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Abstract Book

Spring 2024 Meeting

Saturday, April 6th, 2024

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Conserved periplasmic protein EipA is required for maintenance of *Brucella* cell envelope integrity

Melene A. Alakavuklar,*[1], Aretha Fiebig[1], and Sean Crosson[1]

[1]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA, 48824

The cell envelope provides an important barrier between the interior of a bacterial cell and its external environment and contributes to cell integrity and rigidity. In the alphaproteobacterial genus *Brucella,* the Domain of Unknown Function (DUF)1134 protein EipA promotes cell envelope integrity. EipA has a unique β-barrel structure, localizes to the periplasm, and is important for envelope stress resistance and virulence in both in vitro and in vivo models of infection. In *Brucella abortus,* a strain lacking *eipA* is synthetically sick when genes involved in synthesis of the O-polysaccharide are disrupted. In *B. ovis*, a naturally rough species, *eipA* is essential for viability. Conditional depletion of *eipA* in *B. ovis* leads to a cell chaining and rounding phenotype. Moreover, peptidoglycan sacculi isolated from *eipA*-depleted cells exhibit chaining, indicating that these cells are linked by an uninterrupted cell wall. Cryo-electron microscopy reveals defects in maintaining periplasm size upon *eipA* depletion, suggesting that EipA is necessary for the proper architecture of both the cell wall and the periplasm. A genetic selection approach to identify mutations that alleviate the phenotypes resulting from *eipA* depletion in *B. ovis* uncovered a putative glycoconjugate modification system. Deletion of this locus restores viability and enables proper cell separation when *eipA* is depleted. Steady-state levels of a component of this glycoconjugate modification system are increased when *eipA* is depleted, leading us to hypothesize that elevated protein levels result in uncontrolled modification of a glycoconjugate substrate. DUF1134 proteins are widespread in Alphaproteobacteria, and the expression of *eipA* orthologs from other species including *Agrobacterium tumefaciens* (60% identity) and *Caulobacter crescentus* (40% identity) partially restored the defects caused by *eipA* depletion in *B. ovis*. This result indicates that EipA is executing a conserved cell envelope function across the Alphaproteobacteria.

Assessing Nanoparticle Penetration into *Streptococcus mutans* Biofilms in the Presence and Absence of Dextranase

DeCesaris, Danielle S.*[1], Hayashi, Michael A.L.[1], Rickard, Alexander H.[1], Tenuta, Livia M.A.[2]

1: School of Public Health, University of Michigan, Ann Arbor, Michigan, 48109

2: School of Dentistry, University of Michigan, Ann Arbor, Michigan, 48104

Persistent exposure of dental biofilms to fermentable carbohydrates, such as sucrose, promotes dental caries pathogenesis. Sucrose availability results in a drop in pH within biofilms and production of bacterial extracellular polysaccharides (EPS). An EPS-rich biofilm matrix increases biofilm cariogenicity by enhancing biofilm integrity, favoring microbial adhesion, and potentially reducing the penetration of anticaries treatments like nanoparticle-based therapies into biofilms. The aim of this study was to examine the potential of dextranase, an EPS-degrading enzyme, to improve nanoparticle penetration into *Streptococcus mutans* biofilms. Biofilms of *S. mutans* 3209 constitutively expressing mCherry were developed in 24-well glass bottom SensoPlates over 48 h. Culture media was replaced twice/day to simulate a daily 8 h feast period with 1% sucrose medium and 16 h famine period with 0.1 mM glucose medium. Following development, fluorescent nanobeads were applied to biofilms alongside either a dextranase treatment (10 U/mL, pH 6.5) or a potassium phosphate buffer placebo (pH 6.5). Time-lapse 3D imaging using a confocal laser scanning microscope, after nanobead exposure, enabled the capture of 6 image stacks over approximately 60 minutes (i.e. an image stack every 10 minutes) to show the spatial movement of nanobeads in treatment and control biofilms. Novel quantitative image analysis methods were used to assess percent of biofilm space penetrated by nanobeads in addition to the analysis of signal intensities and overlapping signals from EPS, biofilm, and nanobeads. Results demonstrated nanobead penetration over time was significantly greater in dextranase treated biofilms compared to control biofilms. The results further indicated that significant changes in biofilm structural arrangement occurred over time in treatment versus control biofilms. Based on these findings, this study supports the continued investigation of dextranase as an adjunct to nanoparticle-based therapies for caries control.

Discovery of New Compounds that Kill *Mycobacterium tuberculosis*

Ifeanyichukwu E. Eke*, John T. Williams, Elizabeth R. Haiderer, Veronica J. Albrecht, Heather M. Murdoch, Bassel J. Abdalla, and Robert B. Abramovitch
Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing MI 48823
*All correspondence should be addressed to ekeifean@msu.edu

Tuberculosis (**TB**), caused by *Mycobacterium tuberculosis* (**Mtb**), is a serious respiratory infection that is prevalent in developing parts of the world. Multiple drugs are needed to treat TB, and these come with significant side effects. Treatment failures have also arisen due to the increased evolution of antibiotic-resistant strains. These problems highlight the need for new drugs to treat TB. With the recent approval of pretomanid, nitro-containing compounds have emerged as important agents to treat TB. Our lab conducted a whole-cell high throughput screen for growth inhibitors of Mtb and prioritized some nitro-containing hits for follow-up studies. Three of these hits - HC2209, HC2210, and HC2211 - are nitrofuran-based prodrugs that need the cofactor F_{420} machinery of Mtb for activation. Unlike pretomanid which depends only on deazaflavin-dependent nitroreductase (**Ddn**), these nitrofurans depend on Ddn and possibly another F_{420}-dependent reductase for activation. They also differ from pretomanid in their potent activity against *Mycobacterium abscessus*, a highly drug-tolerant pathogen that causes non-tuberculous mycobacterial (**NTM**) infections. HC2210 was the most potent compound in the series with a whole cell half-maximal effective concentration of 50 nM. In a chronic murine model of TB, HC2210 was orally bioavailable and efficacious in reducing the mycobacterial burden of the infected mice. Overall, these studies show the potential development of nitrofurans as new drugs to treat TB or NTM infections.

Characterizations of AvcD Phage-Defense Cytosine Deaminases

Micah J. Ferrell, Brian Y. Hsueh, Aubree C. Muethel, Christopher M. Waters

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA.

Recently we described the enzyme AvcD (**a**nti-**v**iral **c**ytosine **d**eaminase) and the regulatory sRNA AvcI (**Avc**D **i**nhibitor) as a toxin-antitoxin system from *Vibrio* which results in abortive phage infection through the depletion of cytosolic dCTP pools. Cytosine deamination is a conserved anti-viral defense mechanism in eukaryotes and homologs of *avcD* are found across both prokaryotic and eukaryotic domains of life. AvcD consists of two domains required for deamination activity; a P-loop NTPase and a deoxycytidylate deaminase. While previous attempts to purify active AvcD were unsuccessful we have now developed an optimized expression and purification strategy for purifying active AvcD enzymes from multiple species. Characterization of these purified enzymes have identified cytosolic purine triphosphates as regulators of AvcD activity. We hypothesize that depletion of cellular energy levels during phage infection serves as a signal for activation of this defense system.

Elucidating the Function of a Co-conserved gene pair in iron acquisition in Caulobacter crescentus

Hernandez-Ortiz Sergio*, Fiebig Aretha, Crosson Sean

Michigan State University, East Lansing, Michigan, 48824

Iron acquisition is necessary for cell growth and survival. Though iron is relatively abundant, it forms insoluble Fe^{3+} aggregates in oxygenated environments and is tightly sequestered by plant and animal hosts. Many mechanisms of iron acquisition have been identified in bacteria, but our understanding of this process is incomplete, particularly in soil and aquatic ecosystems. Generally, Gram-negative bacteria utilize siderophores, chelators of Fe^{3+}, and TonB-dependent transporters (TBDT) to acquire iron. *Caulobacter crescentus,* a Gram-negative bacterium, commonly inhabits freshwater and soil environments. *C. crescentus* upregulates four TBDTs (*fiuT, CCNA_00138, CCNA_03023, and hutA*) during iron starvation in complex media (da Silva Neto et al., 2013). HutA has been characterized as a heme/hemin transporter (Balhesteros et al., 2017) while the substrates of the other three TBDTs remain unknown. *fiuT* is co-conserved with *fiuO*, a 2OG-Fe dependent oxygenase, in proteobacteria, suggesting a deep evolutionary connection. To evaluate the role of *fiuO* and *fiuT* in iron acquisition, I tittered strains lacking either gene on complex media. Both mutant strains yielded similar numbers of colonies as WT, but the colonies were smaller. Supplementation with $FeSO_4$-EDTA restored the colony size of both mutants suggesting that iron is the limiting factor. A strain encoding *fiuOT* but lacking the other three iron-regulated TBDTs (*fiuOT*$^{+}$*ΔΔΔ*) grew similarly to WT indicating that *fiuT* is the primary iron-transporting TBDT used by *C. crescentus* in complex media. Importantly, further deletion of *fiuO* (*fiuT*$^{+}$Δ*fiuO* ΔΔΔ) severely reduced growth, akin to a strain lacking all four TBDTs, indicating that *fiuO* works in concert with, and is essential to support the function of *fiuT*. Together, these data support a model in which both *fiuO* and *fiuT* are critical for iron acquisition. Current studies focus on identifying the substrate of FiuT and the role of *FiuO* in iron acquisition.

