Applied Microbiology from the Isolate to the Ecosystem

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Diet Induced Animal Model of Non-Alcoholic Fatty Liver Disease (NAFLD) and Gut Microbiome (GM) Dynamics in Early and Late Stage NAFLD

Jasmine Amirzadegan[1], Patrick M. Gillevet[1], Masoumeh Sikaroodi[1], Swati S. Dalmet[1], Jonathon Marioneaux[2], Ekaterina Smirnova[3], Arun J. Sanyal[2,3], and Rebecca Caffrey[2]

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Background: Host diet modulates bacteria and associated metabolism, thus GM dynamics. High fat and sugar (western) diets (WDSW) may alter bacterial relative abundances (RA), inducing GM dysbiosis, suggesting NAFLD progression and severity. Differential RA (DRA) identifies increased *Clostridiales* and decreased *Lactobacillales*, in the large intestine, bacterial fiber fermentation and liver-associated metabolite production site. GM dysbiosis reflects four NAFLD stages: steatosis, steatohepatitis, fibrosis, and cirrhosis. Hypothesis: WDSW fed DIAMOND™ murine models (DM), known to induce NAFLD progression, shift GM DRA compared to controls fed chow diets and normal water (CDNW). Methods: DM fed WDSW/CDNW were sacrificed at 8/16/24/32 weeks, recapitulating NAFLD stages. 16S rRNA from cecum, colon, fecal pellet, ileum, and jejunum were MultiTag Sequenced. Computationally: Ribosomal Database Project v11 classified bacterial family RA, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States predicted metabolic RA, and Linear discriminant analysis Effect Size identified DRA. Results: Bacterial DRA identified 43 of 122 families, often in cecum and colon. Metabolic RA remained static suggesting system robustness. *Clostidiales* DRA increased in WDSW cecum samples at 08 weeks (*Syntrophomonadaceae*) and 32 weeks (*Ruminococcaceae*, *Peptostreptococceae*, *Natranaerovirga*, *Lachnospiraceae*, *Heliobacteriaceae*, *Clostridiaceae4*). The 24 week WDSW cecum and colon GM was similar to the CDNW GM. In the jejunum, only WDSW samples increased DRA. *Lactobacillales (Carnobacteriaceae)* DRA increased in CDNW cecum and colon at 32 weeks. Conclusions: WDSW bacterial DRA associates with early and late stage NAFLD. Supervised classification and correlation network modelling will investigate intermediate stages and bacteria-metabolite associations. Results indicate GM oscillates consistently with NAFLD progression.

Comparative Antimicrobial Activity of Lactic Acid Bacteria against Pathogenic Microorganisms

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There has currently been continued interest in bacteriocins from an applied perspective as bacteriocins have potentials to be used for natural bio-preservation. Microbial spoilage is a great threat to the safety of the food we consume causing problems such as unpleasant smell, adverse change in taste and bad appearance. Infectious diseases are caused by resistant pathogenic microorganisms which are accountable for increased rate of morbidity and mortality. Nine lactic acid bacteria (LAB) strains previously isolated from yoghurt and cheese were tested against food spoilage and pathogenic microorganisms by disk diffusion method and subjected to comparative analyses. All LAB isolates produced antimicrobial compounds indicating either a clear inhibition zone, turbid zones or both kinds of zones against all twelve indicator strains made up *of Pseudomonas spp.*, *Proteus spp.*, *Micrococcus luteus*, *Serratia marcescens*, *Enterococcus spp.*, *Streptocccus spp.*, *Acinetobacter baumanni*, *Salmonella typhimurium, Klebsiella pneumoniae*. All LAB isolates inhibited a high percentage of the indicator strains showing varying activity with 41% for *Proteus spp.*, 35% for *Micrococcus luteus*, 26% for *Salmonella typhimurium*, 25% for *Pseudomonas spp.* and 16% for *Acinetobacter baumanni*, the zone of inhibition for all indicator strains ranged from (11-41mm). Four of the LAB strains 2A, 3A, AS and CE1 were recorded to show the highest inhibitory properties producing high zone of inhibition on the indicator strains. The results show the potential application of the LAB isolates as bio-preservative.

Genetically Similar Clinical Isolates of Shiga Toxin-producing *Escherichia coli* (STEC) Exhibit Differences in Toxin Production and Virulence

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) has an estimated global diarrheal burden of about 1 million cases and is the leading infectious cause of hemolytic uremic syndrome (HUS). Development of HUS is mediated by Stx, but HUS rates vary by outbreak even when the strains make the same types of Stx. Therefore, we characterized three clinical O157:H7 STEC isolates which produce Stx types 2a and/or 2c for virulence in streptomycin (Str)-treated mice and for toxin levels in vitro and in vivo. Isolates JH2010 (2a+2c+), JH2012 (2a+), and JH2013 (2a+2c+) have similar virulence gene profiles but only JH2010 was 100% lethal in Str-treated mice, with a median survival time (mst) of 5 days. Comparatively, JH2013-infected mice showed significantly delayed virulence with an mst of 8 days, while JH2012 was avirulent. Feces from JH2010-infected animals had significantly higher cytotoxicity compared to feces from JH2013 or JH2012. Because the *stx*s are encoded on lysogenic bacteriophage, toxin levels were measured from cultures grown with and without ciprofloxacin to induce the phage, JH2010 had significantly higher baseline and ciprofloxacin-induced cytotoxicity than JH2012 and JH2013. An stx_{2c} mutant of JH2010 was as virulent as the parental strain and made similar levels of toxin in vitro and in vivo, so the differences between JH2010 and JH2012 were not due to the production of Stx2c by JH2010.Genomic comparisons showed differences in genes that regulate stx_{2a}-phage replication and induction between the two strains. These results suggest that differences in phage induction contribute to variable virulence of STEC.

