analysis revealed significant peaks corresponding to functional groups, with shifts in absorption bands and new peaks emerging as Cd concentrations increased. Common functional groups included $C_2H_2R_2$, aromatic compounds, C_2HR3, P-NH_2, $(RCO)_2O$, R-OH, P=N, C-C, P-NH_2, R_2C=NR or R_2C=NH, C≡C, RCHO, C−H, and P−NH. Unique groups to some strains were 1,4−Disubstituted aromatic compounds, Ar−H, and R_2C=O or RCOOH. Statistical analyses showed differences in IR patterns among the strains at various Cd concentrations. Significant differences ($P < 0.0001$) were observed at 0, 10, and 50 mg/L, but not at 20 mg/L ($P = 0.96$). For L2, significant differences existed between 0 mg/L and all other concentrations, which were similar to each other. For L30, 0 mg/L was different from all other concentrations, which were similar among themselves. Strain R3 showed similarity between 0 mg/L and 50 mg/L, with 20 mg/L similar to all concentrations. Strain R19 had significant differences between 0 and 10 mg/L compared to 20 and 50 mg/L. Overall, L2, L30, and R19 showed significant differences across concentrations ($P< 0.0001$), while R3 had less significant results ($P = 0.008$). These analyses suggest unique molecular responses to Cd exposure among strains. Significant IR changes reflect effective adaptation or stress responses, while nonsignificant changes may indicate limited adaptation.

Testing the Capacity of Environmental Bacteria to Control Ice Formation

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Microorganisms have evolved mechanisms to control the temperature at which ice forms, which can benefit their survival. Current knowledge of the diversity of organisms with this capability is limited. This project evaluates the ability of microbial isolates derived from cold environments to nucleate ice at warmer temperatures than pure water will freeze on its own (-40 ºC). The goals are to determine how bacterial ice nucleation will react to exposure to a range of near 0 ºC temperatures, and to investigate correlations between ice nucleation and physiological responses. To assess this, isolates were incubated at 20ºC for 48 h in liquid medium with the inoculum concentration standardized to the lowest OD600. Standardized inoculum (100µL) was dispensed into each well of a row in a 96-well plate and placed in a -4°C circulating water bath cooled with ethylene glycol solution. Every 10 minutes, the temperature was lowered by 1 degree until all culture wells were frozen. The temperature at which each well froze was recorded. The experiment was then repeated by first acclimating the original culture tubes at 0 ºC to 4 ºC and testing for changes in ice nucleation activity associated with the change in acclimation temperature every 24 hours for 120 hours. Results showed diverse responses to temperature acclimation, with some isolates nucleating ice at temperatures as warm as -5 ºC after 96 hours of acclimation at 1 ºC or 4 ºC. These results demonstrate that ice nucleation is optimized after a controlled temperature stress. These data can contribute to novel applications for managing ice formation in extremely cold environments. Additional studies on the physical properties of these isolates will refine their use in practical applications.

Assessing the antimicrobial activity of hexocannabitriol and its analogs

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Bacterial infections are a leading cause of death globally. Bacterial infections are often treated by some form of antibiotics. However, this does not always stop the infections as antibiotics stop working when the bacteria become antibiotic resistant re This has been happening with more antibiotics as the years pass. Cannabidol (CBD) is the second main component in cannabis. CBD does not have psychoactive qualities. Recently CBD has been shown to have antimicrobial properties. Hexacannabitrol is a derivative of CBD that shows auspicious pharmaceutical qualities. Our collaborators are synthesizing hexacannabitrol as well as analogs; these compounds have not yet been tested for antimicrobial activity. The structure of hexacannabitrol is similar to CBD and we hypothesized that it would also have antimicrobial properties. We assessed the antimicrobial properties of these compounds against multiple bacteria, including *Streptococcus mutans*, *Staphylococcus aureus*, and *Enterococcus faecalis.* in dilution assays to find the lowest inhibitory concentration for the hexacannabitrol and its analogs.

Defining glycerol binding to the *Mycobacterium tuberculosis* protein PPE51

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Mycobacterium tuberculosis (Mtb) has evolved to adapt its physiology to various environmental cues, including changes in pH. A key stage of infection for Mtb is adapting to survive in the acidic environment of the phagosome. When cultured on non-permissive carbon sources (e.g. glycerol) at a pH of 5.7, Mtb restricts its growth in a phenomenon known as acid growth arrest. Previously, a genetic selection was conducted to discover mutants that can grow on glycerol at acidic pH, isolating mutants of *ppe51*. These mutants exhibit a phenotype called enhanced acid growth (EAG). The *ppe51* gene encodes for the protein PPE51; three mutant variants of *ppe51* (S211R, A228D, and E215K) were identified with the EAG phenotype. Additional studies have raised the hypothesis that PPE51 functions to promote glycerol uptake across the impermeable mycomembrane. The goal of this study was to determine the biochemical interactions of PPE51 with glycerol at an acidic pH and the impacts of mutations on these interactions. These findings will allow us to better understand the mechanism of PPE51 and glycerol uptake in Mtb. We hypothesize that the wild-type and mutant variants have differential biochemical interactions with glycerol, leading to the different growth phenotypes. To achieve this goal, I have optimized the expression and purification of the PPE51 recombinant protein from *E.coli*. Current efforts are focused on defining glycerol binding to PPE51 using differential scanning fluorimetry and isothermal titration calorimetry.

Investigating the Role of EipA During Brucella ovis Infection of Macrophage-like Cells

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Brucella ovis is an intracellular pathogen that is the main cause of Brucellosis in sheep. In this research project, we studied the role of the envelope integrity protein A (EipA) in *B. ovis*. *eipA* is an essential gene in *B. ovis* therefore it cannot be deleted from the genome. In order to study the function of EipA we used a conditional EipA depletion strain. EipA is a periplasmic protein with a domain of unknown function (DUF 1134) and it is conserved throughout Alphaproteobacteria. When depleted of EipA, *Brucella* cells appear in chains and rounded as opposed to the wild-type singular coccobacilli form. A driving piece of investigation is to recognize the size and shape differences of cells with and without EipA when within host cells following infection. To model infection in human phagocytic cells, we infected differentiated THP-1 human monocytic leukemia cells. Under this study, MICA fluorescence microscopy was used to observe *Brucella* cells throughout the infection process. *B ovis* labeled with dsRed highlights the presence and shape of the *Brucella* cells within THP-1 cells. We observed wild type *B. ovis* appeared within THP-1 macrophage-like cells as its singular coccobacilli form, while EipA-depleted cells formed chains within the host cell. To see if we can detect EipA within THP-1 cells the *eipA* gene was tagged with mNeonGreen to fluoresce green when viewed under the microscope during infection. Overall, the goal of this project is to understand the role of the essential protein, EipA in *Brucella* cell morphology during the infection process of human phagocytic cells.

Siderophore Production by Multiple Metal-Resistant Bacterial Strains Isolated from Saint Clair River Sediments

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Siderophores are a major class of chelators secreted by microorganisms. While these metal chaperones are specific for iron, they also bind effectively with other metals outside the cell making them an ideal strategy for metal remediation. In this study, 45 LB and 47 R2A isolates retrieved from St Clair River sediments were screened for their ability to tolerate 8 different metals at different concentrations (10-1000 μg/ml) and their ability to produce siderophores in these metal concentrations in the culture media. Growth of the isolates was measured using a plate reader (595 nm) after 24 hours of incubation. Growth of the isolates in metals were in decreasing order from most resistant to least resistant as follows: Pb>Mn>As>Zn>Ni>Cu>Cd>Cr. The R2A isolates tended to have lower growth rates compared to the LB isolates. Siderophore production was more evident in isolates grown in Pb-containing media since the production of siderophores occurred on both LB and R2A isolates. The R2A isolates produced siderophores only when grown in the presence of Pb. In general, the data showed that the higher the concentrations of metals in the media, the less siderophores are produced by the isolates. It also appears that more siderophores were produced when metals were absent in the media because siderophores, by nature, express when there is a lack of iron in the environment. Therefore when the isolates were introduced media without metals, they express more siderophores In conclusion, we can distinguish which isolates encourage the production of siderophores and which metals and metal concentrations, inhibit it.

Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) selects for quinolone resistance in *Vibrio cholerae* DNA repair mutants

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Thiosulfate Citrate Bile-Salts Sucrose (TCBS) agar is commonly used as both a selective and differential medium for isolating marine *Vibrios*, including the aquatic human pathogen *Vibrio cholerae*. While it is valuable as a rapid and inexpensive diagnostic tool, we have observed that certain *V. cholerae* mutant strains grow poorly when cultured on TCBS agar. In particular, certain strains of DNA repair mutant *V. cholerae* are strongly attenuated for growth on TCBS agar. However, after evolving these mutants on TCBS, we identified suppressor mutations in DNA gyrase which result in not only restored growth on TCBS, but resistance to quinolone antibiotics. Our results indicate that the selectivity of TCBS regarding these DNA-repair mutants works in a similar fashion to quinolone antibiotics.