Identification of bacteriophage resistance mechanisms in the dental pathogen, *Streptococcus mutans*

Fatima Herrera [1], Laura A. Young[2], and Joshua J. Thomson[2],
[1]Department of Biology, University of Detroit Mercy, Detroit, MI 48221
[2]Division of Integrated Biomedical Sciences, University of Detroit Mercy School of Dentistry, Detroit, MI 48208

Early Childhood Caries (ECC), in which primary teeth are affected by caries, is a significant public health problem. ECC is highly correlated with the presence of the cariogenic bacterium, *Streptococcus mutans*. Bacteriophage therapy to prevent or reduce disease associated with *S. mutans* has drawn recent interest. However, increasing evidence of widespread resistance to bacteriophage within *S. mutans* complicates its potential utilization. Therefore, we aimed to identify bacteriophage resistance mechanisms employed by *S. mutans* using bacterial strains isolated from saliva and the *S. mutans* bacteriophage, ΦAPCM01. As one potential resistance mechanism, we tested ΦAPCM01-resistant *S. mutans* strains for growth arrest signatures that may indicate the presence of abortive infection (Abi) systems. For this, early-log phase *S. mutans* were mixed with ΦAPCM01 at various multiplicities of infection and growth was observed for 18 hours. If an Abi system was activated in resistant strains, growth curves would abruptly plateau while in the presence of phage. We observed that phage-resistant strains maintained normal growth in the presence and absence of phage, indicating these strains of *S. mutans* do not employ an Abi system. While our work did not identify signatures of Abi systems in phage-resistant strains, these techniques do not fully eliminate their possible presence. For example, alternate phage resistance mechanisms may be triggered prior to Abi systems, as they are generally considered last lines of defense. Therefore, our next steps will focus on discovering other if other phage-resistant mechanisms play a role in resisting phage infection and the point at which the phage replication cycle becomes blocked.

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

Adam P. Kibiloski, Shelby J. Dechow, Robert B. Abramovitch

Abstract

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 mutants 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 transposon mutants that could grow on lactate at acidic pH. Four of the selected mutants had 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 indicated 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. We propose a model where integrating adaptations to acidic pH and carbon source utilization promote Mtb pathogenesis.

Bacterial RNP-bodies organize the pathway of mRNA degradation

Vidhyadhar Nandana[1], Imalka Wanigasekara[1], Ali Hatami[2], Nisansala Muthunayake[1], Bruce Mousseau[3], Luis A. Ortiz-Rodríguez[4], Kaveendya Mallikaarachchi[1], Hiranthi Kulathungage[1], Julie S. Biteen[4], Yingxi Zhu[2], Matthew M. Champion[3], W. Seth Childers[5], Jared M. Schrader[1]

[1]Wayne State University, Department of Biological Sciences
[2]Wayne State University, Department of Biochemical engineering
[3]University of Notredame, Department of Chemistry and Biochemistry
[4]University of Michigan, Department of Chemistry
[5]University of Pittsburgh, Department of Chemistry

Bacteria have a unique challenge to organize their biochemical pathways without the use of membrane bound organelles. Biomolecular condensates are a class of non-membrane bound organelles which can assemble through phase-separation, and Bacterial Ribonucleoprotein bodies (BR-bodies) were the first biomolecular condensates discovered in bacteria. BR-bodies contain the RNA degradosome machinery responsible for mRNA degradation, however, the full complement of proteins enriched within BR-bodies remains undefined. To address this, we conducted enrichment of BR-bodies followed by proteomic analysis using mass spectrometry combined with *in vitro* reconstitutions and *in vivo* co-localization experiments. Our investigation identified 111 proteins enriched in BR-bodies, suggesting that BR-bodies possess a greater complexity than previously assumed. Additionally, our findings indicate that a subset of the enriched proteins undergo RNA dependent phase separation, indicating potential interactions with other ribonucleoprotein condensates. Notably, RNA degradosome protein clients exhibit stronger recruitment to RNase E condensates compared to other ribonucleoprotein condensates, implying that client specificity predominantly arises from direct protein-protein interactions. Finally, we demonstrate that minimally reconstituted BR-bodies containing the RNA degradosome core enhance RNA processing/decay rates *in vitro*. Altogether, this suggests that a complex network of protein-protein and protein-RNA interactions control BR-body phase separation to provide a favorable environment to organize mRNA decay. As BR-bodies have now been observed across various species containing diverse mRNA decay enzymes, and even in mitochondria and chloroplasts, this suggests that BR-bodies mediated mRNA decay may be a universal feature of bacterial cells.

References

1. Al-Husini, N., Tomares, D.T., Bitar, O., Childers, W.S., and Schrader, J.M. (2018). α-Proteobacterial RNA Degradosomes Assemble Liquid-Liquid Phase-Separated RNP Bodies. Molecular Cell *71*, 1027-1039.e14. 10.1016/j.molcel.2018.08.003.
2. Al-Husini, N., Tomares, D.T., Pfaffenberger, Z.J., Muthunayake, N.S., Samad, M.A., Zuo, T., Bitar, O., Aretakis, J.R., Bharmal, M.-H.M., Gega, A., et al. (2020). BR-Bodies Provide Selectively Permeable Condensates that Stimulate mRNA Decay and Prevent Release of Decay Intermediates. Molecular Cell *78*, 670-682.e8. 10.1016/j.molcel.2020.04.001.
3. Nandana, V., Rathnayaka-Mudiyanselage, I.W., Muthunayake, N.S., Hatami, A., Mousseau, C.B., Ortiz-Rodríguez, L.A., Vaishnav, J., Collins, M., Gega, A., Mallikaarachchi, K.S., et al. (2023). The BR-body proteome contains a complex network of protein-protein and protein-RNA interactions. *Cell Reports 42*. 10.1016/j.celrep.2023.113229.

C-di-GMP regulated pathogenesis in *Shigella*

Ruchi Ojha[1], and Benjamin J Koestler[1]

[1] Western Michigan University
Department of Biological Sciences
Kalamazoo, Michigan, 49008, USA

Shigella is a human pathogen that typically is acquired from consuming contaminated food or water and can survive the highly acidic environment in the stomach to establish gastrointestinal infection. *Shigella* preferentially invades the colonic epithelium, resulting in bloody diarrhea. There are approximately 1.1 million cases of shigellosis reported worldwide annually, and currently there is no vaccine for the prevention or treatment of *Shigella* infection.

It is important for gastrointestinal bacteria like *Shigella* to be able to rapidly adapt to changing environments; other bacteria do this using signaling via the molecule cyclic di-guanosine monophosphate (c-di-GMP). C-di-GMP is a bacterial-specific second messenger involved in regulating many processes, such as biofilm formation; a biofilm is aggregates of bacteria encased in sticky substance called extracellular matrix. These aggregates stick to various biotic and abiotic surfaces to form a film that can tolerate stressful conditions such as acid stress or high doses of antibiotics in a host.

C-di-GMP is synthesized by enzymes called diguanylate cyclases (DGC). Bacteria typically have many DGCs encoded in their genome. While *Shigella* is known to encode 4 DGCs, these enzymes have never been studied, and it is unknown if they regulate *Shigella* virulence. The goal of my study is to determine how c-di-GMP synthesizing enzymes impact the ability of *Shigella* to cause disease.

Since DGCs promote biofilm formation in other bacteria, I hypothesized that knocking out the *Shigella* DGCs d*gcC*, *dgcF*, *dgcP* and *dgcI* would decrease biofilm formation in comparison to wild type *Shigella*. I found that individual *Shigella* DGC deletion strains exhibit reduced biofilm formation. I was also interested in how these DGC mutants would invade and spread in human cells. I cultured human epithelial cells and performed an invasion assay to determine the frequency of *Shigella* invasion. I found that the *ΔdgcC* and *ΔdgcF Shigella* strains show a significant reduction in invasion frequency and the *ΔdgcF* knockout strain shows a reduction in cell to cell spread, measured as plaque size. From these data, I conclude that deletion of certain *Shigella* DGCs detrimentally impacts virulence phenotypes.

With this research, I have determined that *Shigella* DGCs contributes to the regulation of biofilm formation and virulence. To further characterize the role of individual DGCs, I have generated null DGC strain by deleting all *Shigella* DGCs in a single strain, and then restored one DGC at a time to study them in isolation. With this approach, I will determine the contribution of each DGC in c-di-GMP synthesis and the regulation of the aforementioned phenotypes. By studying c-di-GMP signaling in *Shigella*, we can learn about how this dangerous pathogen regulates its ability to cause disease using a widely conserved mechanism, and hopefully identify novel targets to develop antibacterial therapies.

Exploring the Role of Sodium Membrane Energetics in *Bacteroides fragilis* Bile Acid Resistance

Matthew Schnizlein*, Timothy Baldwin, Damone Charleston, Aretha Fiebig, Sean Crosson
Michigan State University, East Lansing, MI

As an opportunistic pathogen in the human gut, *Bacteroides fragilis* must adapt to bile acids, which modulate bacterial growth in part by membrane disruption. These disruptions impact the ion motive force used to generate ATP by ATP synthases (ATPase) and maintained using cardiolipin by reducing membrane permeability. While the role of hydrogen (H^+) gradients is well characterized, much remains to be understood regarding how *B. fragilis* uses alternative ion gradients, such as sodium (Na^+) under bile acid stress, and how it modulates membrane composition to minimize leakiness.