Persistent *Chlamydia* forms differ in their interaction with host cells

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Chlamydia trachomatis is the leading cause of sexually transmitted bacterial infections. Although chlamydial infections are treatable with antibiotics, treatment failure is reported to be between 5 and 23%. One potential mechanism of treatment failure may result from *Chlamydia* entering a 'persistent state' following the host response to the infection. Under certain stress conditions, *Chlamydia* species halt cell division, temporarily stalling the microbe's biphasic developmental cycle and preventing production of infectious progeny. It has been proposed previously that this morphological change may signify a persistence mechanism utilized by *Chlamydia* species, allowing these obligate, intracellular pathogens to remain intracellular during unfavorable conditions, such as during an active immune response, and thus confer a level of protection. While it is known that this 'persistent' or 'aberrant' state can be triggered by a number of different mechanisms, relatively little is known about how the induction of persistence affects the interaction of *Chlamydia* with its host cell. In this study, we examined six aberrance-inducing conditions: β-lactam antibiotics, non-ionic osmotic stress, MreB (actin-like filament) inhibition, type III secretion system inhibition, iron depletion, and tryptophan starvation. We found that these persistent forms of *Chlamydia* can be categorized into two broad categories based on physiological properties that directly influence the microbe's interaction with its host cell: the synthesis and release of immunostimulatory peptidoglycan fragments and the secretion of effector proteins that normally coincide with the midway and later stages of the pathogen's developmental cycle.

A Comparative Analysis of Drinking Water Employing Metagenomics

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The microbiological content of drinking water traditionally is determined by employing culture-dependent methods that are unable to detect most microorganisms. High-throughput sequencing now makes it possible to determine the microbiome of drinking water. Thus, the natural microbiota of water and water distribution systems can now be analyzed in significantly greater detail, providing comprehensive understanding of the microbial community of drinking water applicable to public health. In this study, shotgun metagenomic analysis was performed to determine the microbiological content of drinking water and to provide a preliminary assessment of tap, drinking fountain, sparkling natural mineral, and non-mineral bottled water. Predominant bacterial species detected were members of the phyla Actinobacteria and Proteobacteria, notably the genera *Alishewanella*, *Salmonella*, and *Propionibacterium* in non-carbonated non-mineral bottled water, *Methyloversatilis* and *Methylibium* in sparkling natural mineral water, and *Mycobacterium* and *Afipia* in tap and drinking fountain water. Fecal indicator bacteria, i.e., *Escherichia coli* or enterococci, and antibiotic resistance markers were not detected in any of the samples examined in this study. Bacteriophages and DNA encoding a few virulence-associated factors were detected but determined to be present only at low abundance. DNA of opportunistic plant and animal pathogens was identified in some samples and these included bacteria (*Mycobacterium spp.*), protozoa (*Acanthamoeba mauritaniensis* and *Acanthamoeba palestinensis*), and fungi (*Melampsora pinitorqua* and *Chryosporium queenslandicum*). Archaeal DNA (*Candidatus nitrosoarchaeum*) was detected only in sparkling natural mineral water. This preliminary study is the first report of the complete microbiome (bacteria, viruses, fungi, and protists) of selected types of drinking water employing high-throughput sequencing and bioinformatics. Investigation into activity and function of the organisms detected is in progress.

Assessment of Acetylenotrophy within the Genus Bradyrhizobium

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Acetylene (C_2H_2) is a molecule rarely found in nature, with very few known natural sources. Aerobic and anaerobic microorganisms can degrade acetylene and use it as their sole carbon and energy source via acetylenotrophic metabolism. The enzyme responsible for the first step in acetylenotrophy is acetylene hydratase (AH), which catalyzes the hydration of acetylene to acetaldehyde. AH was first characterized in the anaerobic acetylenotroph *Pelobacter acetylenicus*. As of 2018 there were 15 known strains of acetylenotrophs, however we hypothesize that there is an unknown diversity of acetylenotrophs and AH genes. We are assessing this unknown diversity through cultivation and comparative genomics for members of the *Bradyrhizobium* genus. Our lab isolated two acetylenotrophs from the genus *Bradyrhizobium* (strains I71 and I73), which are the first identified acetylenotrophs within Alphaproteobacteria. Strains I71 and I73 exhibit both acetylenotrophy and nitrogen fixation. We also identified several more putative acetylenotrophs from National Center Bioinformatics Information (NCBI) keyword hits using protein prediction software (RaptorX). To date, RaptorX analysis indicated that there are over 20 putative acetylenotrophic strains within the *Bradyrhizobium*. Early analysis of the prevalence of putative AH enzymes within *Bradyrhizobium* indicated that AH is most prevalent in a single clade of the genus. Comparing the genomes of acetylenotrophic and non-acetylenotrophic *Bradyrhizobium sp.* may give clues as to why AH is still prevalent in extant microbes despite the rarity of acetylene in the environment.

Microbial Communities Reveal Groundwater Aquifers in Hawai'i

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Groundwater aquifers of the Hawaiian Islands are poorly understood, and are increasingly vulnerable to anthropogenic stressors. Geological research has disclosed some knowledge about these complex volcanic aquifers however, very little has been done to utilize microbial communities to investigate aquifer characteristics. In this study, we investigated groundwater microbial communities in the Hualālai watershed in Kona, Hawai'i. The Hualālai watershed is unique, it is a tropical semi-arid climate, and groundwater is the sole source of water in an area undergoing rapid urban development. Historically, Native Hawaiians governed the semi-arid watershed by managing land and water resources by elevation topography. We hypothesized that abundances of microbial metabolism genes (e.g. denitrifiers, sulfate reducers), and community structure would reveal information regarding connection and anthropogenic nutrient inputs in these obscure aquifers. Drinking water wells were sampled over a two-year period and microbial communities were analyzed by high throughput sequencing (16S rRNA), and quantitative PCR to assay abundances of indicator metabolism genes. Beta-diversity analysis revealed distinct communities with respect to aquifers at high elevations compared with those at sea-level, but did not distinguish communities separated by the major volcanic rift zone (east vs. west) in the watershed. Aquifer samples differentiated with respect to dissolved concentrations of orthophosphate, silica, and dissolved ions magnesium and sulfate. Overall biomass (16S rRNA) and abundances of denitrifiers were greatest in the aquifer under stress from rapid urban development. Results will be combined with geological data and used in hydrologic models to help community stakeholders make decisions regarding sustaining water resources.