Copper Biosorption by Siderophore-producing Bacterial Strains

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Copper (Cu) is one of the most commonly selected heavy metals for industrial and agricultural applications for centuries. The accumulation of Cu in the environment has led bacteria to develop mechanisms for biosorption or resistance to Cu if levels reach toxicity. Albeit, bacteria require Cu as micronutrient to act as electron carriers, catalysts for redox reactions, and constitute an important feature of cofactor enzymes. Our research focuses on the biosorption effects of Cu on the four bacteria strains R3, R19, L2, and L30 through siderophore production. Various experiments conducted in Luria-Bertani (LB) broth or plates mixed with differing concentrations of Cu were performed to observe these effects and characteristics, such as siderophore assay, minimum inhibition concentration (MIC), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy/transmission electron microscopy (SEM/TEM). 16S rRNA gene PCR amplification was performed to conduct species identification as well. There was a positive presence of siderophores and visible traces of Cu both within and outside cells, and MIC tests showed the optimal Cu concentrations for growth. FTIR described the interaction between the cell wall and Cu. Overall, these strains have shown that these bacterial strains have the capability to adapt to Cu toxicity.

The Use of Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy to Analyze Pb-Induced Changes in Bacterial Strains

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Microorganisms can effectively remove Pb from aqueous solutions through biosorption and the functional groups in their cell walls are responsible for the binding tasks. However, the success of biosorption relies on the diversity of cell wall structures. ATR-FTIR spectroscopy was used to identify the functional groups involved in Pb uptake of four bacterial strains (R3, R19, L2, and L30) grown in different concentrations of Pb (0, 10, 100, and 500 mg/L of Pb). FT-IR profile of Pb-free bacterial strains displayed various peaks corresponding to functional groups. When the cells were treated with Pb, some functional groups shifted, and some new peaks appeared. The number of IR shifts varied between strains. More shifts were observed for R2 and R19 (10-14 IR shifts) than L2 and L30 (8 IR shifts). These functional groups include: $(RCO)_2O$, C-C, $R_2C{=}O$, RCOOH, $C{\equiv}C$, RCHO, $C{-}H$, $RO{-}H$ free, C_2HR_3, monosubstituted, and 1-3 disubstituted aromatic compounds. The appearance of new IR peaks was more evident when cells were grown at 100 mg/L Pb. These IR peaks correspond to functional groups such as $C_2H_2R_2$, C_2H_3R, R-OH, RCOCl, R-OH hydrogen bonded, $R_2C{=}NR$, $R_2C{=}NH$, $C{=}C$, $C{=}C{-}H$, $C{\equiv}C{-}H$, $P{-}NH$, $P{-}NH_2$, P=N, Ar-H, 1-2 and 1-4 disubstituted aromatic compounds. This study showed significant changes in the functional groups when exposed to Pb which might be responsible for the biosorption of Pb by the strains.

Regulation of biofilm and motility with znuABC deletion in *Vibrio Cholerae*

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Vibrio cholerae is a gram-negative bacterium known for living in aquatic environments and causing diarrheal diseases. Cyclic di-GMP is an important secondary messenger located within *V.cholerae* that controls the switch between the microorganism's motile and stationary - marked by biofilm buildup – states, with high levels leading to more biofilm formation and low levels leading to greater motility. This messenger is produced by diguanylate cyclases (DGC) and broken down by phosphodiesterases (PDE). Previous findings show that znuABC deletion in the N16961 strain results in high c-di-GMP levels and that zinc inhibits a few DGCs that help make c-di-GMP. Because of this, we intend to explore if other *V.cholerae* mutant strains with znuABC deletions – such as E7646 and C6706 – also exhibit this trend of high biofilm formation and low motility. As a control, the mutant strain E7646 Δ12 DGC – which lacks several DGCs crucial for c-di-GMP synthesis and biofilm formation – will be used. So far, we have made mutant strains of E7646 and C6706 and will perform motility and biofilm assays on all three mutant strains.

Developing a Random Bacteriophage Transposon Library

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This novel project aims to develop a strategy for creating 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 bacteria host, 2) identification of bacteriophage-specific selectable markers, and 3) isolation and propagation of transposon-containing bacteriophage. Because diaminopimelate (DAP) is an essential precursor in peptidoglycan synthesis required for the construction of bacterial cell walls, the loss of the *dapA* allele (Δ*dapA*) results in a conditional lethal phenotype in *Escherichia coli*. We engineered a plasmid (pAAL01) containing a transposable element encoding the dapA allele, which fully complements DAP auxotrophy in Δ*dapA* by restoring cell viability. By utilizing Δ*dapA* as a host and the *dapA* allele as a selectable marker, we hypothesize that bacteriophages whose genomes carry a transposon encoding *dapA* will exhibit higher fitness in the ΔdapA host compared to bacteriophages lacking this allele, thus addressing all three major hurdles to generating our desired mutant libraries. To test this, we are propagating bacteriophage on Δ*dapA* pAAL01 to facilitate transposition of the *dapA* allele into bacteriophage genomes, isolating phage progeny, and comparing the relative infectivity of pAAL01 exposed bacteriophage to un-exposed bacteriophage on a naive ΔdapA host. We are using growth curve analysis and plaque-forming unit (PFU) counts as measures of infection efficiency. Based on these results, we hope to inform the development of a universal technique for constructing bacteriophage transposon mutant libraries.

Identifying Cell Wall Genes in *Candida albicans* and *Streptococcus mutans* that form Carious Biofilms

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The human mouth is filled 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 of the most common diseases in the human mouth including cavities (caries) and periodontal disease. *Candida albicans* and *Streptococcus mutans* are among some of the microorganisms that have long been associated with dental caries. One of the major mechanisms that microorganisms use to cause caries, which can lead to infection, is multi-organismal biofilms, which can be defined as microbial communities that bind to a substrate and are encased by an extracellular matrix. This research aims to identify which cell wall genes are required for the *C. albicans* and *S. mutans* biofilm formation with the goal that these genes could be targeted with new drugs to decrease the adherence frequency. This research was conducted through plating of the mutant yeast strains and a control strain, inoculation, and spectrophotometer analysis.

Zinc and quorum sensing regulates cyclic di-GMP in *Vibrio cholerae* via a genomic island-encoded phosphodiesterase

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The El Tor biotype of *Vibrio cholerae*, a bacterial pathogen responsible for the diarrheal disease cholera, is responsible for the ongoing 7th cholera pandemic. El Tor differs from previous classical pandemic strains by two acquired genomic islands, VSP-I and VSP-II. Zur, the major repressor that regulates adaptation to zinc in bacteria, represses the *vc0512-vc0515* operon in the VSP-II genomic island in the presence of zinc. This operon encodes a cyclic di-GMP phosphodiesterase (*vc0515*). Phosphodiesterases (PDEs) degrade intracellular levels of c-di-GMP, a signaling molecule, which regulates biofilm formation and motility and contributes to bacterial infection in *V. cholerae*. We determined that the *vc0515* relative fold expression in Δzur mutants is higher than wild type, indicating that Zur represses *vc0515*. The *vc0515* upstream region includes a predicted *hapR* binding site that represses the promoter of *vc0515*. HapR, the quorum sensing central regulator in *V. cholerae*, is induced at high-cell density (HCD). We observed that *vc0515* expression was induced at low-cell density and repressed at HCD, suggesting HapR represses *vc0515*. Genome analysis of geographically diverse 7th pandemic strains showed that the *vc0512-vc0515* operon and *hapR* binding sites are prevalent in several El Tor strains, which indicates evolutionary significance and importance for adaptation. Several EAL phosphodiesterases are present in *Vibrio cholerae*, which collectively regulate intracellular zinc levels. We are currently investigating the roles of other phosphodiesterases and the impact of zinc on their regulation influencing cyclic di-GMP levels. Our results highlight the importance of zinc as a signal in regulating cyclic di-GMP levels in *V. cholerae*.

Mechanism Cd Absorption and Removal by *Klebsiella* sp. and *Raoultella* sp. in Aqueous Solutions

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Due to urbanization and pollution, cadmium (Cd) is widely present in aquatic environments and is causing toxicity to aquatic organisms. Microorganisms have developed resistance to Cd and mechanisms remove Cd through the absorption process. However, the mechanism of Cd biosorption by bacteria in aqueous solutions is still not well understood. Thus, the goal of this study was to better understand the mechanism and how bacteria play a role in the removal of this metal from aqueous solutions. Experiments such as minimum inhibitory concentration (MIC) to Cd, siderophore production in the presence of Cd, SEM (scanning electron microscopy), and TEM (transmission electron microscopy) was performed on four bacterial strains (R3, R19, L30, and L2). The MIC of the R3 and R19 strains were lower (20 mg/L) than the L2 and L30 strains (75 mg/L). Siderophore production was observed at 20 mg/L for strain L30, at 50 mg/L for L2, L30, and R19, and at 10 mg/L for R3. No siderophores were produced in strain R3 in the presence of Cd at any given concentration. TEM showed images that demonstrated that among the eight metals examined, Cd is the most dominant metal found not just on the cell wall but also in the cytoplasm, suggesting that the strains have mechanisms to transport Cd within the cell. The study provides a comprehensive understanding of Cd-bacteria sorption reactions as well as practical applications in the remediation of heavy metals.