Through a combination of barcoded transposon- and RNA sequencing, we identified genes for two cardiolipin synthases and a conserved Na^+ ATPase complex important for *B. fragilis*' stress response to the microbially-modified bile acid deoxycholate. We made clean gene deletions (Δ*clsA*, Δ*clsB*, and Δ*vtpK*) and characterized their impact on *B. fragilis* growth under deoxycholate, Na^+ and H^+ stress, and intracellular ion levels through inductively coupled plasma mass spectrometry (ICP-MS).

Using *in vitro* growth curves, *B. fragilis* Δ*clsA* and Δ*clsB* strains each had longer lag phases and decreased max optical density under deoxycholate stress as well as high Na^+ and H^+. Δ*clsA* and Δ*clsB* strains also had marked losses of intracellular Na^+. The Δ*vtpK* strain was less fit under deoxycholate stress but not under other osmotic stress. *vtpK* deletion did not affect intracellular Na^+, suggesting that other enzymes help maintain Na^+ homeostasis in its absence.

Further work will characterize how the Na^+ ATPase harnesses membrane ion gradients to generate or consume ATP, particularly whether the ATPase functions primarily as a Na^+-exporting pump or as an ATP generating synthase. I will also compare the effect of membrane cardiolipin levels on ion homeostasis by using ICP-MS and targeted lipidomics. These findings suggest the importance of these widely conserved enzymes in *Bacteroides* fitness in the gut and will inform future efforts to treat *B. fragilis* infections.

The role of ATP-dependent chromatin remodelers in autophagy in budding yeast

Cole Tracy, Uzair Khan, Krystal Cvetkovski, Chhabi K. Govind

Department of Biological Science, Oakland University, Rochester, Michigan-48309

Autophagy is a cellular process utilized for recycling and degrading cellular components. This mechanism is highly conserved across species. Defects in autophagy have been linked to many human diseases. In budding yeast, *S. cerevisiae*, autophagy can be triggered by starvation stress. A significant research effort focuses on comprehending the core machinery involved in the process and how specific proteins or organelles are targeted for degradation by autophagy. However, transcriptional regulation of this process is poorly understood. To understand changes in gene expression during autophagy, we performed Chromatin Immunoprecipitation (ChIP-seq) for RNA polymerase II (Pol II). Autophagy was induced by transferring cells to a media lacking amino acids and nitrogen (SDN-). Wildtype (WT) cells can survive many days in this media by degrading and recycling cytoplasmic components via autophagy. To investigate changes in transcription, we conducted ChIP-seq to measure Pol II occupancies at different timepoints ranging from 0 minutes to 7 days in SDN- media. Additionally, we determined Pol II occupancies after depleting the ATPase subunits of chromatin remodeling complexes SWI/SNF and RSC. We focused on these complexes because we observed that depletion of SWI/SNF within 24 hours of autophagy induction resulted in cell death and significantly reduced autophagy induction. Collectively, we present the first detailed analysis of gene expression changes during autophagy and also implicate chromatin remodeling complexes in this process.

Using a barcoded mutant library to identify genes important for *Vibrio cholerae* physiology and gene regulation

Alex J. Wessel*, Drew Johnson, Aretha Fiebig, Christopher M. Waters
Department of Microbiology, Genetics, and Immunology, Michigan State University, East Lansing, MI 48843

For more than a decade, transposon insertion site sequencing (TnSeq) has been a powerful tool for identifying gene function and contributions to growth in a range of bacterial species and conditions. TnSeq-generated mutant pools can be assayed for specific phenotypes of interest so long as the screening method employed is adequately sensitive. The next generation of TnSeq uses randomly barcoded transposons (RB-TnSeq) in which each insertion site is tagged by a 20nt barcode. By mapping each barcode to the mutant's Tn insertion site, we can more rapidly measure mutant fitness after selection simply by PCR amplifying, sequencing, and counting each barcode's abundance.

I am developing RB-TnSeq for the human pathogen *V. cholerae* and to this end have constructed a library of ~36,000 barcoded mutants. I have performed screens using this RB-TnSeq library to identify mutant strains that fail to grow on the selective *Vibrio* medium thiosulfate-citrate-bile salts-sucrose (TCBS) agar. While TCBS is routinely used to isolate *Vibrios*, its capacity to inhibit the growth of other gram-negative bacteria suggests that there are physiological requirements for survival and sustained growth. In support of this idea, we have identified multiple DNA repair mutants that are attenuated for growth on TCBS agar. In addition to defining the genetic requirements for *V. cholerae* growth on this selective medium, I have also competed the library of mutants in a variety of rich liquid mediums to probe the diversity of the mutant pool during exponential growth. The barcodes from these competitions have all been sequenced and are currently being analyzed. Lastly, we are in the process of pairing RB-TnSeq with fluorescence-activated cell sorting (FACS) to conduct genetic regulatory screens of cells encoding fluorescent transcriptional reporters. Thus, the development of this library has set the stage for multiple avenues of further systems level exploration of *V. cholerae* physiology and transcriptional regulation.

Development of a small shuttle plasmid for use in oral *Veillonella* and initial appraisal of potential for fluorescence-based applications

M. Paula Goetting-Minesky[1], Jordan Kim[2], Duane T. White[2], Michael Hayashi[2], Alexander H. Rickard[2] and J. Christopher Fenno[1]

[1]Department of Biologic and Materials Sciences and Prosthodontics, School of Dentistry, University of Michigan, Ann Arbor, MI 48109 USA. [2]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan 48109, USA

Oral *Veillonella* species are among the early colonizers of the human oral cavity and contribute to dental plaque development. The aim of this work was to evaluate the utility of a small single-selectable-marker *Veillonella* shuttle plasmid system by examining its potential use in transforming different oral *Veillonella* strains and to explore the use of a gene encoding oxygen-independent fluorescent protein to generate a fluorescent *Veillonella parvula* strain. Given that tetracycline resistance is common in *Veillonella*, genes encoding ampicillin- and tetracycline-resistance in a previously described shuttle plasmid (pBSJL2) were replaced with a chloramphenicol acetyltransferase gene. The resulting plasmid was successfully introduced into three strains of *V. parvula* and one *V. atypica* strain by either natural transformation or electroporation. We further modified this plasmid to express a gene encoding an oxygen-independent fluorescent protein in *V. parvula* SKV38. Determined from microplate-based fluorimetry experiments, the resulting fluorescent strain yielded a signal intensity approximately 16 times higher than the wild-type. While cell suspensions, colonies, and biofilms of fluorescent *V. parvula* SKV38 could also be visualized and imaged using fluorescence microscopy, photobleaching was a significant issue. In conclusion, we anticipate that this genetic system and the supporting information presented here will enable enhanced future studies to explore the properties and behavior of different oral *Veillonella* species.

Differential nutrient availability during acute lung injury enhances growth of *Pseudomonas aeruginosa*

Jennifer M. Baker[1]*, Thomas L. Flott[1], Laura A. McLellan[1], Nicole R. Falkowski[1], Gary B. Huffnagle[1], Kathleen A. Stringer[1], Robert P. Dickson[1]

[1] University of Michigan, Ann Arbor, MI, 48104

A modified version of this abstract was presented at the 2024 Gordon Research Conference on Biology of Acute Respiratory Infection.

Secondary bacterial pneumonia is a frequent complication of acute lung injury, and injured lungs likely vary from health in their availability of nutrients for respiratory microbiota. We hypothesized that alveolar fluid from healthy and injured lungs differs in bacterial nutrient availability, enabling pneumonia pathogens to flourish in the injured lung microenvironment. We investigated the growth dynamics and nutrient uptake of *Pseudomonas aeruginosa* in the injured lung microenvironment via *ex vivo* bacterial culture. Using an established model of oxygen-induced lung injury, we obtained bronchoalveolar lavage fluid (BALF) from mice exposed to 0 (healthy) or up to 96 h (severe injury) of 95% FiO_2. Acellular BALF aliquots from healthy and injured mice were inoculated with *P. aeruginosa*, bacterial growth was assessed via spectrophotometry, and metabolomic profiles of BALF before and after growth were acquired via ^1H nuclear magnetic resonance spectroscopy. We found that growth of *P. aeruginosa* in BALF from injured murine lungs was enhanced relative to healthy lungs and correlated with abundance of preferred metabolites. Maximum growth rate and total bacterial density were elevated for *P. aeruginosa* grown in BALF from injured mice. Similarly, BALF from injured lungs initially contained increased concentrations of metabolites preferred by *Pseudomonas* (branched-chain amino acids, ketones), and these preferred metabolites decreased in concentration after 10 hours of bacterial growth. In contrast, glucose was used by *P. aeruginosa* in healthy BALF only, suggesting that in healthy lungs, it relies on non-preferred metabolites due to low nutrient availability. These data indicate that enhanced *Pseudomonas aeruginosa* growth in BALF from acutely injured lungs is attributable to increased availability of preferred bacterial nutrients. These findings support a model of secondary pneumonia in which the altered ecology of the alveolar microenvironment promotes selective bacterial outgrowth.