Engineering metagenomic *Escherichia coli* standard strains by CRISPR targeting of *lacZ*

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Metagenomic next generation sequencing (mNGS), a shot-gun whole genome sequencing (WGS) approach, offers an attractive means to detect clinical pathogens that are rare, in low-abundance, or refractile to isolation and *in vitro* culture. While mNGS is considered a relatively unbiased microbial sampling method, steps within mNGS workflows can result in a biased and non-standardized dataset, reflecting a distorted microbial community population ratio and preventing accurate, standardized comparisons with other clinical datasets. Sequence population bias can arise from varied sample collection procedures, irregular DNA extraction efficiencies, non-uniform conversion of RNA to DNA, incomplete coverage of DNA libraries, and non-standardized bioinformatics methodologies. Internal "spike-in" standards permit monitoring and correcting of these biases. Standards are differentiated from the parental species by unique, synthetic genomic markers and can serve as an unbiased reference for mNGS by duplicating the steps of the mNGS approach from start to finish. Here, we demonstrate the generation of novel spike-in standards using *E. coli,* strain MG1655. The genomic marker was introduced into the non-essential, conserved genomic target site (*lacZ*) using a plasmid-borne CRISPR/Cas9 delivery system in conjunction with ectopic expression of l-red recombinase complex. We demonstrate the high specificity of the single guide RNA (sgRNA) and the significant editing efficiency of the sgRNA:Cas9 complex. The genetic marker was readily incorporated into the target site and detected via PCR. Minor modifications of this approach that effect ribonucleoprotein (RNP) delivery may form the basis for a platform to rapidly generate spike-in controls.

Bacterial metabolism in infection-relevant oxygen gradients with label-free fluorescence lifetime and hyperspectral imaging

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Background: Understanding bacterial physiology in real-world environments is a challenging, yet necessary endeavor to effectively treat infection. Cystic fibrosis (CF) sputum is characterized by steep oxygen gradients, yet the effect of hypoxia on opportunistic pathogens is not taken into account in clinical microbiology lab assessments of antibiotic sensitivity. We sought to determine the impact of oxygen gradients on CF bacterial physiology and antibiotic-tolerance.

Methods: *Pseudomonas aeruginosa* was grown as biofilms or in CF sputum. Hyperspectral and fluorescence lifetime imaging microscopy can visualize metabolite production and metabolic shifts, independent of fluorescent labels. Hyperspectral fluorescence was used to measure the spectral emission of bacteria (2-photon excitation=740 nm, emission=400-690 nm). Fluorescence lifetime imaging of NADH was used to track changes in respiration (emission filter=442/46 nm).

Results: Hyperspectral imaging of bacterial auto-fluorescence captured the production of constituent molecules, including NADH, and condition-specific molecules, such as *Pseudomonas*-derived pyoverdine and pyocyanin. Notably, the fluorescent lifetime of *P. aeruginosa* shifted with biofilm depth, and the shift in fluorescence lifetime correlated with higher cell density and pyocyanin (measured by hyperspectral fluorescence). Using two-photon fluorescence with the DIVER microscope, a custom-made FLIM platform at the LFD, we resolved bacteria up to 2 mm deep in sputum.

Conclusion: Our results indicate that oxygen as a function of biofilm depth is an important driver of bacterial physiology. FLIM shows promise for studying bacterial structure and metabolism in vitro and in vivo.

Inosine Monophosphate Dehydrogenase Inhibitors Halt Cell Division in the Lyme Disease Pathogen, *Borrelia burgdorferi*

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Lyme disease is the most commonly reported vector-borne illness in the United States. The causative agent, the spirochete *Borrelia burgdorferi*, has lost the ability to synthesize purine nucleotides de novo, and relies entirely on tick and mammalian hosts to provide the purines necessary to meet its basic growth requirements. In particular, guanosine monophosphate (GMP) is essential for DNA and RNA synthesis. *Borrelia* encodes Inosine-5'-monophosphate dehydrogenase (IMPDH, also referred to as GuaB), which allows it to effectively convert adenine and hypoxanthine-based nucleotides to GMP. IMPDH inhibitors have previously been shown to effectively decrease the function of recombinant *B. burgdorferi* IMPDH in vitro. In this study, we utilized an IMPDH inhibitor [Q161] for its ability to attenuate the growth of B. burgdorferi strain B31 in vitro. Actively growing B. burgdorferi cultures were treated with 100μM Q161, bacterial cells were examined by structured-illumination microscopy for morphological changes. Viability after Q161 treatment under starvation conditions in a survival curve was determined by colony forming unit counts. Borrelia cells treated with Q161 exhibited cell division and permeability defects, as evidenced by pronounced cell elongation and retention of propidium iodide staining. Under nutrient restriction, treatment with Q161 promoted death. Bacterial growth was restored upon the addition of 1mM guanine to culture media, indicating that its bactericidal activity was not due to off-target effects. This study demonstrates that IMPDH-inhibitors can effectively kill *B. burgdorferi* suggesting IMPDH is a promising target for antimicrobial development.

Development and validation of quantitative real-time PCR for detection of *Alteromonas macleodii* from environment.