Diverse Aminoglycoside Gene Cassettes *(aad*A1, *aad*B, and *aac*A4) in Class 1 Integrons in *Escherichia coli* Strains Isolated from Three Urban Watersheds

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Gene cassettes within class 1 integrons in bacterial chromosomes are crucial for spreading antibiotic resistance. We analyzed 66 *E. coli* strains isolated from three watersheds for class 1 integrons with aminoglycoside resistance genes using PCR amplification for class 1 integron variable regions, followed by sequencing with 3CS and 5CS primers. Of the 66 strains, 43 contained Int1: Rouge River (23 strains), Lake Saint Clair (11 strains), and Saint Clair River (9 strains). Sequencing and analysis of these Int1-containing strains revealed the presence of 11 aminoglycoside gene cassettes. The *aac*A4 gene cassette exhibited resistance to multiple aminoglycosides, such as gentamicin, tobramycin, and streptomycin, suggesting it may be linked to several resistance mechanisms through horizontal gene transfer (HGT). Certain combined cassettes, such as '*aad*A1-*aac*(3) IV', '*aad*A1-*aac*A4', and '*aad*B-*aac*A4-*aac*(3) IV', exhibited a wider range of drug resistance, illustrating the complexity of antimicrobial resistance mechanisms within these strains. For instance, the '*aad*A1-*aac*(3) IV' cassette provides resistance to streptomycin, rifampin, tobramycin, and nitrofurantoin, with some strains also resistant to ampicillin, ciprofloxacin, and ceftriaxone. However, the lack of resistance to chloramphenicol and imipenem, and limited resistance to trimethoprim/sulfamethoxazole, suggests potential therapeutic vulnerabilities. These findings suggest that class 1 integrons are likely responsible for antibiotic resistance in the *E. coli* strains isolated from the three watersheds. The selection of specific gene cassettes within these integrons occurs over time, indicating a dynamic process where certain resistance genes are favored under selective pressures, highlighting the critical role of class 1 integrons in the dissemination and persistence of antibiotic resistance.

Does the way *Candida auris* differs from *Candida albicans* in one protein family relate to its drug resistance or emergence as an urgent threat to human health?

Presenting Author: Carson Noyes

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Candida auris is a fungus and an opportunistic pathogen that is inherently drug resistant. There are increasing cases of *C. auris* infections that are causing deaths in immunodeficient patients. *C. auris* is closely related to a more studied microbe, *Candida albicans.* We have found that overexpressing the gene *PHO15* in *C. albicans* can impair the organism's ability to filament or create biofilms in certain growth conditions. This study aims to clone the *C. auris* ortholog of gene *PHO15* and over-express it in *C. albicans.* Then, analyze the change in morphology and antifungal resistance to see if the *C. auris* ortholog gene behaves similarly in *C. auris*, furthering the understanding of the microbe. Preliminary results indicate that the *PHO15* ortholog gene conserves some function between *C. auris* and *C. albicans* because overexpression of both versions reduces hypha formation in YPD medium and increases tolerance to caspofungin.

Electron Microscopy Techniques and Energy Dispersive X-ray Spectrometry as Tools to Characterize Biosorption of Lead by Bacterial Strains from an Urban Watershed

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For many bacteria, biosorption is a mechanism that allows heavy metals like lead (Pb) to be removed from bodies of water. To understand the mechanism of this process, this study was conducted to determine the biosorption capacity of Pb uptake of two bacterial strains, *Klebsiella sp.* (R19) and *Raoultella planticola* (L30), isolated from the St. Clair Lake. Multiple techniques were employed to understand the biosorption process and the structural and biochemical characteristics of the strains, including: FTIR spectroscopy, STEM-EDS assay, MIC assay, and siderophore assay. The FTIR analysis indicated that biosorption of Pb is achieved through binding on the cell wall's functional groups. The STEM-EDS analysis showed that biosorption of Pb can also occur via extracellular polymeric substances (EPS) secreted from microbes as well as intracellularly. Both of the strains were resistant to Pb as both required a relatively high concentration of Pb before showing signs of growth inhibition (MIC = 800 mg/L) and were also observed to produce more siderophores with increasing Pb concentrations. The EPS and cell wall facilitated ion exchange and metal chelation-complexation by virtue of the existence of ionizable functional groups such as carboxyl, sulfate, and phosphate present in the protein and polysaccharides on the cell surface. The siderophores facilitated the accumulation of Pb in the cytoplasm. This highlights the potential application of bacteria for bioremediation of Pb from multiple metals through biosorption and the use of STEM-EDS as a powerful tool to determine the location of Pb in the cell during Pb uptake.

Biosorption mechanisms of metal removal by locally isolated bacterial strains

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Rapid industrialization and urbanization have resulted in widespread contamination of metals in aquatic ecosystems. In view of this, the present study examines the metal tolerance and biosorption characteristics of four bacterial strains isolated from Saint Clair River sediments. These strains were found to remove metals (As^{3+}, Pb^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cr^{6+} and Ni^{2+}) in mono- and multi-metal solutions. While *Klebsiella* sp. R3 and *Klebsiella* sp. R19 were less tolerant to metals than *Serratia* sp. L2 and *Raoultella* sp. L30, they demonstrated the ability to efficiently remove more metals. This result suggests that the efficiency of metal removal does not rely solely on metal tolerance but also on the cation binding ability of the strain. Metal resistance was found to be associated with decreased uptake and/or impermeability, which reduced the overall metal uptake of strains L2 and L30. FT-IR analyses indicated that strains R3 and R19 strains possess more accessible carboxyl and amide functional groups than L2 and L30, which are the most important metal binding sites. SEM analysis showed reduction in size and changes in cell morphology demonstrating the toxic effects of the metal. The cells were also aggregated together. STEM results indicated that the metals were fixed at the cell surface and in the cytoplasm. This study confirms that the simultaneous presence of an aqueous solution may cause a mutual inhibition in the adsorption of each metal to the EPS resulting in reduced metal uptake emphasizing the need to select specific bacterial strains for a given metal-containing effluent.

Investigation of an innovative microplate-based method to study coaggregation between oral bacteria

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Background and aim: Coaggregation, the recognition and adhesion of genetically distinct bacteria, is well documented to occur between oral bacteria and is proposed to contribute to dental plaque development. Many methods to detect coaggregation between oral bacteria rely on methods that are semi-quantitative, logistically challenging to perform, or lacking high throughput. The aim of this work was to evaluate an innovative 24-well microplate-based method, previously used to evaluate coaggregation between freshwater bacteria, to quantitatively measure coaggregation using two types of laboratory buffer. **Methods:** The coaggregating oral bacteria *Streptococcus gordonii* DL1 and *Actinomyces oris* T14V were grown in Schaedler broth at 37°C in 5% CO2 and suspended in "coaggregation buffer" or buffered KCl prior to coaggregation assays. Coaggregation ability was subsequently evaluated in a tube-based visual aggregation assay, a cuvette-based spectrophotometry assay, and a recently published novel spectrophotometric-based microplate-based assay using a 24-well microplate containing dome (convex) - shaped wells. **Results:** *S. gordonii* DL1 and *A. oris* T14V demonstrated strong coaggregation ability using the visual tube-based assay and the microscopic assay. These results were similar when suspending cells in both coaggregation buffer and the buffered KCl. Using the cuvette-based method, compared to coaggregation buffer, coaggregation between this pair of bacteria in buffered KCl yielded the greatest change in optical density. Also, for the microplate method, buffered KCl yielded the greatest change in optical density. **Conclusions:** All the assay methods demonstrated that coaggregation occurred between *Streptococcus gordonii* DL1 and *Actinomyces oris* T14V. However, as opposed to coaggregation buffer, bufffered KCl was most suited to detecting coaggregation, especially using the microplate assay. Given the quantitative nature of the microplate method (compared to the visual aggregation assay), the potential of high-throughput studies (compared to using cuvettes), there are clear benefits to using modified microplates with dome shaped wells to study coaggregation between oral bacteria.