Discovery of a Type IV restriction system encoded on the *Vibrio cholerae* Pathogenicity Island-II

Jasper B. Gomez and Christopher M. Waters

Phage therapy, which is increasing in importance due to antibiotic resistance, can be inhibited by various molecular bacterial defense systems that target phage. Using a *Vibrio cholerae* genomic library in *Escherichia coli*, I discovered a unique cosmid encoding *V. cholerae* DNA that protected against T2, T4, T5, T6, and secΦ18 infection. Further analysis showed this 25kB fragment is part of the Vibrio Pathogenicity Island-II. Transposon and deletion mutagenesis revealed that two genes, *vc1767* and *vc1766*, are required for protection against T2, T4 and T6 coliphage. *vc1767* and *vc1766* are homologous to *gmrSD*, a Type IV restriction system (Type IV RS), which targets foreign DNA that has unusual base modifications. I hypothesize that *vc1767* and *vc1766* act as a Type IV restriction system. Using experimental evolution, I isolated T2 mutants that are resistant to *vc1767* and *vc1766.* These resistant phages had mutations in *agt*, the gene encoding for an alpha glycosyl-transferase that adds a glucose to the 5-hydroxy-methyl-cytosine of phage DNA, a modification present in all T-even coliphages. Additionally, a T4 mutant deleted for both *agt* and *bgt*, a beta-glycosyl-transferase, is resistant to defense encoded by *vc1767* and *vc1766.* Both T2 and T4 mutants are susceptible to *mcrABC*, a restriction dependent system that targets un-glucosylated 5-hmC. These studies uncover a novel Type IV restriction system in *V. cholerae*, increasing our understanding of the evolution and ecology of *V. cholerae* while highlighting important mechanisms by which bacteria can resist phage therapy.

Title -Phase separation of bacterial translation initiation factor provides resistant to temperature sensitivity

Authors: Aishwarya Ghosh*[1], Vidhyadhar Nandana[1], Jared M. Schrader[1]
Wayne State University, Detroit, Michigan-48202 [1]

Bacterial cells have a key challenge to organize biochemical reactions in their cytoplasm which typically occurs without the use of membrane-bound organelles. Biomolecular condensates are non-membrane organelles that have been found to be broadly distributed in eukaryotes and bacteria and are generated through phase-separation. Biomolecular condensates have been found in the machinery of DNA replication, transcription, and mRNA decay, but have not yet been found with the mRNA translation machinery. We hypothesized that bacteria may have translation machinery condensates, and we performed a bioinformatic screen to identify possible candidates. Bioinformatic analyses of the bacterial translation machinery identified that translation initiation factor 2 (IF-2) has a conserved large intrinsically disordered region, a hallmark of phase-separating proteins, which indicated it may be able to phase separate. To test whether this protein can phase separate into a biomolecular condensate, we have investigated the *Caulobacter crescentus* and *Escherichia coli* IF-2 proteins with both *in vivo* imaging and *in vitro* reconstitution approaches. Purified *E. coli* or *C. crescentus* IF-2 could phase separate *in vitro* when incubated at their physiological concentrations, suggesting they have the intrinsic capacity to drive phase separation. Together, these data suggest that IF2 from different bacteria can phase separate, suggesting that this may be a conserved biomolecular condensate. To investigate the potential cellular and biochemical functions of IF-2 biomolecular condensates, we investigated how different temperature impact phase separation and phase separation property of IF-2 rescue *Escherichia coli* from cold sensitivity. Overall, this work demonstrates that bacteria contain biomolecular condensates of the translation machinery (IF-2), and therefore have condensates of the machinery of each process in the central dogma. As these are essential processes in bacteria, it is possible that they may be promising new targets in the field of antibiotic development.

Analyzing *Treponema denticola* protease complex (dentilisin) in clinical isolates

M. Hassanein, M.P. Goetting-Minesky, W. Zheng and J.C. Fenno
[1]School of Dentistry, Dept. of Biologic and Materials Sciences & Prosthodontics; [2]School of Medicine, Department of Computational Medicine and Bioinformatics

Treponema denticola is one of several oral microbes implicated as "keystone" pathogens in periodontal disease. Its surface-localized acylated protease complex (dentilisin) contributes to dysregulation of tissue homeostasis, driving the disease process. While PrtP-encoded protease activity is well-characterized, the mechanisms by which dentilisin's three lipoproteins interact to form a stable complex are not known. To identify potential interacting domains in the proteins comprising dentilisin (PrcB, PrcA and PrtP), we are examining sequence variations in the dentilisin operon in *T. denticola* clinical isolates.

Using PCR and DNA sequencing, we examined *prcB, prcA* and *prtP* in over >60 *T. denticola* clinical isolates. We grouped isolates according to variations in PrcB and the PrtP C-terminal domain. Molecular models of PrcB and the C-terminal domain of PrtP were generated using the D-I-TASSER algorithm. We are currently amplifying, cloning and sequencing the remainder of the dentilisin locus (5' region of *prcB* through 3' region of *prtP*) in these strains. The PrtP C-terminus fell into (11) groups and showed considerable interstrain variability, consistent with its predicted extracellular exposure and antigenicity. PrcB sequences were >95% conserved in all strains. We identified 11 PrcB groups, plus 4 strains that did not fall in any group. Preliminary AlphaFold modeling showed predicted interacting domains of each protein.

Our results support the hypothesis that the PrtP C-terminal domain is a surface-exposed antigenic domain distinct from the conserved proteolytic domain of PrtP. High conservation of PrcB is consistent with our previous report of PrcB requirement for expression of PrtP protein. This study is part of a larger project including molecular modeling and protein-protein interaction studies.

Abstract

Dynamics of the host microbiome, PTI-associated defense gene expression, and decay in post-harvest apple (*Malus* x *domestica*) fruit

Roselane Kithan-Lundquist [1], Sheng Yang He [2,3], and George Sundin [4]

[1] Department of Microbiology, Genetics and Immunology, Michigan State University; [2] Department of Biology, Duke University; [3] Howard Hughes Medical Institute, Duke University; [4] Department of Plant, Soil and Microbial Sciences, Michigan State University

There has been a shift in the life sciences in the past few decades, in which the microbiome is now viewed as a driver of the physiological capabilities of eukaryotic hosts. The plant microbiota confers fitness advantages to the plant host that promote essential processes including growth, health, abiotic stress tolerance, nutrient uptake, and pathogen resilience. However, insights on how microbiome influence post-harvest fruit rot and vice versa are almost wholly lacking. Post-harvest losses of fruits represent an enormous drain on the global food supply. Fruit microbiome studies could potentially result in a new paradigm shift in our understanding of postharvest biocontrol and postharvest health and physiology. To improve our understanding of the fruit microbiome, we are studying the model apple fruit, the most consumed fruit worldwide. Recently, it has been found that Pattern Triggered Immunity (PTI) is an important component that acts as a host barrier to control the level of commensal microbes (and possibly pathogenic and opportunistic microbes) from excessive proliferation in the host tissue for optimal plant. In light of the above findings, it seems likely that post-harvest losses of many fruits could be the result of a breakdown in the relationship between fruit immune response, the microbiome, and fruit health, comparable to what is observed in the immune-compromised *A. thaliana* mutant. Hence, the overall goal of this research is to characterize the relationship between fruit immunity and the fruit microbiota and the influence of that relationship on fruit health. Our preliminary data shows that the microbiota composition significantly differs across time points at post-harvest suggesting that there is a shift in microbial community composition as the apple fruit ages at post-harvest. We also observed a change in relative abundance of bacterial and fungal taxa at a genus level at each time point. Overall, *Sporobolomyses* was the most prevalent genera among fungi in healthy apples. However, we observed that there was a gradual decrease in abundance of *Sporobolomyses* and increase in *Alternaria*, a fungal pathogen, as the fruit started to rot. Similarly, in bacteria, genus belonging to *Caulobacter, Hymenobacter, Cupriavidus*, and *Pseudomonas* (beneficial microbes in plants) were abundant in healthy apples but steadily declined in relative abundance over the course of post-harvest with increase in pathogen such as *Gluconobacter* and *Acetobacter* at rotting stage. Interestingly, we observed a steep decline in the fruit PTI-defense response (as monitored by *FLS2* and *BAK1* expression) around 42 days post-harvest which coincides with the timing of the emergence of pathogenic microbes. Therefore, we propose that there is a crosstalk between the host immune response and the microbiota. A proper understanding on the correlations between host immune response and microbial community composition and how fruit microbiomes are established and maintained can facilitate a potentially powerful, untapped approach to enhance post-harvest fruit health, defense, and productivity

Transient Interaction with *E. coli* Chaperone Protein CsgC Delays Fibril Formation of Functional Amyloid CsgA

Divya Kolli[1], Anthony Balistreri[1], Yilin Han[1], Sanduni W. Jayaweera[2], Emily Goetzler[1], Lily Kalcec[1], Rachel Alessio [1], Anders Olofsson [2], Brandon Ruotolo[1], Matthew R. Chapman[1]

[1]University of Michigan, Ann Arbor, MI 48109, USA

[2]Umeå University, 901 87 Umeå, Sweden.