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Alteromonas is a globally distributed marine Gammaproteobacteria found in a variety of habitats. It was previously reported as one of the early colonizers of copper-treated marine vessels. Our lab isolated a strain of *A. macleodii* from copper test coupons that grows at high (3mM) copper levels. To understand their role in surface colonization and biofilm formation, a quantitative method for detecting and quantifying *A. macleodii* is required. A real-time PCR assay was developed targeting a region of the DNA gyrase (*gyrB*) gene specific to *A. macleodii*. The primer optimization was carried out to increase assay sensitivity. Absolute quantification using an external calibration curve was used to quantify the *gyrB* gene copies. The standard curves were prepared via a dilutions series of a PCR-amplified and cleaned gyrB gene product as the template, ranging from 50 copies/µl to 50×10^7 copies/µl. The standard curve possessed an amplification efficiency of 94.3%, and a limit of detection up to 50 copies/µl. The assay specificity was validated by melt curve analysis, followed by sequencing. The assay was specific to *A. macleodii* strains- CUKW, MIT1002, J912, EZ55, W12, J589, OCN004, V450, 5003, MR32A, PC21ay, BT3 and did not amplify other marine genera. The assay was then used to screen a range of environmental samples for *A. macleodii*. This assay will be used to track *A. macleodii* growth and biofilm formation over time upon exposure to a range of copper conditions, and in conjunction with quantitative reverse-transcription PCR assays to measure gene expression.

GED: Now is the Best Time to Learn about Genetic Engineering Detection

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Biological research, specifically genetic engineering, has opened up new frontiers to explore. However, as research progresses, the barrier of entry to using genetic engineering (GE) for bio-weapons production gets lower. Some bad actor can insert a harmful gene, using a tool like CRISPR, from a pathogenic or toxin-producing organism into the genome of an otherwise innocuous organism and release it. Here, I propose a tool that can detect GE organisms in a metagenomic sample. Our tool, called GED (Genetic Engineering Detector), can use various methods to label the organism of origin on either side of the GE junction and accumulate evidence for a GE event, as well as the details about the organisms involved. It is implemented as a software plug-in to PanGIA, a new first-in-its-class metagenomic taxonomy classifier for routine biosurveillance monitoring. GED uses a database of genomes from very common and harmless to pathogenic organisms, including the most serious bio-threats. This database is used to identify as many genomic reads as possible in a metagenomics sample. Reads spanning the splice junction of an engineering event will not map well to any singular reference genome in the database. For example, it can accumulate evidence for chimeric genomes by looking at paired-end reads and identifying pairs whose mates appear to map best to different organisms, which can be a signature of an engineered genome. We tested the performance of counting mismatched paired-end read labellings as a way of detecting chimeric reads. We applied this method to synthetically generated paired-end Illumina reads, generated with DWGSIM, for Bacillus Anthracis (BA), Bacillus Subtilis (BS) and simulated genomes that combined genomic information from the two organisms. Different amounts of read-pairs were generated from synthetic GE reads and a background metagenome produced from BA/BS and GE reads. These were analyzed to determine how many GE reads would be present before a signal above background was detected. The results show that positive detection of GE BA/BS can be done with as few as 500,000 GE derived reads in a background of BA/BS (for a total of 10^7 paired-end reads). In the event of a novel disease case or the presentation of a disease without any known cause, this tool can potentially also provide forensic evidence for the origin of the engineered organism. This ability to characterize novel, emerging engineered threats will be essential for safe-guarding the health and security of our armed forces and our country.

Similarity in Subsurface Microbial Community Dynamics at Two Crude Oil Spill Sites

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Microbial community structure was compared at two crude oil spills in glacial outwash aquifers located near Bemidji and Cass Lake, Minnesota. We characterized subsurface microbial communities across a gradient of both oil spills (including uncontaminated areas, within the oil, and within the hydrocarbon plume) to understand the microbial responses to oil spills and potential processes contributing to oil degradation. Bacteria and Archaea communities clustered into three groups independent of oil spill site, but corresponded to contamination characteristics: (1) oily, (2) plume, and (3) uncontaminated sediments. Uncontaminated sediments were dominated by *Nitrospirae* and *Thermotogea*. Oily sediments were dominated by Proteobacteria. Plume sediments were dominated by specific groups such as *Chlorobi*, *Planctomycetes*, Candidate Divisions, and *Bacteroidetes*. At the Bemidji site, oil has spatial variability in degree of degradation, whereas oil at all Cass Lake locations is highly degraded. Geochemistry indicates that biodegradation of the oil body is occurring through sulfate reduction, methanogenesis, iron reduction, and dissimilatory nitrate reduction to ammonium (DNRA). We are currently comparing how shifts in bacterial and archaeal abundances relate to shifts in degradation processes across the different contamination characteristics. This research is expected to aid in understanding the interplay between the microbial community in contaminated sediments and use them as a fingerprint to assess the geochemistry and degradation state from crude oil spills.

Characterization of the role of pstB1 in *Enterococcus faecalis* membrane stress and biofilm formation

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Enterococcus faecalis is a Gram-positive GI commensal that has emerged as a leading healthcare-associated pathogen. Clinical isolates are often resistant to antibacterial agents and readily form biofilms, making infections difficult to treat. Understanding the genetic mechanisms responsible for *E. faecalis* biofilm formation is critical for finding novel treatments. We used recombination-based in vivo expression technology (RIVET) screens to identify promoters that are up-regulated in three biofilm conditions: rabbit foreign-body abscess, rabbit endocarditis, and in vitro-grown. A putative promoter upstream of pstB1 was identified in all three conditions. pstB1 is located within the pst-phoU locus, a putative operon that encodes a well-conserved inorganic phosphate (Pi) importer. Pi homeostasis contributes to virulence and biofilm formation in many bacteria. The roles of Pi and pst-phoU in *E. faecalis* biofilms are not understood. We have generated a ΔpstB1 deletion mutant strain and begun phenotypic characterization studies to evaluate the role of pstB1 in membrane stress and biofilm formation. When compared to wild-type OG1RF, ΔpstB1 exhibited increased susceptibility to bile salts and detergent on solid medium. This phenotype was not recapitulated with strains grown in broth culture. To determine the role Pi plays in *E. faecalis* biofilm formation, we generated a reduced-phosphate BHI broth. OG1RF did not produce as much biofilm in the reduced-phosphate media compared to normal BHI. Interestingly, the ΔpstB1 strain produced equal amounts of biofilm regardless of the media. These findings demonstrate that Pi plays an important role in *E. faecalis* biofilm formation and that deletion of pstB1 contributes to membrane instability.