Antibiotic Resistant Gene Profiles in an Urban Watershed

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In recent decades, environmental pollution has increased, with pollutants such as runoff from farms entering rivers and potentially inducing antibiotic-resistant mutations in microorganisms. This study aimed to analyze the presence of 87 antibiotic-resistant genes in microbes isolated from 6 different sites (Canton, Rouge Park, Venoy, Dearborn, Inkster, and Ford Field) along the Rouge River. Water samples were collected from these sites and filtered to isolate the microbial content. Genomic DNAs (gDNAs) of the microbes on sterivex filters were then isolated, extracted, and quantified to ensure sufficient gDNA (~500 ng) for quantitative PCR (qPCR). The qPCR analysis provided Ct values for each antibiotic-resistant gene (ARGs) at respective sites. Statistical analysis highlighted differences, particularly the Inkster site, which exhibited a significantly different mean compared to other sites. The cluster analysis indicates closer similarity between the Dearborn and Inkster sites, although all sites shared overall similarities. All sites showed presence of ARGs, Venoy site leads with the highest percentage (30%) with Rouge Park site the lowest (9%). The consistent presence of fluoroquinolone-resistant gene AAC (6)-Ib-cr across all sites underscores the widespread nature of certain ARGs in this urban watershed. However, the absence of some ARGs, such as those for erythromycin, suggests a complex and varied resistance profile. These findings underscore the need for continued monitoring and further studies to assess whether the levels of ARGs increase over time. This study provides compelling evidence of the significant presence of ARGs in the Rouge River, emphasizing the impact of environmental pollution on microbial resistance.

Identification of Gene Function in the *Mycobacteria* Phage Xeno

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Bacteriophages are viruses that target bacteria. The demand for studying bacteriophages has increased due to it's importance within the field of therapeutic phages. Because of the bacteriophages' high specificity in aiming for their target bacteria,this process can help us develop more effective treatments for bacterial infections. One limiting factor to understanding and developing better phage therapy is that only 25 % of sequenced genes have a known or even hypothetical function. The research will consist of molecular cloning, phenotypic assay, and interaction assay. So far, our group has built the foundation of identifying the functionality of these genes within the phage Xeno through processes such as polymerase chain reaction, gel electrophoresis, chemical transformations, and isothermal assembly.
Xeno is a siphovirdae bacteriophage that targets Mycobacterium smegmatis. Its genome is 42395 nucleotides long and was calculated to contain 69 different genes.
We have tested 30 out of the 69 genes, with 18 out of the 30 tested being functional.
The purpose of this research in identifying the functionality of the gene, is to proceed in completing the cytotoxicity, as well as the defense assays to begin the cloning required for the hybrid experiments.

SEM-EDS and TEM-EDS Analysis of Zn Biosorption Characteristics of *Klebsiella* sp. and *Raoultella* sp.

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This study investigates the biosorption characteristics of zinc (Zn) by two bacterial strains, *Klebsiella* sp. and *Raoultella* sp., using Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDS) and Transmission Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (TEM-EDS). The biosorption potential of these bacterial strains is of particular interest due to their potential application in bioremediation and environmental sustainability. The experimental procedure involved the cultivation of the strains in media enriched Zn-enriched growth media, followed by harvesting and preparation of the biomass samples. SEM-EDS was utilized to examine the surface morphology and elemental composition of the bacterial cells before and after Zn biosorption, enabling visualization of the binding sites and distribution of the adsorbed Zn. TEM-EDS analysis provided further insights into the cellular internalization of Zn and its intracellular distribution. Preliminary results indicate that both *Klebsiella* sp. and *Raoultella* sp. exhibited significant Zn biosorption capabilities, with variations observed in their biosorption mechanisms and patterns. The SEM-EDS analysis revealed the Zn precipitates on the bacterial cell surface, while TEM-EDS uncovered evidence of intracellular Zn localization within distinct organelles. This study contributes to better understanding of the Zn biosorption process, shedding light on the potential mechanisms involved in their interaction with Zn. These results have important implications for the development of eco-friendly bioremediation strategies and open avenues for further exploration of the biotechnological applications of these bacterial strains in environmental remediation. However, more comprehensive investigations are warranted to elucidate the underlying molecular and cellular processes driving the observed biosorption characteristics.

Metabolic Function and Surface Waters and Sediments in Urban River and Lake Watersheds

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Microbial communities are a fundamental part of aquatic ecosystem's biochemical processes. The goal of this research is to compare how different carbon sources are utilized by the microbial communities in the surface water and sediments from Lake Saint Clair and the Saint Clair River. To achieve this, specific sites within the river (SC1, SC2, and SC3) and lake (LC1, LC2, and LC3) were selected for the Biolog Ecoplate assay. There was a significant utilization difference between surface and sediment communities ($P \leq 0.0001$) but no significant carbon use difference between the river and lake communities (P=0.957). Surface water and sediment communities showed the highest average carbon utilization in the polymer and carbohydrate groups, respectively. Within these groups, surface water communities used D-Xylose, I-Erythritol and Glucose-1-phosphate the least, while the sediment communities used D-Xylose, I-Erythritol the least. Surface water communities utilized Tween 80 (>6%) and L-Asparagine (>6%) more, whereas sediment communities more D-mannitol (>10%) and glycogen (~3-8%).The observed carbon utilization patterns align with established behaviors; oxygen-rich surface communities prefer simpler carbons like Tween 80 due to rapid metabolism, while anaerobic sediment communities favor more complex carbons due to slower metabolism. The data has applications for monitoring impacts of pollution on aquatic ecosystems. Sudden shifts in the carbon utilization pattern implies fundamental changes in biogeochemical cycling of the ecosystem which could impact the system's resiliency.

Identifying novel phage defense systems in *Vibrio cholerae*

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Since the discovery of antibiotics, antibiotic resistance has been rapidly evolving, leading to one of the greatest public health threats. Phage therapy 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 infection. To develop phage therapies, we must understand the mechanisms of phage defense systems. To address this, we use *Vibrio cholerae* as a model due to its constant interaction with phage in its environment. *V. cholerae* encodes two pathogenicity islands (VSP-1 and VSP-2) where only two phage defense systems known as CBASS and AvcID have been identified. Thus, we hypothesize that *V. cholerae* still harbors unknown phage defense systems. 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 *vca0483* was involved in protection against T2. After creating an overexpression plasmid, we found that *vca0483* was sufficient for protection. Curiously, we found that overexpression of *vca0483* is toxic to the cell. A prior study showed a link between *vca0483* and *vqmA*, a post-transcriptional inhibitor of biofilm formation in *V. cholerae*. I am currently investigating the mechanism and regulation of *vca0483*. Overall, understanding the mechanisms bacteria have evolved against phage infections is paramount to developing more effective phage therapies.

Exploring *Vibrio cholerae* Biotype Differences Using the Zebrafish Model

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Cholera is an acute diarrheal disease which largely affects areas of the world characterized by poor sanitation and lack of adequate infrastructure. *Vibrio cholerae* are the gram-negative bacteria that emerge seasonally in endemic areas to cause cholera outbreaks. *V. cholerae* as a species includes both pathogenic and nonpathogenic strains that vary in their virulence gene content and surface O antigens. The O1 serogroup that can cause epidemic cholera is further subdivided into two biotypes, classical and El Tor. The El Tor strains have globally displaced the classical biotype both in clinically affected populations as well as the environment. They also persist significantly longer in the host even after the symptoms have cleared. We wanted to understand the host factors that may contribute to this prolonged survival phenotype.

Zebrafish (*Danio rerio*) are useful as a natural host model for *V. cholerae* infection as the entire disease cycle can be recapitulated in the presence of an intact intestinal microbiome and immune responses. Previous unpublished studies from our lab suggest that *V. cholerae* El Tor strains can colonize adult zebrafish for up to two weeks, but classical strains get cleared by 3 days post-infection (dpi). Although both biotypes caused dysbiosis of the intestinal microbiome during infection in adult zebrafish to some extent, the microbiome composition reverted back by 3 dpi in the fish infected with the classical biotype but not the El Tor. We hypothesized that a combination of gut microbiome perturbation and differential immune responses to classical and El Tor biotypes contribute to the colonization duration differences observed in zebrafish.

When larval zebrafish hatch, they have a full arsenal of innate immune factors. However, it takes about 4-6 weeks for development of the adaptive immune system. Therefore, using larvae for this experiment will help us assess innate immune response independently of the adaptive immune responses. This would also give us an insight into the duration of *V. cholerae* colonization for larvae and how their microbiome develops as a result of infection.

Zebrafish larvae were infected via immersion at 5 days post-fertilization (dpf), since that is when the gut lining opens, and they are able to take up exogenous substances. Every day, the excretion water was collected, and appropriate dilutions were plated to determine the number of colony forming units. This allows us assess *V. cholerae* shedding over time, without having to euthanize too many larvae. Washing, feeding and complete water changes were performed every 24 hours for 90 days or until *V. cholerae* was no longer detected. Larval homogenate samples were also collected on different days during the course of the infection for microbiome analysis.