Bacterial biofilms display enhanced protection from environmental stressors, predation, and antibiotics. *E. coli* forms an extracellular matrix composed of cellulose, extracellular DNA, and curli fibrils that is critical for biofilm development. Curli fibrils are assembled on the cell surface by functional amyloid protein called CsgA along with a nucleator protein CsgB. CsgA and CsgB are secreted from the cell in an intrinsically conformation. An anti-amyloid chaperone protein called CsgC discourages CsgA from inappropriately aggregating into an amyloid inside the cell. *In vitro,* CsgC potently inhibits CsgA amyloid formation at sub-stoichiometric levels. Additionally, CsgC can maintain CsgA in an intrinsically disordered form *in vitro*, delaying the formation of the characteristic β-sheet rich amyloid fold. Therefore, we hypothesized that CsgC interacts transiently with pre-nuclear CsgA delaying the initiation of amyloid formation. We utilized Thioflavin-T assays, ion-mobility mass spectrometry, and a modified NHS column to characterize CsgC-mediated inhibition. Thioflavin-T assays demonstrated that *E. coli* CsgC and homologs significantly extend the nucleation/lag phase of CsgA fibril formation. Additionally, supplementation of pre-formed CsgA fibril seeds only partially accelerated fibril formation, indicating that CsgC's inhibitory effect may be pre-nuclear. We showed that CsgA eluted from a modified NHS column containing CsgC-linked agarose beads displayed a delayed lag phase, indicating that transient interaction between CsgA and CsgC is sufficient to observe an inhibitory effect. Lastly, we detected a low abundance heterodimeric species of CsgA:CsgC via ion-mobility mass spectrometry indicating that a sub-population of CsgA monomers are bound to a CsgC monomer at a given time. Here, we investigated chaperone activity on functional amyloid CsgA and show preliminary evidence indicating that transient interaction between monomeric CsgC and monomeric CsgA is sufficient to extend the lag phase of fibril formation.

BR-bodies are Broadly Conserved Across Bacterial Species and Play a Critical Role in Plant Root Colonization and Symbiosis in *Sinorhizobium meliloti*

Mallikaarachchi, Kaveendya S.[1*], Chen, Joseph C.[2], Schrader, Jared M.[1]

[1]Wayne State University, Detroit, MI 48201

[2]San Francisco State University, San Francisco, CA 94132

Eukaryotic cells utilize membrane-bound organelles and membrane-less biomolecular condensates to organize biochemical pathways. While bacteria generally lack membrane-bound organelles, recent findings revealed the widespread utilization of biomolecular condensates in bacteria. However, studies have been so far limited to model bacteria like *Escherichia coli* and *Caulobacter crescentus*. *C. crescentus* BR-bodies were the first biomolecular condensates discovered in bacteria, and they assemble through phase separation. RNase E is the major scaffolding protein that drives BR-body formation in *C. crescentus*. Intrinsically disordered C-terminal domain of RNase E, with alternating charge patterning is critical for BR-body assembly. RNase E's critical role as the primary mRNA decay nuclease in numerous bacteria, and high conservation of phase separation driving features in all of them suggests the widespread utilization of BR-bodies across bacterial species as a subcellular organization mechanism. To test the phylogenetic breadth of BR-bodies, we performed *in vivo* recombinant expression of different RNase Es from different bacterial species in *E. coli* where we identified all five out of five RNase Es tested were able to form foci, suggesting they can make BR-bodies. We then performed direct experimental validation using the α-proteobacterial symbiont *Sinorhizobium meliloti*. While *S. meliloti* BR-bodies exhibit similar properties to *C. crescentus* BR-bodies, including droplet fusion and dissolution, a *S. meliloti* BR-body null mutant demonstrates longer mRNA half-life and reduced mRNA decay rates, emphasizing the functional significance of BR-bodies. Moreover, the *S. meliloti* BR-body null mutant displays impaired plant colonization and fails to promote plant growth, further confirming physiological importance of these broadly conserved BR-bodies in bacterial systems.

Keywords: BR-bodies, subcellular organization, membrane-less organelles, host colonization, mRNA decay rate

Streptococcus mutans Adhesion on Orthodontic Archwires After Surface Challenges

Sundoz Kesbeh, Mack Powell, **Olivia Manzano**, Mary Larson, Jayanth Nalluri, Sami El-Nachef, Claudia Larochelle, Danya Taleb, Ania Dadkhah, Joseph Ferracciolo, Laura Young, and Joshua J. Thomson

University of Detroit Mercy, School of Dentistry, Division of Integrated Biomedical Sciences

Objectives: The aim of this study was to determine if physical and chemical challenges impact adhesion of *Streptococcus mutans* to stainless steel orthodontic archwires.

Methods: Stainless steel orthodontic archwires (STSS2020, G&H Orthodontics) were cut to 30.0 mm strips with a cross-section of 0.020” x 0.020” and subjected to different challenges: [1] physically roughened using #200 grit sandpaper; [2] submerged in solution of 10% bleach (for 24 hours); or [3] submerged in solution of 30% H_2O_2 (for 24 hours). Bacterial adhesion and biofilm accumulation were assessed using *Streptococcus mutans* in various conditions by colony count. Data was analyzed using one-way ANOVA and Tukey’s posttest (α=0.05) or Kruskall-Wallis and Dunn’s posttest (α=0.05).

Results: Increases in bacterial adhesion were observed when sandpaper-roughened wires were exposed to *S. mutans* in BHI or artificial saliva for 2.5 hours in the absence of sucrose. This increase in adhesion was lost when grown in the presence of 0.05% sucrose and if adhesion occurred for 18 hours in BHI. Pre-treatment of wires in 10% bleach wires trended towards reduced adhesion in artificial saliva and approached statistical significance in 2.5-hour and 18-hour adhesion and reached statistically reduced adhesion in artificial saliva plus 0.05% sucrose. Additionally, treatment of sandpaper roughened wires with 10% Bleach significantly decreased adhesion of *S. mutans* and sandpaper roughening of previously bleach treated wires returns bacterial adhesion levels to that of sandpaper-roughened wires.

Conclusions: Change in surface roughness is not the only factor that can impact bacterial adhesion to surfaces. Treatment of wires physically and chemically had differential impact on surface roughness and changes in bacterial adhesion depended on media composition and adhesion time.

The General Stress Response System is Necessary for Detergent Resistance in a *Brucella ovis* Mutant Lacking the HWE Histidine Kinase, BOV_1602

Perez, Emily*. Chen, Esther. Crosson, Sean. Fiebig, Aretha.

Bacterial responses to the environment involve a diverse set of gene regulatory systems. Two-component systems are a conserved type of regulation that – as the name indicates – are typically composed of two protein components: a sensor histidine kinase and a response regulator. Within the *Alphaproteobacteria*, an atypical family of histidine kinases called the HWE kinase family are common. These environmental sensor kinases remain largely uncharacterized but have been implicated in regulation of the general stress response (GSR) in diverse *Alphaproteobacteria*. An HWE kinase that contains a photosensory LOV domain (i.e. LovK) has been demonstrated to control the general stress response in *Brucella spp.* through distinct molecular mechanisms, but it is not known if other HWE-family kinases directly regulate the GSR system. Therefore, we are using a genetic and physiological approach to test whether other HWE kinases regulate GSR in the intracellular ovine pathogen, *Brucella ovis*.

The *Brucella ovis* HWE kinase, BOV_1602, is encoded from a locus adjacent to the core GSR regulators, *phyR* and *rpoE1,* suggesting potential involvement in regulation of the GSR. Deletion of *BOV_1602* resulted in enhanced resistance to detergent (SDS) stress. Deletion of *BOV_1602* in combination with the core GSR system genes demonstrated that the SDS resistance phenotype of *ΔBOV_1602* requires *phyR* and *rpoE1* but does not require the HWE kinase, BOV_1607. RNA sequencing analysis of Δ*BOV_1602* and Δ*phyR* mutants demonstrated a positive correlation between transcriptomes, implicating 1602 as a positive regulator of the GSR system. A genome-scale comparison of the transcriptome in *ΔBOV_1602* to SDS-treated cells revealed some congruence, suggesting that *ΔBOV_1602* cells have characteristics of WT detergent stressed cells. Thus *ΔBOV_1602* may be primed to mitigate detergent stress. Future characterization of HWE kinase functions and *Brucella ovis* will advance our understanding of mechanisms of two-component signal transduction and stress adaptation in *Brucella spp.*

Salmonella enterica Typhi activates the macrophage integrated stress response through GCN2

Zachary M. Powers[1], Mack B. Reynolds[1], Basel H. Abuaita[1], Olivia Harlow[1], Hanna S. Hong[2], Luiza Antunes de Castro Horge[1], Katherine R. Spindler[1], Costas A. Lyssiotis[2], Mary X. O'Riordan[1].

1 Department of Microbiology & Immunology, University of Michigan, Ann Arbor, MI. 2 Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI.