CURE-based discovery of genetic interactions governing PdhS-DivK-CtrA pathway activity in *Agrobacterium tumefaciens*

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Throughout the Alphaproteobacteria, including *Agrobacterium* species, the PdhS-DivK-CtrA regulatory pathway influences multiple developmental phenotypes including the cell cycle, attachment to surfaces, and swimming motility. The facile nature of generating and screening for suppressor mutations affecting swimming motility and exopolysaccharide production makes this system ideal for participation and completion by undergraduate researchers. Using sensitized PdhS-DivK-CtrA pathway mutant strains in the context of a course-based undergraduate research experience (CURE), we have identified novel genetic interactions governing pathway activity and phenotypic outputs. Newly uncovered pathway components include the *A. tumefaciens* CpdR2 homologue (Atu3663), the *A. tumefaciens* SpbR homologue (Atu0923), a dual-function diguanylate cyclase-phosphodiesterase (Atu3207), and a two-component system (Atu0970/Atu0971). Importantly, validation of these newly isolated pathway components extends to phenotypes beyond those used for screening. These results highlight that use of an upper-level undergraduate laboratory course for mutant generation and phenotypic screening allows for accelerated discovery of mechanisms regulating the PdhS-DivK-CtrA pathway and its outputs.

Investigating the potential for host selection in the establishment of the microbiota among multiple axenic mosquito species.

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We are just beginning to appreciate the complexity of interactions between metazoans and their associated microbiota. To address unknowns, we have developed an axenic model for three mosquito species (*Aedes aegypti*, *Aedes albopictus* and *Ochlerotatus triseriatus*), which we believe is the first axenic system to incorporate multiple species. Having this system now allows us to examine the dynamics of microbiota colonisation between individuals, within and among species. Preliminary observations suggested that the mosquito-associated microbiota primarily serves a nutritional role, thus we hypothesised that bacterial composition of the inoculation source, rather than host species would be the primary driver influencing microbiome composition. In this study, we controlled both the environment and diet of the larvae to investigate how and which bacteria are recruited from the aquatic environment. Using bacterial culture and 16S rRNA gene sequencing, we have found that when raised in the same conditions, bacterial abundance between species are similar. Yet, initial sequencing results suggest that when the species are raised in isolation, while there are some shared bacterial isolates across species, both *Ae. aegypti* and *Oc. triseriatus* harbour unique isolates despite sharing the same inoculation source. Similarly, the two Aedes mosquitos had no bacterial isolates in common despite sharing the same initial bacterial species pool. These results suggest that host phylogeny has a more significant influence on bacterial colonisation in mosquitoes than initially thought and that there is potentially a role for host selection in the establishment of the microbiome.

Sulforaphane as an Adjunctive Epigenetic Therapy for Gonorrhea and Chlamydia

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Gonococcal and chlamydial infections are the two most frequently reported infections to the CDC. They are caused by the Gram-negative bacterium *Neisseria gonorrhoeae* (Ng) and the obligate intracellular bacterium *Chlamydia trachomatis* (Ct). In the absence of effective vaccines, controlling the spread of these STIs is primarily limited to safe-sex counseling and identification and treatment of infected individuals and their sexual partners. Treatment is constantly challenged by rapid evolution of antibiotic resistance in Ng, which has necessitated global surveillance programs and frequent changes in treatment guidelines. One approach for combating infections is to harness host innate defenses that can challenge pathogens. Recently, the use of histone deacetylase inhibitors (HDACi) as epigenetic modulators that affect the innate immune response has received attention as a potential anti-infection therapy. In this study, we used sulfuraphane (SFN), an isothiocyanate from cruciferous plants, as a model HDACi. We report that SFN reduces the adhesion/internalization of Ng and Ct by ME-180 human endocervical cells, and it induces secretion of factors that dramatically reduce the viability of Ng and Ct, in a dose- and time-dependent manner. Additionally, we identified some of these factors by RNAseq, and confirmed their expression changes by qPCR. Furthermore, we demonstrated that there is synergy between SFN and several anti-Ng antibiotics in vitro (ME-180) and in vivo (Ng murine infection model). Given these findings and the demonstrated safety of SFN in humans and laboratory animals, SFN is a promising candidate for adjunctive epigenetic therapy against these common sexually transmitted pathogens.