We observed that the larvae were able to clear the classical infection within 32 dpf/27 dpi whereas the El Tor infection cleared around 55 dpf/50 dpi. This timeline for clearance is consistent with previously conducted rounds of the long-term colonization experiment.

In Vivo redox monitoring of LctR in *Campylobacter jejuni*

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The foodborne pathogen *Campylobacter jejuni* is estimated to cause 1.5 million cases of gastroenteritis each year, with the rise of antibiotic-resistant strains posing a significant threat. In our previous study, we showed that *C. jejuni* utilizes lactate as a carbon source, and the lactate uptake and metabolism (*lctP*) operon is essential for *C. jejuni* growth during the acute stage of infection in ferrets. We also found a redox regulator *LctR* which can regulate the expression of lctP in presence and absence of oxygen. To evaluate the redox state of *LctR* in vitro, *LctR* extracts were incubated with or without the reducing agent DTT for 60 minutes at 37°C, followed by separation on a 10% SDS-PAGE gel using a non-reducing loading buffer. Conformational changes in the protein were assessed through Coomassie staining or western blot analysis using an anti-*LctR* polyclonal antibody. Both methods revealed that under reducing conditions, *LctR* exists as a monomer (~37.5 kDa), whereas under oxidizing conditions, it forms higher molecular weight species, suggesting dimerization or oligomerization. For *in vivo* assessment, *C. jejuni* were cultured under aerobic, microaerobic, and anaerobic conditions, then lysed via sonication. Proteins were precipitated by adding trichloro acetic acid to the cell lysates, separated by SDS-PAGE, and analyzed via western blot. The *in vivo* western blot result showed that *LctR* exists only at ~70 kDa and ~110 kDa, indicating that *LctR* does not exist as a monomer *in vivo*, but mainly in a dimer and oligomer state. AlphaFold predictions show the presence of three surface exposed cysteine residues in *LctR* that are likely to allow the protein to oligomerize through the formation of disulfide bridges. Based on this data, we propose a model for the redox regulation of the *lctP* operon, where *LctR* binds to the *lctP* promoter as a reduced dimer under low-inflammation conditions in the gut and dissociates when oxidized as inflammation and oxygen levels rise. Future research will focus on identifying the role of open cysteine residues in *LctR* oligomerization under oxidative conditions and the effects on *lctP* regulation when these residues are removed.

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.

Norovirus alters host metabolism for efficient virus replication

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Human noroviruses (HNoVs) are single-stranded, positive-sense RNA viruses that are the leading cause of acute non-bacterial gastroenteritis worldwide. Despite the devastating public health impact of HNoV infections, neither vaccines nor therapeutics exist, underscoring the need for further investigations to better understand NoV biology. Viruses hijack host metabolic pathways, creating more favorable intracellular environments to ensure optimal reproduction. However, little is known about the ability of NoVs to reprogram host metabolism. Our published metabolomic and quantitative flux analysis revealed that murine norovirus 1 (MNV-1) upregulates and relies on glycolysis and glutaminolysis for efficient virus infection. Mechanistic investigations demonstrate that the activity of glutaminase, the rate limiting enzyme in the glutamine catabolic pathway, is upregulated during MNV infections and these changes are mediated via the non-structural protein NS1/2. Importantly, studies in intestinal epithelial cells revealed that MNV upregulates central carbon metabolism in CD300lf-expressing murine intestinal enteroids (MIEs) and in murine tuft cell cultures. Similar to infected macrophages, MNV's efficient reproduction in MIEs depended on both glycolysis and glutaminolysis. Extension of metabolic investigations to HNoV, revealed its efficient replication in human intestinal enteroids (HIEs) also required both glycolysis and glutaminolysis. Early mechanistic investigations indicated that HNoV NS1/2 also increases glutaminase enzymatic activity. Current studies aim to determine the full range of metabolic alterations in HNoV-infected intestinal epithelial cells and uncover the underlying mechanisms. Collectively, these data demonstrate the importance of multiple host metabolic pathways for productive NoV infection and the ability of a noroviral protein to alter the activity of a metabolic enzyme.

Investigating the Antimicrobial Resistance Profiles and Genetic Diversity of Clinical *Shigella* spp. Isolates from Michigan

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Shigella spp. are bacteria that are the causative agent of shigellosis, a diarrheagenic illness. Symptoms of shigellosis include diarrhea, fever, and stomach pain, and can range from mild to severe, with certain species of *Shigella* causing deadly disease. The global burden of disease is immense, with 80-165 million infections and 600,000 deaths each year, making shigellosis a global health concern. Furthermore, the rapid rise of antibiotic resistance (ABR) in *Shigella* spp. associated with clinical infections, including the emergence of multi- and extensively drug-resistant isolates, is a major threat to public health. Therefore, the Centers for Disease Control and Prevention (CDC) added *Shigella* to their National Antimicrobial Resistance Monitoring System (NARMS) to collect data about the prevalence and distribution of ABR *Shigella* infections. NARMS collaborates with state health departments, which submit every 20th *Shigella* spp. isolate they receive to the CDC for ABR testing. Therefore, it is likely that NARMS underestimates the actual burden of ABR *Shigella* spp. in the US. The Michigan Department of Health and Human Services (MDHHS) Bureau of Laboratories began collecting *Shigella* spp. isolates from patients in Michigan with shigellosis for further analysis. We received 83 of these isolates to phenotypically and genotypically characterize to assess whether the NARMS estimates of ABR in *Shigella* reflect the current state in Michigan, as well as determine the genetic diversity present in Michigan's *Shigella* population. To do this, we obtained whole genome sequencing data for each isolate and searched the genomes for ABR genes, and determined the susceptibility of the isolates to 13 antibiotics that NARMS monitors. Overall, we found that ABR was more prevalent among the Michigan isolates than what is reported by NARMS. Furthermore, the isolates carried a broad spectrum of ABR genes in their genomes and belonged to two distinct genotypes. These data highlight the importance of continuous surveillance efforts to monitor the population of *Shigella* linked to clinical infections at both the state and national levels.

α-ketoglutarate and Citrate Transporters Regulate *C. jejuni*-Mediated Commensal Colonization of Chickens

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The foodborne pathogen *Campylobacter jejuni* (*C. jejuni*) is the leading bacterial cause of gastroenteritis globally, primarily transmitted through poultry. As a common inhabitant of the chicken gut, it makes poultry meat a major source of human campylobacteriosis. *C. jejuni* burden in chickens must be reduced to minimize transmission; furthermore, alternatives to prophylactic antibiotic use in chickens must be developed to combat the rise of multidrug-resistant strains. Developing such approaches requires a deeper understanding of *C. jejuni* colonization strategy in the chicken gut. Previously we identified an α-ketoglutarate transporter (*kgtP*) mutant has a fitness defect in chicken gut by transposon mutagenesis analysis, suggesting that α-ketoglutarate (AKG) acts as a major carbon source during *C. jejuni* colonization. To validate this observation we created a *kgtP::kan* mutant strain by insertional inactivation with kanamycin cassette. When grown in AKG+ minimal media, we observed a significant growth defect in the mutant compared to the WT across all growth phases. Partial growth recovery in the late phase suggests the presence of an alternative AKG transporter. We identified citrate transporter CitA in *C. jejuni* similar to citrate transporter CitP of *Lactobacillus lactis* which imports AKG. A double mutant *C. jejuni* strain (*kgtP::kan/citA::cm*) in AKG+ minimal media had a complete growth defect. We completed a competition assay between WT and three mutants (*kgtP::kan*, *citA::cm,* and *kgtP::kan/citA::cm*). These mutants demonstrate a competition defect when compared to wild type strain on day seven of post-infection. Analysis of the KgtP structure identified twelve transmembrane domains, a probable AKG binding site, and a periplasmic region where iron may serve as a stabilizing cofactor. The evidence suggests AKG's significance to growth and poultry colonization has exerted selective pressure for *C. jejuni* to develop redundant uptake mechanisms.