Many pathogens induce cellular stress during infection, including viruses, parasites, and intracellular bacteria. The Integrated Stress Response (ISR) combines signal inputs from four kinase sensors that monitor ER stress, cytosolic dsRNA, and nutrient availability. In addition to activating cellular repair and adaptation pathways, the ISR plays a key role in metabolic reprogramming and shaping the innate immune response to infection. We find that the facultative intracellular bacterial pathogen, Salmonella enterica, activates the ISR during macrophage infection through the ISR kinase GCN2, a sensor of amino acid starvation and ribosomal dysfunction. Macrophages play complex roles during S. enterica infection, serving as a survival niche as well as a driver of innate and adaptive immune responses. This research aims to identify the role of GCN2 and the ISR in macrophage innate immune responses to S. enterica infection, and the mechanisms by which bacterial infection actively triggers GCN2 activation. Understanding ISR modulation during bacterial infection of macrophages may lead to the identification of intervention points for future therapeutics and lay the foundation of this emerging subfield of host-pathogen interactions.

Characterizing the Microbial Communities of Diverging Hydrocarbon Pools

Sell, Jakob A.*; Beaver, Carol L.; Rossbach, Silvia

Western Michigan University, Kalamazoo, MI 49008

Since the industrial revolution, humanity has done untold damage to our environment. From mining to agriculture to commerce, much of our activity has negative effects on other living things. In the modern age one of the biggest environmental problems needing to be solved is that of oil spills. When massive amounts of hydrocarbons are released into an unexpecting environment, the resulting damage lasts decades, potentially even centuries. Despite the environmental harm oil spills do, they also create a unique environment where microbes that feed off these hydrocarbons can thrive. One such site is in Bemidji, Minnesota where a pipeline burst in 1979 spilled over a million barrels of oil onto the surrounding landscape. Much of this oil was cleaned up, but approximately 400,000 liters sank beneath the sandy soil to sit atop the water table. Over the decades the hydrocarbon pool separated and settled into three separate pools. Since the 1980's this site has been used to study the breakdown and progression of oil spills over time. Although much work has been done on the site, most of the microbial research has been done on just one of these pools, known as the north pool. Recently our lab has begun to study the microbial community of one the other pools, the south pool. Preliminary DNA work on the south pool has shown that although similar to the north pool, its microbial community is unique. One of the most prominent features of the south pool is an uncultured genus of methanogenic archaea distinct from the *Methanoregula* found at the north pool. Our research has shown that since these pools separated from each other, their microbial communities have diverged. This gives new insights into how these microbes engage with hydrocarbon degradation over time.

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

Hadi Yassine[1], Luis Ortiz-Rodriguez[2], Julie Biteen[2], Jared Schrader[1]
Wayne State University[1], Detroit, MI University of Michigan[2], Ann Arbor, MI

BR-bodies are phase-separated membrane-less organelles found in Caulobacter crescentus (Cc) that help process mRNA decay in the logarithmic (log) growth phase. In bacteria, mRNA has short half-lives. While actively growing, BR-bodies quickly assemble to shuttle in mRNA for degradation. Upon mRNA cutting, BR bodies rapidly disassemble. RNase E is the key protein for BR-bodies assembly. RNase E's CTD is sufficient and necessary to trigger its assembly. We have generated an RNEΔCTD strain that cannot form BR-bodies. When comparing its growth rate to the wild-type NA1000 strain, RNEΔCTD has no significant growth defects at the log phase. However, when stationary phase cells are provided with fresh media, RNEΔCTD tends to regrow much slower than NA1000 into the log phase. This suggested that BR-bodies play a prominent role in the stationary phase to allow a shorter lag phase and faster regrowth upon nutrient replenishment. Additionally, fluorescent images and FRAP analyses revealed that BR-bodies lose their log-phase dynamic properties at the stationary phase and adopt more of a static state. However, upon nutrient replenishment at the stationary phase, solid-like BR-bodies disassemble and allow cells to grow and replicate. Translation initiation labeling (BONCAT) demonstrated that upon adding fresh media to stationary phase cells, cells that dissolve BR-bodies tend to have higher translation activity than cells that haven't disassembled their BR-bodies yet. 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.

Understanding the Role of *Vibrio cholerae* Biofilms in Cholera Transmission

Tasnim Tamima* and Withey Jeffrey H.

Wayne State University School of Medicine, Detroit MI 48201

Cholera is a severe human disease that affects millions of people each year. Currently, we are in the seventh cholera pandemic, caused by the *Vibrio cholerae* El Tor biotype, which is more persistent during infection and in the environment as compared to the Classical biotype. Research indicates important contributions of *V. cholerae* biofilms in cholera transmission. Previous studies showed that *Vibrio* polysaccharide protein R (VpsR) and VpsT regulate *V. cholerae* biofilm extracellular matrix proteins (RbmA, RbmC and Bap1). Hence biofilm specific genes have been found to play critical role in the intestinal colonization during infection. A protocol has been established in our lab to use zebrafish as an animal model that offers several advantages over using mice model. Therefore, we hypothesize that *Vibrio cholerae* El tor strain N16961 (wild type=WT) that forms biofilm may colonize adult zebrafish guts more efficiently in comparison with the biofilm knockout (KO) strain N16961 Δ*vpsR*. We also predict the biofilm mutant will have defects in hyperinfectivity and transmission after 24 hours infection. The major goal of this study is to determine if biofilm-specific genes are important for colonization and hyper-infectivity of *Vibrio cholerae* during zebrafish natural host infection. We performed a competitive index assay between the WT and a Δ*vpsR* strain of *Vibrio cholerae* to assess gene defects during colonization and transmission. The competitive index study between the WT and Δ*vpsR* strains showed a significant ($p<.0001$) defect in cholera transmission, which indicates the importance of biofilm genes in cholera transmission in the aquatic environment.

Poster Title: Water Quality and Coliform Contamination on the Huron River near Peninsular Dam
Students name: Evan Veenhuis & Zainab Almayahi
Faculty mentor: Dr. Daniel Clemans

The presence of Gram-negative enteric bacteria such as *Escherichia coli* found within the Huron River, is an indicator organism of environmental fecal contamination. This study aimed to assess the concentration of fecal coliform and other coliform contamination in the Huron River and the antibiotic sensitivity profiles of bacterial strains. It was hypothesized that with the removal of the Peninsular Park Dam, microbial loads behind the dam will be released downstream. Water and sediment were sampled at seven sites spanning from just below Superior Dam to Lower Huron Metropark, with emphasis on Peninsular Park and Frog Island Park. Cultural-dependent techniques utilized CHROMagar® to quantify fecal coliforms, other coliforms, and other Gram-negative bacteria. Molecular methods were employed to analyze specific isolates, and antibiotic sensitivity testing (AST) was utilized to examine susceptibility to six antibiotics. The presence of pathogens and antibiotic resistance poses significant challenges to public health, the efficacy of current treatment strategies, and the potential for increased difficulty in managing infectious diseases.

Genomic and Patient Features Associated with Non-Susceptibility to Meropenem-Vaborbactam & Imipenem-Relebactam Before Their Market Introduction

Kyle J Gontjes MPH[1*], Ali Pirani MS[1], Jennifer Han MD[2], Pam Tolomeo[3,4], Ellie JC Goldstein[5], Ebbing Lautenbach MD[3,4,6], Evan Snitkin PhD[1]

1. Department of Microbiology & Immunology, University of Michigan Medical School, Ann Arbor, MI
2. GSK, Collegeville, Pennsylvania
3. Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania
4. Department of Biostatistics, Epidemiology and Informatics, University of Pennsylvania Perelman School of Medicine, Pennsylvania
5. R.M. Alden Research Laboratory, Santa Monica, California
6. Department of Medicine, Division of Infectious Diseases, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania

Background

Knowing what drives the emergence and spread of resistance to antimicrobials before clinical deployment could inform strategies to slow resistance development. We evaluated the associations between patient characteristics and bacterial genotypes with resistance to two β-lactam/β-lactamase inhibitor agents before their clinical availability.

Methods

Antimicrobial susceptibility testing and whole-genome sequencing of 415 carbapenem-resistant *Klebsiella pneumoniae* sequence type 258 isolates collected from 2014-15 across 20 US long-term acute care hospitals. To identify features associated with resistance emergence and spread, we evaluated the fraction of non-susceptibility explained by carbapenem resistance-associated genotypes, executed an unbiased genome-wide association study (GWAS), and performed regression modeling of patient characteristics.

Results

Fifty-eight isolates were non-susceptible to a novel β-lactam/β-lactamase inhibitor agent. Resistance differed across clades: increased baseline minimum-inhibitory concentration and non-susceptibility were enriched in clade I. Variants in known carbapenem resistance-associated genotypes partially explained non-susceptibility (29%), with clade-specific differences detected (clade I, 13%; clade II, 63%). The GWAS improved the explanation to 69%, with marked improvement in clade I (69%). Examination of GWAS results supported an association between elevated minimum-inhibitory concentration in clade I with a GD insertion in loop 3 of *ompK36*. Subsequent transitions above clinical breakpoints were often associated with elevated KPC copy number, with one plasmid with increased KPC copy number enriched in non-susceptible strains that spread among patients. After distinguishing patients acquiring non-susceptibility via putative *de novo* evolution from cross-transmission, single-agent carbapenem exposure was positively associated with resistance evolution, but not spread.

Discussion

Our study supports a role for genetic background in developing non-susceptibility to β-lactam/β-lactamase inhibitor agents, and that clinical exposures and resistance genotypes differ among patients acquiring resistance via *de novo* evolution or cross-transmission. Pre-introduction molecular surveillance in clinically relevant populations can identify potential drivers of resistance that could inform efforts to prolong the efficacy of novel antimicrobials.