Characterization of lysogenic bacteriophages in soybean nodulating bradyrhizobia genomes

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Bacteria are known to carry both functional and defunct viruses in their genomes. These prophages (integrated viruses) are known to impact the fitness of the bacteria, participate in horizontal gene transfer and affect bacterial abundance in a population. In the University of Delaware Bradyrhizobia culture collection (UDBCC) containing 354 soybean *Bradyrhizobium spp.*, bacteria that nodulate the roots of soybean and fix atmospheric nitrogen, about 69% produce phages spontaneous and on chemical induction. While much research is being done on prophages and its impact in different bacteria, almost no research is focused on the impact of these prophages on ecology and biology of bradyrhizobia. To answer this question, first, all accessions of UDBCC were classified according to phenotypic, genotypic and phage production data. Later, multiple approaches were used to characterize the prophages from four spontaneous phage producers including host genome sequencing, phage fraction sequencing (mapping to host genome to identify prophage regions), and transmission electron microscopy for morphological assessment. Three sequenced bacterial genomes (USDA122, S10J and S06B) contained one prophage region and USDA 76 contained two prophages. Two of the identified prophages had synteny similar to a Gene Transfer Agent (GTA) observed in other alphaproteobacteria indicating the involvement of these phages in horizontal gene transfer. Initial qPCR experiments showed phage capsid gene expression in planta. Further experiments are being planned to study in planta prophage gene expression in these bacteria and involvement in horizontal gene transfer both of which may impact symbiotic efficiency and consequently, the billion-dollar soybean industry.

Assessing the Impacts of Oil and Gas Wastewater Dumping in the Permian Basin on Soil Microbial Communities

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In southeastern New Mexico, the Bureau of Land Management identified over 39 illegal oil and gas (OG) wastewater dumps which started in November 2017 and released approximately 4,000 barrels of OG drilling and production wastewaters onto desert soils. Despite reports of livestock and wildlife kills from exposure to spilled OG wastewater, little is known about the environmental health impacts and risks of such spills on arid lands. We are evaluating these impacts by analyzing the responses of soil microbial communities to changes in soil geochemistry due inputs of OG wastewater. Approximately 4 months after the dump sites were initially identified and mapped, soils from a total of 7 dump sites were sampled at the surface level, as well as cores of variable depth. The geochemistry of soils across all dump zones differed compared to areas outside of the dump zones, reflecting the composition of local OG wastewater (e.g., enriched in Na and Cl). Using Illumina 16S rRNA gene sequencing and downstream bioinformatics analyses, microbial communities were characterized in spill and control soils. To date, multivariate analyses show that microbial communities in dump zones had lower diversity and shifts in composition compared to control sites. Significant shifts in microbial taxa in spill-impacted soils were also observed, including an increase in halophilic taxa. Further work will assess potential microbial community functions, e.g., hydrocarbon degradation, affected by OG wastewater inputs. This study will help elucidate the role of microorganisms as natural markers for and their potential to naturally attenuate OG wastewater spills.

Characterizing the Zoo-Managed Elephant Gut Microbiome

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Zoo managed African elephants (*Loxodonta africana)* and Asian elephants (*Elephas maximus)* suffer from low reproductive rates, obesity, and gastrointestinal (GI) issues. Ovarian acyclicity, or the lack of a regular reproductive cycle, afflicts nearly half of the captive female African elephants and a quarter of female Asian elephants. Acyclicity has been monitored in zoo elephants, but is not well understood. My research aims to examine the relationship between the elephant gut microbiome and health issues in captivity including acyclicity, obesity, and GI issues. The gut microbiome has been tied to numerous health conditions such as obesity and GI issues, and gut microbes have been found to regulate and respond to host hormones in model species. To assess the elephant gut microbiome, I leveraged fecal samples and health records from a large Elephant Welfare Project conducted across North American zoos in 2012. Fecal samples from 69 African and 48 Asian elephants across fifty zoos were characterized using Illumina sequencing of the 16S rRNA bacterial gene. My results indicate the gut microbiome differs between African and Asian elephants and is influenced by zoo institution. The gut microbiome does not appear to have a relationship with reproductive cycling status, obesity, or the frequency of GI issues in captive elephants. Studying the gut microbiome of captive elephants builds our knowledge of how bacterial communities vary between species, how the environment influence the gut microbiome, furthers our understanding of disease etiology, and has the potential to influence captive management to improve elephant welfare.

"Microbi-omics" of the NIST Dish to Measure a Model Sourdough Community

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The ecological forces that guide the assembly and stability of microbial communities remain unresolved. Thus, there is a need to integrate multi-omic techniques in microbiology or "microbi-omics" to better quantify natural microbial communities and predict the fate of engineered ones. The focus of this project is to provide a new measurement platform that will improve the field's ability to characterize complex microbial systems. Current techniques are also largely qualitative and are hampered by a lack of reproducibility. We have transformed the classic petri dish into in-house fabricated microwell arrays (NIST-dish) to serve as arrayed microscopic petri dishes. The NIST-dish was utilized to study a model sourdough community consisting of two bacteria and two yeast species. High throughput, automated imaging, in addition to mass spectrometry based metabolomic measurements, and genomic sequencing were utilized to characterize community interactions. The integration of the NIST dish and the accompanying "microbi-omics" is a revolutionary advancement to the quantitative measurement of microorganisms and has the potential to advance multiple industries including food, medicine, and biotech, in which reproducibly engineered microbial communities are crucial.

Exploring metagenomic assembly graphs to identify novel microbial variants contributing to human and environmental health

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Sequence variation within metagenomes imparts important information about microbial changes in human and ecological health. However, many existing methods for variant detection are reference-dependent and limited to single nucleotide polymorphisms, missing more complex functional and structural changes. Our lab developed MetaCarvel, a novel scaffolding and assembly graph-based variant detection tool that identifies insertion/deletion events, simple and complex strain-level differences, plasmids, and inter- and intra- genomic repeats within complex microbial communities. We have applied MetaCarvel to almost 1,000 metagenomes from the Human Microbiome Project, identifying over nine million variants that were involved in bacterial proliferation and defense mechanisms, including bacteriocin biosynthesis and antibiotic resistance genes. Within indels and interspersed repeats, MetaCarvel identified known and potentially novel phage that had integrated into a host bacterial genome. Our work highlights the utility of using graph-based variant detection to capture biologically significant signals in microbial populations.