Regulation of the *Legionella pneumophila* effector SidL by its Cognate Metaeffector

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Legionella pneumophila is a Gram-negative, freshwater, intracellular bacterial pathogen. It has coevolved with amoeba in the environment for billions of years, and the virulence strategies employed by *L. pneumophila* to parasitize amoebae permits its replicate in mammalian macrophages, causing Legionnaires Disease or Pontiac Fever. To successfully infect host cells, *L. pneumophila* utilizes over 300 effector proteins to establish the Legionella-containing vacuole, a replication-permissive compartment evades fusion with lysosomes. *L. pneumophila* also utilizes proteins to bind and regulate these effector proteins, called metaeffectors. Previous work has shown that disruption of the interaction between an effector/metaeffector pair can lead to attenuation of *L. pneumophila* intracellular replication. Effector/metaeffector pairs have a central role in subverting host mRNA translation, freeing needed amino acids for *L. pneumophila* replication. One predicted effector/metaeffector pair is SidL/LegA11, an effector protein that inhibits host translation, and LegA11, SidL's predicted metaeffector. We utilized protein pulldowns, *in vitro* translation assays, and actin polymerization assays to show that LegA11 is a metaeffector of SidL. We show that LegA11 regulates SidL, via a direct protein-protein interaction and that disruption of this interaction prevents LegA11 regulation of SidL. We found that LegA11 leads to a fitness disadvantage in *L. pneumophila* in macrophages. Δ*legA11* mutants show a fitness advantage in primary C57BL/6 *Nlrc4*$^{-/-}$ bone marrow-derived macrophages (BMDMs) while no fitness advantage is seen in *Acanthamoeba castellanii*, a natural host for *L. pneumophila.* Different phenotypes in BMDMs and amoeba suggest that LegA11 may be involved in effector-triggered immunity. Future work will uncover the mechanism and role by which SidL and LegA11 regulate mRNA translation and influence L. pneumophila host-pathogen interactions.

Phosphate restricts *Mycobacterium tuberculosis* growth on lactate at acidic pH

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Mycobacterium tuberculosis (Mtb) has evolved to replicate in the acidic environment encountered during growth in macrophage phagosomes. Mtb cultured in minimal medium at acidic pH arrests growth when provided specific non-permissive single carbon sources, including glycerol and lactate. We previously selected for mutants with a gain-of-function enhanced acidic growth (EAG) phenotype when cultured on glycerol as a sole carbon source. The selected mutants had missense mutations in *ppe51,* a gene that promotes glycerol uptake to enable growth. However, none of the *ppe51* EAG mutations enabled growth on lactate, leading us to hypothesize that a different mechanism is driving growth arrest on lactate at acidic pH. To test this hypothesis, a genetic selection was conducted to identify loss-of-function transposon mutants that could grow on lactate at acidic pH. Four of the selected mutants had transposon insertions in *phoT* and one had an insertion in *pstC2*. Both *phoT* and *pstC2* encode for components of a phosphate ABC transporter. When tested for growth on a panel of non-permissive carbon sources, *phoT* mutants only grew in lactate. Dose-responses to phosphate were evaluated in both WT and the *phoT* mutant, when grown on lactate as a sole carbon source at pH 5.7. The *phoT* mutant was insensitive to phosphate, growing at all tested phosphate concentrations, while WT Mtb and the *phoT* complemented strain grew well at low phosphate and arrested growth on increasing amounts of phosphate. RNA sequencing was performed and indicates that type VII secretion and other phosphate transport systems are upregulated in the *phoT* mutant. These results taken together suggest that phosphate uptake is associated with lactate utilization. Notably, higher concentrations of lactate are observed in activated macrophages and the Mtb granuloma, and we propose a model where integrating adaptations to acidic pH and carbon source utilization promote Mtb pathogenesis.

Antibacterial effect of *Oregano vulgare* essential oil on multidrug resistant *Campylobacter jejuni* and isolation of the active antibacterial compound.

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Campylobacter jejuni is the foremost cause of foodborne bacterial gastroenteritis in humans worldwide. *C. jejuni* exhibits significant strain-to-strain variability, which can result in differences in virulence potential, clinical presentations, and antimicrobial susceptibility. Emergence of multidrug-resistant C. *jejuni* due to antibiotic misuse has resulted in ineffective treatment outcomes and escalated healthcare expenses, necessitating the search for alternative treatments. One approach is to explore active compounds in medicinal plants. Medicinal herbs derived from nature are utilized globally, particularly in developing nations. Our study is aimed at determining the *in vitro* antimicrobial effect of *Oregano vulgare* essential oil on MDR *C. jejuni* and isolation of the active antibacterial compound. We are investigating five isolates of *C. jejuni*, all from humans; two multiple-drug resistant (strains 16607 and 16398) and three antibiotic sensitive (strains 11168, 81-176 and 16420). We screened *Oregano* essential oil (EO) against these isolates by disc diffusion, followed by broth microdilution to determine MICs and MBCs. The disc diffusion zone of inhibition (ZOI in mm) assay showed complete inhibition of all five strains at 10^-1 to 10^-3 EO dilutions. Subsequently, at 10^-4 EO dilution, the strains recorded 43-75 mm ZOI. *Oregano* EO exhibits a wide range of activity against the *C. jejuni* isolates in the microdilution assay, with 0.23 µg/mL MIC and 0.46 µg/mL MBC for strain 11168. Work is still ongoing for the other strains, as well as determining the mechanism of killing and characterizing the secondary metabolite(s) present in the plant with active antibacterial potential.

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

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Bacterial RNP-bodies (BR-bodies) are biomolecular condensates that play a crucial role in post-transcriptional gene regulation by organizing several RNA metabolic and regulatory processes. The mRNA decay enzyme RNase E (Rne) contains a C-terminal intrinsically disordered region (IDR) that is required for BR-body assembly. The IDR is conserved across Alphaproteobacteria including *Brucella* species, which cause the widespread zoonotic disease brucellosis. Here, we investigate whether *Brucella* forms BR-bodies and explore their role in stress responses and infection biology. *Brucella ovis* cells expressing fluorescently labeled RNase E (*rne*-msfGFP) or Rne lacking the IDR (*rne*ΔIDR-msfGFP), were imaged using phase contrast and fluorescent microscopy. The cells were then exposed to different environmental conditions and pictures were taken at 3 and 24 hours to measure the variation in formation of fluorescent foci. To assess the contribution of the IDR to envelope stress survival, wild type (WT) and ΔIDR *B. ovis* cells were plated in a dilution series on TSA blood agar +/- stress conditions, and colony forming units (CFUs) were enumerated. In a macrophage infection model, a gentamycin protection assay was performed on THP-1 macrophage-like cells infected with WT and ΔIDR *B. ovis*. The THP-1 cells were then lysed, and *B. ovis* CFUs were enumerated at 2, 24, and 48 hours post infection. Through fluorescence microscopy, we show that Rne forms foci within *B. ovis* cells, while a ΔIDR mutant does not. We also demonstrate that the fluorescent profile of rne-msfGFP fluctuates over time in response to environmental changes. Additionally, a *B. ovis* ΔIDR mutant is more sensitive to envelope stressors compared to WT and has fewer CFUs within THP-1 cells. Together, these results demonstrate that the IDR domain of RNase E is sufficient for the formation of BR-bodies in *B. ovis* and that they are dynamic. Moreover, we illustrate that formation of BR-bodies contributes to cell survival under envelope stress and increases fitness in the intracellular niche. Our data provide evidence that BR-bodies play a critical role in *Brucella* physiology and infection biology.

Functional Differences Provides Insights into the Pathogenic Potential of *Escherichia marmotae*

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Escherichia marmotae is an emergent member of the *Escherichia* cryptic clades that has gained scientific prominence recently due to its association with human infections, including human sepsis, and urinary tract infections (UTIs). *E. marmotae* was called "cryptic" because it could not be distinguished from *E. coli* by standard diagnostic tests, existing matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), except that it was clearly different genetically by 10%! We hypothesize that *E. marmotae*, like *E. coli*, has unique adaptations that enable some strains of *E. marmotae* to cause human infections.

Here, we investigated potentially different properties of *E. marmotae* compared to *E. coli*, including the presence of virulence and antibiotic resistance genes, temperature-sensitive motility, and biofilm formation that distinguish *E. marmotae* from *E. coli*. We developed an identification technique using qPCR primers and TaqMan assays targeting the *uidA* and *uidB* genes, as well as MALDI-TOF MS biomarker peaks in the 7260-7268 m/z range, bridging a critical diagnostic gap. Our findings showed that *E. marmotae* shares infection-related genes with *E.coli*, such as motility and biofilm formation, which may contribute to its pathogenicity. However, these traits were temperature-dependent, with increased motility and biofilm production observed at 28°C compared to 37°C. Reduced expression of motility-associated genes *motA, flhD, fliA, fliC*, and *flgM* was observed in *E. marmotae* compared to *E.coli* . Variant calling analysis (VCA) identified at least two missense mutations in genes related to motility and biofilm formation, a possible reason for differences in functional impacts and phenotypic traits.

In conclusion, *E. marmotae* exhibits functional differences in key pathogenic traits, with some motility-related genes possibly being nonfunctional, raising questions about its role as a pathogen.