Simulating Low pH Cycles in an *In Vitro* Dental Biofilm Model System

Dickinson Madeleine, A[1*], Blinkiewicz Hailey, E[1], Tenuta Livia, MA[2], Rickard Alexander, H[1]

1 Department of Epidemiology, School of Public University of Michigan, Ann Arbor

2 Department of Cariology, Restorative Sciences and Endodontics, School of Dentistry, University of Michigan, Ann Arbor

Dental biofilms rich in *Streptococcus mutans* are strongly associated with the development of dental caries. Different *in vitro* models have been developed for fundamental and applied studies of *S. mutans* biofilm development and behavior. Specifically, continuous flow model systems are particularly useful when applied to studies of feast and famine cycles to which dental biofilms are exposed daily. Recently, a 3D-printed *in vitro* model system was developed that provides continuous flow of liquid media over developing *S. mutans* biofilms. The model system also contains multiple hydroxyapatite coupons for biofilm growth that can be analyzed via confocal microscopy to examine biofilm architecture over time. The objective of this study is to determine if this model can mimic a typical Stephan curve of pH decline in a dental biofilm after exposure to sucrose. *S. mutans* UA159 biofilms were grown for 22 h within the 3D-printed model in flowing 25% Tryptic Soy Broth (TSB) (without dextrose) supplemented with 0.1% sucrose to allow initial biofilm attachment and growth. Subsequently, 25% TSB (without dextrose) continuously flowed through the model system for 20 h with the addition of intermittent 13 minute pulses of sucrose containing medium (0.5% sucrose and 25% TSB without dextrose) every 4 hours. During this period, pH was continuously assessed using a microelectrode fixed within the model flow channel. Afterward, Live/Dead staining was performed to assess biofilm growth and architecture. A typical Stephan curve of pH decline after sucrose exposure was repeatedly observed. Initial sucrose pulses generated smaller pH drops, suggesting that *S. mutans* biofilm is continuously growing within the model system, thus enhancing its acidogenic potential over time. Confocal microscopy revealed biofilm architectural differences between those formed on glass and hydroxyapatite surfaces. In conclusion, the 3D-printed continuous flow model generated biofilms and yielded typical Stephan curves in response to sucrose, making it useful for the study of dental caries and anticaries treatments.

Title: My-shield® Broad Spectrum Disinfectant Provides Long-term Protection against Bacterial Contamination

ABSTRACT

Aims: The aim of this work was to evaluate the efficacy of My-shield® Broad Spectrum Disinfectant, a commercially available antimicrobial formulation, against microbial surface contamination.

Methods and Results: My-shield® was tested *in vitro* for its long-term persistence on surfaces and effectiveness against *Staphylococcus aureus* biofilms in comparison to 70% ethanol and 0.1-0.6% sodium hypochlorite. Field testing was also conducted at the University of Michigan Athletic Center. *In vitro* studies demonstrated the log reductions achieved by My-shield®, 70% ethanol, and 0.1% sodium hypochlorite were 3.6, 3.1, and 3.2, respectively. My-shield® persisted on surfaces after washing and scrubbing, and pre-treatment with My-shield® prevented *S. aureus* biofilm formation on surfaces for up to 30 days. In comparison, pre-treatment with 70% ethanol and 0.6% sodium hypochlorite were not protective against *S. aureus* biofilm formation after seven days. The field test demonstrated that weekly application of My-shield® was more effective at reducing surface bacterial load than daily application of a control product.

Conclusions: My-shield® confers greater long-term protection against bacterial growth and biofilm formation by *S. aureus* than ethanol and sodium hypochlorite, requiring less frequent application while still maintaining a high level of antimicrobial activity.

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.

Analysis of Freshwater Bacterial Coaggregation Using a Newly Developed Microplate Spectrophotometry Method

MacLeod, Brenna L.*, Hayashi, Michael A.L., Rickard, Alex H.

University of Michigan School of Public Health

Ann Arbor, Michigan, 48130

Bacterial coaggregation, the specific recognition and adherence of genetically distinct bacteria, is proposed to enhance multi-species biofilm formation. Approaches to detect and quantify coaggregation are often semi-quantitative in nature, do not allow the study of coaggregation kinetics, and are low throughput. The aim of the work presented here was to compare a previously developed quantitative 24-well microplate-based spectrophotometric assay system to a new higher-throughput 96-well format microplate assay system for the assessment of coaggregation kinetics of selected freshwater bacteria.

Coaggregation between *Blastomonas natatoria* 2.1, *Micrococcus luteus* 2.13, and *Pseudomonas aeruginosa* 2.19 were studied using four methods: a semiquantitative visual tube assay, a quantitative cuvette-based spectrophotometric assay, a quantitative 24-well microplate-based spectrophotometric assay, and a quantitative 96-well microplate-based spectrophotometric assay. Confocal laser scanning microscopy was also performed to evaluate the structure of coaggregates.

Results from the four methods demonstrated were generally congruent with regards to detecting coaggregation: *B. natatoria* 2.1 and *M. luteus* 2.13 showed strong coaggregation, *B. natatoria* 2.1 and *P. aeruginosa* 2.19 displayed weak coaggregation, while *M. luteus* 2.13 and *P. aeruginosa* 2.19 exhibited no coaggregation. Coaggregation strengths in the 96-well microplate

were positively correlated with 24-well microplate, cuvette, and visual coaggregation assay scores (Pearson correlation coefficient = 0.68, 0.69, 0.75, respectively).

Our findings are consistent with previously reported coaggregation patterns among the studied species and support the utility of the 96-well plate method. This study enhances the methodological toolkit available to study bacterial coaggregation which has relevance to environmental, medical, dental, and industrial microbiology.

MI - ASM Spring 2024

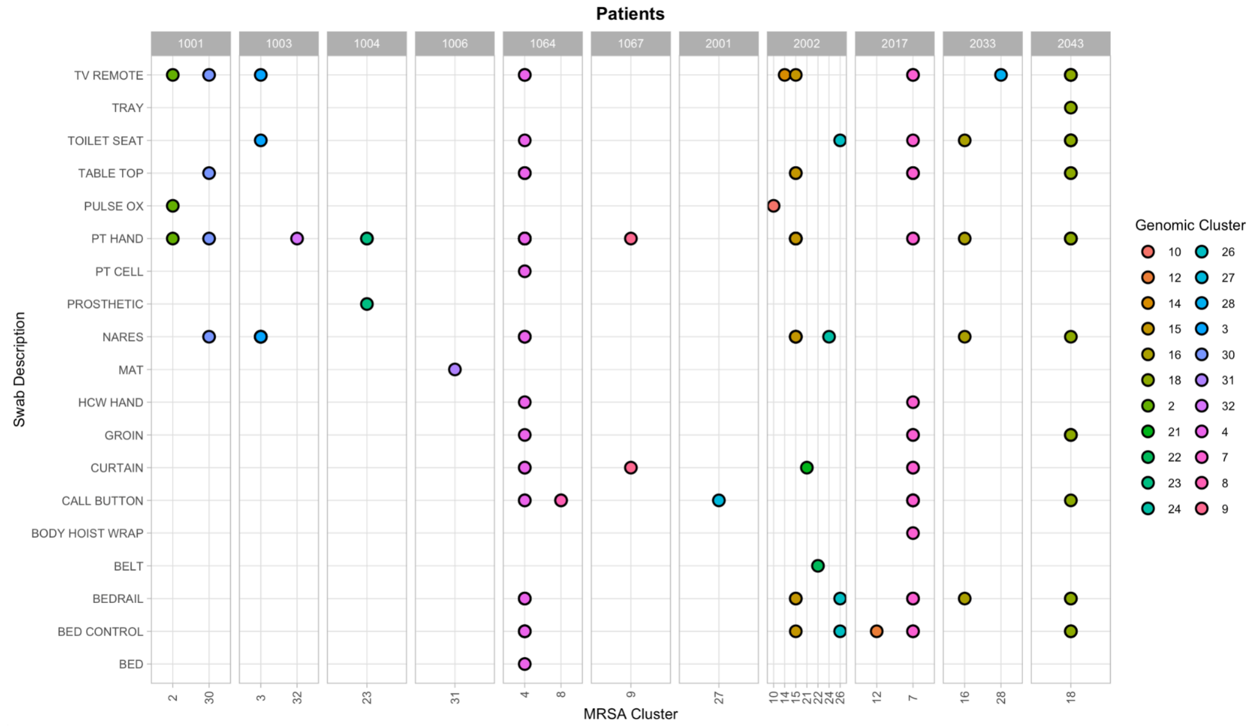
Title: Capturing MRSA Diversity by Integrating Genomic and Epidemiological Data of Patients and their Spaces

Background:
Methicillin-resistant S. aureus (MRSA) is known to cause frequent and severe infections in community living centers and can cause significant mortality for elderly patients. More research is needed to understand how to utilize genomic and epidemiological data to understand characteristics that may lead to increased transmission. We hypothesized that combining genomic and epidemiological information to sample patients and their environments over a 30 day period will be able to capture a diverse set of MRSA strains.