Novel insights for biosurveillance of bat-borne viruses

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Bats are rich reservoirs of viruses, including several high-consequence zoonoses. In this study, high throughput sequencing is used to characterize the virome through a longitudinal study of a captive colony of fruit nectar bats in Singapore. This study utilized viral RNA extracted from swabs of four body sites per bat per timepoint. Swabs of the exterior of the bat (head and body) were used to evaluate virus populations and demonstrate utility as a sample site for future surveillance to extrapolate population-level infection. Through unbiased shotgun and target-enrichment sequencing, we identify both known and previously unknown viruses of zoonotic relevance and define the population persistence and temporal patterns of viruses from families that have the capacity to jump the species barrier. We observed population persistence of zoonotic-related viral families that are known to be associated with spillover from bats to humans. Noninvasive surveillance methods that target the body of bats not only detect viruses shed within the colony but can also represent viral populations dispersed throughout the entire colony. New knowledge of persistent viral families should inform future directions for biosurveillance of viruses that have the potential to cross the species barrier from bats to humans or other amplifying hosts.

Crosstalk between prokaryotic replication initiation and acidic phospholipids

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The role of cellular membranes in bacterial DNA replication has been speculated on for over five decades. In *Escherichia coli*, biochemical studies indicate that acidic phospholipids, specifically phosphotidylglycerol (PG) and cardiolipin (CL), stimulate the conversion of replicatively inactive ADP-DnaA to replicatively active ATP-DnaA, the initiator protein for chromosomal replication. In vivo studies also suggest a crosstalk between inner membrane acidic phospholipids and the machinery for initiating replication. However, the physiological mechanism by which acidic phospholipids regulate the DnaA-mediated process of initiation of replication is still unclear. Separately, acidic phospholipids are also involved in the maturation of the major outer membrane lipoprotein, Lpp. Studies have shown that depletion of acidic phospholipids adversely affect the maturation of lipoprotein, which leads to accumulation of immature intermediates at the inner membrane, causing growth-arrest. In this study we investigate whether there is a link between the state of *E. coli* membranes and DnaA-mediated chromosomal origin (oriC)-dependent initiation of DNA replication. We assessed the growth restoration capacity of: 1) ectopic expression of the DnaA(L366K), a point mutation in the membrane binding amphipathic helix of DnaA, and 2) genome modification of the bacterial orisome in cells with immature Lpp intermediates accumulated at bacterial inner membranes, but with the physiological levels of acidic phospholipids. Our results confirm that: 1) the ectopic expression of DnaA(L366K), and 2) deletion of the gene encoding for Fis (Factor of Inversion Stimulation), which inhibits untimely initiation of the chromosomal replication at the oriC, rescue growth in cells with altered bacterial inner membranes that have accumulated immature Lpp. We propose a dynamic interplay between DnaA, Fis and acidic phospholipids in regulating initiation of the chromosomal replication.

Cool Science Sans Lab Coat (Suit Coat Likely Necessary)

Michael Patterson

Quantitative Scientific Solutions LLC, www.QS-2.com

For scientists who are driven by curiosity and the need to make a difference away from bench research, there are many career options available; however, identifying and thriving in these environments can be a challenge. I currently work as a technical Science Engineering and Technical Assistance (SETA) contractor to the Intelligence Community and have also served as an advisory and assistance (A&AS) support for the DoD. This variety of roles have offered extensive exposure to multiple government and commercial cultures, systems, and missions. These positions have many titles, all with similar responsibilities across government, commercial, and industrial sectors including: program/project manager, science officer, A&AS contractor, and SETA contractor. Although cultures may vary across the USG, the role and expectations are similar: technical expertise, commitment to mission success, and the ability to bring a technical insight to a high-level program impact (i.e. seeing the forest instead of the trees). These roles initially require a clear technical background but also allow, and often expect, individuals to expand to other scientific fields. Scientific knowledge and curiosity are critical for success but communication (written and oral), organizational skills, and leadership traits are similarly important and necessitate constant practice and cultivation. There are incredible benefits and challenges in this field, both personal and organizational, for which early forethought and preparation is beneficial. The goal of this poster is to highlight several career paths in hopes of encouraging and helping more scientists to pursue this fulfilling role.

A Suppressor Mutation of Eep-Mediated Lysozyme Resistance Leads to Permanent Alterations of the *Enterococcus faecalis* Cell Surface

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Enterococcus faecalis is an opportunistic pathogen that is resistant to lysozyme, an important antimicrobial of the host innate immune system. Lysozyme resistance is stimulated through a signal transduction cascade that involves activation of the alternative sigma factor SigV via cleavage of the anti-sigma factor RsiV by transmembrane metalloprotease Eep. Our lab has isolated suppressor mutants of Δeep (Δeep lysR) that regain the ability to thrive in the presence of lysozyme. The goal of this study is to elucidate the molecular mechanisms that confer the Δeep lysR phenotype. Using confocal microscopy, we observed that the cell envelope of Δeep lysR biofilms stained with an Alexa Fluor 594-wheat germ agglutinin conjugate fluoresced more intensely than that of similarly-labeled WT and Δeep cells. Further examination of Δeep lysR cells by transmission electron microscopy demonstrated that the cell surface of these isolates lacked the electron dense structures observed on the surface of WT and Δeep cells, suggesting changes in the bacterial cell envelope. Whole genome sequencing analysis of Δeep lysR cells revealed that a teichoic acid biosynthesis gene, OG1RF_11713, was mutated in these strains. Deletion of OG1RF_11713 in the Δeep background restored the lysozyme-sensitive phenotype to that of the parent Δeep strain. Interestingly, strains lacking OG1RF_11713 were more susceptible to detergents and polymyxin B, and exhibited a net increase in cell surface charge. In conclusion, in the absence of functional Eep protein, Δeep lysR suppressor mutants utilize an alternate mechanism of lysozyme resistance that is associated with stable alterations to the cell surface.