Implementing a CRISPR Gene Editing System in *Sinorhizobium meliloti* to Investigate Transport of Extracellular Lanthanide

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While primarily recognized for its well-studied nitrogen-fixing relationship with alfalfa, *Sinorhizobium meliloti* was recently discovered to possess a methylotrophic characteristic involving the expression of a methanol-metabolizing enzyme, XoxF. XoxF, a periplasmic, Lanthanide (Ln)-dependent methanol dehydrogenase, is unique in its utilization of Ln as a cofactor. Prior to characterization of this protein, Ln, a rare earth-element, was not known to have a role in any biological processes. The discovery of the *xoxF* gene in *S. meliloti* has quickly led to subsequent research in outlining the methanol metabolism pathway in this species, however, it is unclear how extracellular Ln is transported into the cell. Through RNAseq analysis, several genes which encode an ATP-binding cassette (ATP)-transporter have since been identified with potential roles in Ln transport. To confirm this transport pathway, the editing efficiency of CRISPR/Cas12k, a transposon-mediated system, will be harnessed via the CRISPR/Cas12k Genetic Engineering Toolkit (C12KGET). Once constructed with the custom gene-targeting sgRNA fragment, and transformed into *S. meliloti* 1021, this dual-plasmid system will allow for versatile, efficient gene knockout. Resulting mutants will then be cultured in media containing MeOH as the sole carbon source and supplemented with Ln to assess for resulting methylotrophic growth.

Peptide Supplementation Inversely Regulates Motility and Adherence of *Clostridium butyricum*

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Clostridium butyricum is an anaerobic bacterium that inhabits diverse environments including the mammalian gut. Interest in studying *C. butyricum* includes chemical production, probiotic development and in limited cases its pathogenicity. Biofilm formation by *C. butyricum* has been reported, however the nutritional signals regulating sessile growth have not been identified. We previously observed that *C. butyricum* colony formation on minimal media required peptide supplementation. Nutritional signals controlling motile versus non-motile behavior will likely impact bacterial colonization of a host. I hypothesize that peptide availability acts as a signal for the transition to motile planktonic growth by *C. butyricum*. To test the effect of media and/ or growth phase of the inoculum on the behavior of *C. butyricum*, I monitored biofilm production, starch adherence, swimming motility and growth kinetics. Inoculum used for all assays was cultivated on defined or complex media and subcultured with inocula grown to exponential or stationary phase. While no significant biofilm formation was observed, cells cultivated on media lacking peptides adhered better to starch than cells cultivated on complex media. In contrast, both exponential phase inocula and peptide supplementation stimulated swimming motility. This effect was not solely dependent on growth rates, as faster growth rates occurred when cells were subcultured into medium supplemented with peptides versus conditions that included peptides in both inocula and subculture medium. Peptide supplementation and growth phase of inocula were determined to inhibit adherence, but stimulate swimming motility and growth rates. These results highlight the importance of developing standardized methods to monitor bacterial behavior.

Differential Effects of Iron on Methanogenesis as Both an Accelerant and a Depressant

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Greenhouse gases such as methane have been continuously accumulating in our atmosphere since the industrial age began. Despite methane's outsized impact on the warming of our planet, much is still unknown about the microbes that produce methane, methanogens, and how they interact with their environment. The recent discovery of methanogens' ability to reduce iron and on the other hand, the impact of the iron mineral magnetite on methane production rates has raised many questions about methanogens' relationship with iron. To investigate this relationship, I designed an experiment examining the impacts of different iron supplements and carbon sources on the methane and reduced iron production of soil microbes gathered from a field site. The results of this experiment have confirmed that not only does magnetite accelerate methane production, but also that methanogens prefer iron reduction over their namesake metabolism. Furthermore, our results seem to indicate that the methanogens are converting iron into magnetite. These results will promote our understanding of how methanogens interact with the minerals in their environment and how those minerals in turn impact the methane output into our atmosphere.

Antimicrobial Effects of Indole Against Drug-resistant *Campylobacter jejuni*

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Campylobacter jejuni is one of the leading causes of bacterial gastroenteritis in both developed and developing countries. Campylobacteriosis can present with diarrhea, fever, and severe abdominal pain lasting two to five days after exposure, commonly through contaminated poultry. While *C. jejuni* is often a commensal in the gastrointestinal tracts of animals, its emergence as a multidrug-resistant pathogen of humans requires new and innovative solutions to prevent disease dissemination. With the growing rates of antibiotic resistance, scientists must look to new approaches to combat infections. Previous studies with other pathogenic bacteria such as Enterohemorrhagic *E. coli*, *Vibrio cholerae*, and *Pseudomonas aeruginosa* have demonstrated inhibition of virulence in the presence of indole. This heterocyclic aromatic compound is naturally found in the human gut ranging from 0.25 to 1mM in concentration. Our study investigates the effect of indole on growth, respiration, and morphology of *C. jejuni*. Preliminary data suggests indole inhibits *C. jejuni* growth through reduction in respiration and intracellular ATP levels. We hypothesize these effects result from indole influencing membrane potential through the electron transport chain. Our preliminary results also demonstrate an effect of indole on the helical morphology of *C. jejuni*. We hypothesize this is due to indole-induced differential expression of proteins that regulate peptidoglycan structure in the cell. Lastly, preliminary *in vitro* studies reveal inhibition by indole of the acetogenesis pathway, a necessary metabolic pathway for ATP generation. Overall, our data highlight the potential of indole as a therapeutic agent against multi-drug resistant *C.jejuni*.

Autoinducer 2 (AI-2) Mediated Signaling in *Shigella*

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Shigella is a gram-negative pathogen that has evolved from *E. coli* and utilizes various strategies to invade and persist within host cells leading to shigellosis. This research aims on understanding the impact of AI-2 signaling in *Shigella*, an important signaling molecule that plays a critical role for bacterial communication and the regulation of virulence. *Shigella* has undergone gene loss and gain to adapt to its unique environment, having nearly 20% of the *E. coli* genes lost or in the form of pseudogenes. AI-2, a small signaling compound used in bacterial quorum sensing, allows bacteria to perceive the density of their population. In *Shigella*, the *luxS* gene initiates production of AI-2, while sensing and uptake are controlled by the *lsr* genes. The *Shigella* AI-2 quorum sensing system is different from that of *E. coli*. While LuxS generates AI-2 by breaking down S-adenosylmethionine (SAM) into 4,5-dihydroxy-2,3-pentanedione (DPD), which spontaneously converts to AI-2, three of the AI-2 pathway genes (*lsrB, lsrK, and lsrFG*) are pseudogenes. Thus, *Shigella* is unable to utilize AI-2 as much as *E. coli*. But we have seen that some pseudogenes are still functional in *Shigella* like the DGCs. By investigating AI-2-mediated signaling pathways in *Shigella*, this research aims to bridge the gap between understanding the functionality of pseudogenes and uncovering how gene expression is regulated in their absence. Additionally, it seeks to determine the full potential of AI-2 production and its broader implications for *Shigella's* intracellular adaptation.

BR-bodies switch from mRNA decay to mRNA storage in stationary phase

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Bacterial Ribonucleoprotein bodies (BR-bodies) are phase-separated condensates found in *Caulobacter crescentus* (Ccr) that help organize mRNA decay machinery during exponential growth phase. During exponential growth, BR-bodies quickly assemble to promote mRNA decay on the 1-4 minute timescale, and upon mRNA decay, BR bodies rapidly disassemble. Interestingly, mRNA decay rates drop in stationary phase of many bacteria, yet it has remained unclear what role BR-bodies play in the deceleration of mRNA decay in stationary phase. Time-lapse microscopy and FRAP revealed that BR-bodies become dynamically arrested upon entry into stationary phase, resembling a solid-like state. We observe that as BR-body dynamics become gradually arrested, there is a corresponding reduction in mRNA decay rates. However, upon nutrient replenishment, solid-like BR-bodies melt back into a dynamic liquid-like state, which occurs prior to when the cells resume growth. Translation rates assayed by amino acid incorporation (BONCAT) demonstrated that upon the addition of fresh media to stationary phase cells, we find an anti-correlation of translation activity and BR-body assembly, suggesting that solid-like BR-bodies may be sequestering poorly translated mRNAs. RNase E together with RNA drive BR-bodies phase separation, where its intrinsically disordered C-terminal domain (CTD) is necessary and sufficient to drive phase separation. To investigate the physiological roles of BR-bodies in stationary phase, we generated an RNEΔCTD strain that cannot form BR-bodies. When comparing its growth rate to the wild-type, RNEΔCTD has only subtle growth defects in log phase. However, when stationary phase cells are provided with fresh media, RNEΔCTD tends to have an extended lag-phase phenotype compared to wild-type. This suggested that BR-bodies promote faster entry into log-growth upon nutrient replenishment. Overall, we propose that BR-bodies shift their function from mRNA decay to mRNA storage when cells enter the stationary phase, and upon nutrient availability, BR-bodies dissolve and release the stored mRNA to promote fast regrowth.

Intracellular parasite *Toxoplasma gondii* triggers inflammasome-mediated Sterile Immunity

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Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite capable of infecting almost all warm-blooded animals, including humans. Although infection in immunocompetent individuals are often asymptomatic, the parasite poses severe risks for immunocompromised individuals, pregnant women, and fetuses. While *T. gondii* is able to establish a life-long chronic infection in most of the hosts, the Lewis (LEW) rat mounts a sterile immune response that leads to complete parasite clearance. Previous studies from our group and others show that *T. gondii* infection activates the host intracellular pattern recognition receptor, NLRP1 inflammasome, in infected macrophages isolated from the LEW rat resulting in the activation of Caspase-1. Caspase-1 activation further mediates the release of active interleukin (IL)-1β and initiates a form of programmed cell death called pyroptosis. However, it remains unclear whether this inflammasome activation occurs in vivo and whether the inflammasome is the essential restriction factor for anti-*T. gondii* sterile immunity.

In this study, we used the Caspase-1 inhibitor VX-765 to block inflammasome activation prior to infecting LEW rats with *T. gondii*. Peritoneal fluid and cells were collected 24 hours post-infection (p.i.) to quantify IL-1β secretion and assess cell viability. At 30 days p.i., whole brain of the rats were isolated to measure the load of *T. gondii* tissue cysts (latency form of the parasite). We found that parasite infection in the VX-765-treated rats induced a significantly lower proportion of dead peritoneal cells. Additionally, VX-765 treatment resulted in reduced levels of secreted IL-1β levels in the infected groups. Most importantly, we were able to detect tissue cysts in the brain of rats infected with the parasites and treated with vx-765. Our findings demonstrated that *T. gondii* infection in LEW rats induces inflammasome activation *in vivo*, and this activation plays a critical role in controlling parasite infection. Future studies will focus on understanding the mechanism employed by *T. gondii* to activate LEW rat NLRP1 inflammasome *in vitro* and *in vivo*.

Norovirus Surveillance and Whole-Genome Sequencing in Wastewater

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Background: Norovirus is a leading cause of acute gastroenteritis worldwide, responsible for an estimated 685 million infections annually and 200,000 deaths worldwide due to its rapid contagion. Effective monitoring strategies are essential for early detection and outbreak mitigation given its widespread transmission and substantial public health burden. Wastewater-based surveillance (WBS) has emerged as a critical tool for monitoring norovirus epidemiology particularly due to the virus's high concentration in feces even among asymptomatic individuals. In this study, we employed droplet digital PCR (ddPCR) and whole-genome sequencing (WGS) to investigate Norovirus occurrence and genomic diversity in wastewater. **Methods:** Wastewater samples were collected from four sites in Mainstee, MI, including a wastewater treatment plant, over a 16-month period. Samples were precipitated with polyethylene glycol (PEG), and subsequently, total RNA was extracted from each sample using QIAamp Viral RNA Mini Kit. Genotypes GI and GII-specific genes were amplified using commercial kits from GT-Molecular and quantified on RT-ddPCR. We will further apply WGS on the Nanopore platform (MinION) to achieve complete or near-complete genome recovery of Norovirus GI and GII genogroups. **Results and conclusion:** We analyzed a total of 260 wastewater samples, out of which 185 (71%) and 226 (87%) tested positive for Norovirus GI and GII, respectively. We observed an increase in Norovirus GI gene copies between March and June 2024 in all sites, while Norovirus GII showed a notable increase starting in December 2024 and continuing into early 2025. Our data highlight the value of WBS in tracking norovirus occurrence and temporal trends as WBS provides real-time surveillance insights into its presence in the community. The observed variations in GI and GII prevalence underscore the need for continuous monitoring to detect emerging outbreaks and assess viral dynamics. Whole-genome sequencing will further enhance our understanding of Norovirus genetic diversity and evolution, supporting public health interventions and outbreak preparedness efforts.

Keyword: Wastewater-based surveillance; RT-ddPCR, NGS; WGS; Norovirus

The Post-antibiotic Effects of MmpL3-targeted and Nitro-containing Compounds in *Mycobacterium tuberculosis* and *Mycobacterium abscessus*

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Mycobacterial infections are difficult to treat for multiple reasons, including the prevalence of a nonreplicating state (*Mycobacterium tuberculosis; Mtb*), the formation of biofilms (*Mycobacterium abscessus; Mab*), high levels of drug resistance (*Mtb* and *Mab*), and differences in the host immune response. A recently employed regimen for *Mtb* has shortened treatment times from 6 to 4 months; however, the standard for *Mab* has not seen such improvement, and involves two phases totaling 1-2 years of treatment. Thus, there is a clear need for enhanced treatment regimens for both of these pathogens.

Our lab has characterized several compound classes that exhibit *Mtb* and *Mab* growth inhibition. These include compounds targeting the mycolic acid transporter MmpL3 and nitro-containing compounds. Among other factors, we seek to prioritize analogs that have extended post-antibiotic effects (PAEs), and, ideally, compounds that exhibit synergism both in terms of efficacy and PAE.

Using the charcoal agar resazurin assay (CARA) established by Gold et al. (2015, *AAC*), we have examined the PAEs of *Mtb* and *Mab* inhibitors. This assay relies on the use of charcoal to absorb test compounds, and the metabolism of resazurin to the fluorescent resorufin as a measure of mycobacterial growth. Initial CARA results show that a minimum of 3-4 days of exposure to our MmpL3 inhibitors is needed to see a PAE in *Mab*. This is in contrast to control drugs amikacin and bedaquiline, which exhibit a pronounced PAE in *Mab* with only one day of exposure. Notably, in *Mtb* extended PAEs can be seen with shorter exposure times (4-24 h) to standard of care compounds (bedaquiline, isoniazid, and rifampicin), nitro-containing compounds, and MmpL3 inhibitors. Current experiments are combining the CARA with the DiaMOND (diagonal measurement of n-way drug interactions) method to examine the impact of drug combinations on PAE. Taken together, these data will allow us to prioritize drug combinations that are synergistic both in terms of efficacy and PAE. Furthermore, results will inform future animal studies, compound development and SAR, and eventually, *Mtb* and *Mab* treatment strategies.

Investigating the Prion-Like Domain of the Budding Yeast Ty1 Retrotransposon

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Retrotransposons and retroviruses shape genome evolution and can negatively impact genome function. *Saccharomyces cerevisiae* and its close relatives harbor several families of LTR-retrotransposons, the most abundant being Ty1 in several laboratory strains. The cytosolic foci that nucleate Ty1 virus-like particle (VLP) assembly are not well understood. These foci, termed retrosomes, contain the Ty1 Gag protein and the Ty1 mRNA destined for reverse transcription. We recently reported a novel intrinsically disordered N-terminal prion-like domain (PrLD) within Gag that is required for transposition. This domain contains amino acid composition similar to known yeast prions and is sufficient to nucleate prionogenesis in an established cell-based prion reporter system. Deleting the Ty1 PrLD results in dramatic VLP assembly and retrotransposition defects but does not affect Gag protein level. Ty1 Gag chimeras in which the PrLD is replaced with other sequences, including yeast and mammalian prionogenic domains, display a range of retrotransposition phenotypes from wild type to null. We examine these chimeras throughout the Ty1 replication cycle and find that some support retrosome formation, VLP assembly, and retrotransposition, including the yeast Sup35 prion and the mouse PrP prion. Our interchangeable Ty1 system provides a useful, genetically tractable in vivo platform for studying PrLDs, complete with a suite of robust and sensitive assays. Our work also invites study into the prevalence of PrLDs in additional mobile elements.

Characterizing an N4-like phage that infects *Caulobacter crescentus*

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Bacteriophage or phage (i.e. viruses that infect bacteria) are the most abundant organisms with ~10^{31} phage on the planet. Phage will bind, infect, and replicate in their bacterial hosts before lysing them to release the phage progeny into the environment. Due to rising antimicrobial resistance rates, phage are increasingly being used as an alternative treatment for recalcitrant bacterial infections and biofilms. It is therefore critical to examine identify and characterize new phage in order to better understand the interactions between bacteria and phage. We have recently isolated a novel phage from a local, freshwater lake (Lake Lansing) that infects the model organism *Caulobacter crescentus*. Genomic analysis of this phage, hereby named Circe, indicates that it is an N4-like *Schitoviridae* family phage that encodes a dsDNA genome that is 73,793 bp with 106 predicted ORF and 2 tRNA. Phage Circe encoded all seven of the hallmark genes for N4-like phage, including a large, viral RNA polymerase. To identify putative receptors for the phage, we performed BarSeq experiments in which we infected a barcoded transposon library with phage Circe and examined fitness throughout the course of infection. Mutants with disruptions in genes that are known or predicted to control smooth lipopolysaccharide (LPS) biosynthesis were observed to have increased fitness during infection. Consistently, we observed that deletion of genes identified from the BarSeq experiments made strains resistant to phage infection. Together, this suggests that smooth LPS plays a critical role in infection of *C. crescentus* by the N4-like phage Circe.