Making sense of unclassified viral proteins through ORF network analysis

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Microbes play an important role across all ecosystems and viruses influence these communities by acting as ecological drivers while also providing a large source of genetic diversity. Deep sequencing across whole microbial communities has yielded a reservoir of information; however, results often show a lack of homology among environmentally sampled sequences when compared to known databases. This lack of annotated viral genes has been classified as viral dark matter and can occur in more than half of the surveyed sequences. Here, we aim to shed light on unclassified metagenomic viral proteins' functions by assuming gene neighbors often share complementary function. The overall concept is driven by a network-based analysis on predicted peptide open reading-frames or (ORFs) that are positioned along assembled contiguous reads (contigs). By clustering all ORFs at a 40% sequence identity, analysis can then be made on the interactions between these ORFs and neighboring clusters that fall within a specified proximal range. Each cluster and interacting neighbor is mapped onto a network and various statistical methods are used to associate unassigned proteins to known neighbors therefore, implicating possible functional attributes. The ribonucleotide reductase class 1 alpha and beta subunits were used as a foundation to explore previously overlooked unknown ORFs within metagenomic data sets. This approach has identified a number of unclassified ORFs through multiple layers of validation and illustrate viral dark matter as more than just random noise.

Novel field-based interactions between soybean and symbiotic root nodule bacteria

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Symbiotic nitrogen-fixing bacteria associated with legumes can provide soybean with up to 95% of its nitrogen demands. Some studies have reported presence of non-nitrogen fixing bacteria inside nodules of legumes, including soybeans. While specificity of soybean-diazotroph interactions has long been established, little is known about the impact of presence non-diazotrophs on soybean traits. Additionally, nitrogen-fixation is sensitive to environmental perturbations such as soil water-status. A knowledge gap exists regarding environmental interactions of the soybean root-nodule microbiome. To address this deficit, we planted nine diverse cultivars of soybean at Virginia Tech's Kentland Farm in 2014. We subjected them to natural rainfall and irrigation treatments. Then, we harvested the root nodules, extracted DNA, amplified the 16S rDNA gene as a marker of the overall bacterial community, and iron nitrogenase nifH gene as a marker of diazotrophs. We also profiled the amino-acid composition of the nodules. Results revealed a surprising nodule microbiome bacterial diversity that was cultivar specific. Families such as *Pseudomonadaceae* and *Enterobacteriaceae* contributed up to 45% of the nodule microbiome. Unsurprisingly, the diazotroph population were exclusively composed of *Bradyrhizobium* sp. However, they were more sensitive to the variation of water status than the other bacteria, as expected. Functional changes in the nodules were attributed to both the cultivar and water-status. While the role of these bacteria is still unknown, these results can be exploited towards sustainable agriculture, and have the potential to change soybean breeding, and crop management practices. Future directions will involve associating microbiome changes with the plant genome traits.

Infection-elicited microbiota enhances colonization resistance

Apollo Stacy

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A crucial function of the microbiota is to shield the host against infections, a process termed colonization resistance. Previously, we showed that even a brief pathogen exposure can "scar" the microbiota, but how infection-elicited microbiota affects colonization resistance remains unexplored. To address this question, we developed a gut infection model where mice are transiently infected first with the food-borne pathogen *Yersinia pseudotuberculosis* (Yptb) then with the hospital-acquired pathogen *Klebsiella pneumoniae* (Kp). Using this model, we found that mice previously infected with Yptb are more resistant to Kp. Using metagenomics, we identified the species most negatively correlated with Kp to be *Bilophila wadsworthia* (Bw). Bw preferentially consumes the sulfur-containing amino acid taurine. In the gut, taurine is supplied through bile acids, and indeed, we measured higher gut levels of taurine and bile acids in previously Yptb-infected mice. From taurine, Bw generates sulfide, an inhibitor of respiration that may thereby protect against Kp. Supporting this, transposon mutant fitness profiling revealed that the ability of Kp to respire with 1,2-propanediol is blocked in previously Yptb-infected mice. Remarkably, supplementing mice with taurine alone could also promote resistance against Kp. As many pathogens depend on respiration to colonize the gut, taurine may represent a natural microbiota-directed therapy. In our working model, 1) prior Yptb infection alters host bile metabolism, 2) elevated intestinal taurine drives an expansion of Bw, and 3) Bw-derived sulfide inhibits Kp. Together these findings suggest that "memory" of infections by the microbiota, analogous to the immune system, can bolster resistance against pathogens.

Understanding Methane Oxidation in Freshwater Mineral Wetlands

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Wetlands are a large source of atmospheric methane, a potent greenhouse gas. Understanding when and why wetlands are a source of methane is thus important in the context of climate change. Methane biogeochemistry in wetland soils is largely determined by microbial metabolism, with methanogens producing methane that can be oxidized by methanotrophs, preventing it from reaching the atmosphere. These metabolic processes are driven by environmental factors, but questions remain about how exactly these factors shape subsequent microbial activity, including how important the response of microbial community structure is for subsequent biogeochemical function. This is especially true in wetlands where environmental factors have been altered, including restored wetlands which can often experience altered soil structure and hydrology. In this study we aimed to assess both the capacity for methane oxidation and the associated microbial community, in a set of three restored and three natural freshwater mineral wetlands. We predicted that restored sites would show reduced capacity for methane oxidation and an altered microbial community with reduced diversity and lower abundances of methanotrophs. We also predicted decreased soil macro porosity and increased bulk density would correlate with loss in microbial function associated with restored sites due to the importance of soil structure in shaping how the microbial community experiences the soil habitat. We performed soil incubations with a methane headspace to assess methane oxidation potential rates. We also sequenced the universal marker gene 16S rRNA to assess microbial community structure. Edaphic variables and soil structure were assessed in situ and through lab analysis.