Methods:
This study included genome sequencing of patient and environmental samples from 11 patients within the VA Ann Arbor Healthcare System from May 4, 2021- November 16, 2022. All 11 patients tested positive for MRSA during their stay (mean days = 31). Patient and environmental samples were taken throughout their stays, screened for MRSA, and whole-genome sequenced. Single nucleotide variants (SNVs) were identified by mapping reads and calling variants against strain-specific reference genomes. We used ape v5.6-2 in R v4.2.2 to analyze and infer evolution, acquisition, and transmission events based on pairwise SNV distances. Genomic clusters were determined using stats v3.6.2 and a SNV distance of 20.

Result:
Samples that were collected from patient bodily sites were able to reveal 20 distinct genomic clusters of MRSA (patient hands: n = 10, nares: n = 7, groin: n = 3). Environmental samples from patient environments also revealed distinct genomic MRSA clusters (tv remote: n = 9, toilet seat and bed rail: n = 6, table top, bed control, and call button: n = 5, bed curtain: n = 4, pulse ox = 2, cell phone, tray, pulse ox, mat, body hoist wrap, bed: = 1)

Conclusion:
The identification of various genomic clusters from patients and their environmental reservoirs suggests intrahost variation that can only be captured by using a more holistic approach of integrating epidemiology and genomic sequencing. Developing studies that incorporate genomic data, various environmental sources, and multiple isolates over time within community living centers can increase our understanding of strains that are more likely to transmit, survive on living and non-living surfaces and therefore lead to improved recommendation for infection prevention interventions and drivers of endemicity.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TodK controls biofilm formation of *Myxococcus xanthus* by perturbation of A-signaling

Mataczynski, Christopher R.*, Glaser, Maike, Higgs, Penelope I.

Wayne State University, Detroit, Michigan 48202

Approximately 80% of bacteria on Earth's surface exist in a biofilm. Biofilms are microbial communities attached to surfaces and encased in a self-produced extracellular matrix (EPS). Biofilms pose a health and food safety risk, can induce corrosion by biofouling, and ultimately have a multitrillion dollar economic significance. Complete removal of biofilms is often difficult because of an increased tolerance to physical and chemical treatments. To develop control mechanisms, it is important to understand biofilm regulatory mechanisms.

We use *Myxococcus xanthus* as a model organism to investigate biofilm regulation. In the *M. xanthus* specialized biofilm, starvation induces cells to aggregate into mounds which mature into fruiting bodies filled with environmentally resistant spores. To determine if population density is sufficient to warrant biofilm formation, cells utilize extracellular population sensing signals: peptides, amino acids, and proteases collectively referred to as A-signal. A-signal is required for proper accumulation of at least two transcription factors, MrpC and FruA, which are essential for biofilm production. We have identified the TodK histidine kinase as an important regulator of biofilm formation in *M. xanthus*. Interestingly, overproduction of TodK (*todK*$^{++}$) completely prevents biofilm formation. Analysis of MrpC and FruA accumulation patterns in the *todK*$^{++}$ strain suggests there may be a defect in the reception or production of A-signal. Addition of exogenous A-signal to *todK*$^{++}$ did not restore biofilm production, suggesting this strain is incapable of sensing A-signal. Surprisingly, experiments to examine whether *todK*$^{++}$ produces A-signal revealed that *todK*$^{++}$ secretes an inhibitory factor that prevents biofilm formation in the wild type. Together, these results suggest TodK participates in the A-signal production pathway and overproduces a biofilm inhibition factor. We are currently investigating the nature of this factor and ascertaining if this is a universal biofilm inhibitor.

Lanthanide-dependent Methanol Metabolism in *Sinorhizobium meliloti*

Pathak, Enish*, Walser, Olivia N., Singh, Pragati, Rossbach, Silvia

Western Michigan University, Kalamazoo, Michigan, 49008

Lanthanides were thought to be biologically inert; however, the discovery of XoxF, a lanthanide-dependent methanol dehydrogenase a decade ago, shed new light on the specific roles of lanthanides in biology. A homolog of *xoxF* is also present in *Sinorhizobium meliloti*. *S. meliloti* is a soil bacterium that undergoes a symbiotic relationship with alfalfa plants by fixing atmospheric nitrogen within root nodules. In this study, we demonstrate that *S. meliloti* can grow using methanol as a sole carbon source in the presence of lanthanide ions and we observed that a *xoxF* mutant strain was unable to grow under these conditions. Through complementation of the *xoxF* mutant we successfully restored the wild-type phenotype, indicating that XoxF is the only methanol dehydrogenase enzyme in *S. meliloti*. RNAseq studies from wild-type strains showed the upregulation of genes involved in methanol metabolism when grown in the presence of lanthanides. A putative lanthanide transport pathway in *S. meliloti* is proposed in this study. A deeper understanding of lanthanide-dependent methylotrophy in bacteria would enable us to use these bacteria for carbon capture or recovery of rare earth elements, such as lanthanides from electronic waste.

Selenium nanoparticles enhance antioxidant and antimicrobial signaling in bacterial endophthalmitis.

Authors: Ahmad Zeeshan, Kolge Henry, Singh Sukhvinder, Ashok Kumar

Affiliations: Department of Ophthalmology, Vision and Anatomical Sciences, Wayne State University School of Medicine, Detroit, Michigan.

Purpose: Bacterial endophthalmitis is a debilitating infectious disease caused by bacterial pathogens introduced into the eye during surgery or trauma, leading to blindness. Our recent study showed that during endophthalmitis, the antioxidant signaling mediated by glutathione peroxidase 4 (GPX4) is significantly diminished, resulting in increased retinal cell death through ferroptosis. Given that selenium is an essential component of GPX4, we tested the hypothesis that supplementing with selenium nanoparticles (SeNPs) could restore GPX4 activity and promote cell survival during infection.

Method: Selenium nanoparticles (SeNPs) were synthesized and tested alone or in combination with the antibiotic vancomycin. In vitro studies were conducted using mouse bone marrow-derived macrophages (BMDMs) or retinal Müller glia cells challenged with *Staphylococcus aureus.* The direct antimicrobial activity of SeNPs was evaluated using broth dilution and disc diffusion methods against methicillin-resistant S. aureus (MRSA) and the laboratory strain RN6390. In vivo studies were performed by intravitreal injection of SeNPs in a mouse model of S. aureus endophthalmitis. The progression of the disease was evaluated using non-invasive methods such as electroretinography (ERG), as well as invasive methods including assessment of bacterial burden and cytokine levels

Results: Our nanoformulations revealed that the SeNPs had a size range of 40-60 nm with a negative zeta potential. The loading of vancomycin onto these nanoparticles was found to be 92%. Our data demonstrated that Selenium nanoformulation exhibited potent antibacterial activity against S. aureus, including MRSA and RN6390 strains, with a minimum inhibitory concentration (MIC) of 0.5ug/ml. Furthermore, SeNPs treatment restored GPX4 levels, reduced lipid peroxidation, and inhibited bacterial-induced ferroptosis in retinal cells.

Conclusion: Our findings demonstrate that SeNPs loaded with vancomycin effectively kill bacterial strains and enhance antioxidant signaling by increasing GPX4 expression. Thus, this dual-acting nanoformulation holds promise as a therapeutic agent for bacterial endophthalmitis.

Title: *Staphylococcus aureus* metallophores contribute to virulence in inducing endophthalmitis

Authors: Susmita Das[1] and Ashok Kumar[1]

Affiliations: [1]Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University School of Medicine

Purpose: Transition metals are essential nutrients needed for the growth and virulence of bacterial pathogens. In *Staphylococcus aureus*, metal ions such as zinc (Zn), cobalt (Co), nickel (Ni), iron (Fe), etc. contribute to several processes such as metabolism, DNA synthesis, regulation of virulence factors, and defense against oxidative stress. Vertebrate hosts sequester these transition metals to fight bacterial infection, and this innate immune response is called "nutritional immunity." To infect the host, *S. aureus* must overcome host sequestration of these critical nutrients. In the present study, we aim to find these virulence factors of *S. aureus* contributing to endophthalmitis.

Methods: USA300 JE2 wild-type and its isogenic mutant strains encoding for Zn-family metallophores were ordered from BEI resources. Growth curves were compared between the wild-type and mutant strains. In vitro studies were performed to assess the adhesion, invasion, and intracellular replication behaviors of the different strains in cultured human retinal pigment epithelial cells (ARPE-19), human Müller glial cells (MIO-M1) and human leukocyte monocyte cells, differentiated to macrophages (THP1). Endophthalmitis was induced by intravitreal injections of the wild-type and mutant strains in C57BL/6 mice. Disease progression was monitored by eye exam using slit-lamp microscopy, bacterial burden enumeration, assessment of inflammatory mediators to compare between wild-type and mutants. The expression of inflammatory mediators was also evaluated in MIO-M1 and ARPE-19 cells via qPCR and ELISA post bacterial challenge. LDH assay was done to determine the cytotoxic effects of the strains in the cell lines.

Results: Our data showed no difference in growth rate between the wild-type and mutant strains (∆*aur,* ∆*cntA,* ∆*cntD,* ∆*cntM, and* ∆*cntL)*. In vitro infection studies revealed that ∆*aur*, encoding for a biofilm synthesis protein aureolysin was significantly reduced in adhesion in both ARPE-19 and THP1 cells. However, ∆*cntD*, and ∆*cntL* encoding for the metallophore staphylopine showed significant reduction in their adhesion to THP-1 cells. Although, the intracellular replication assay in ARPE-19 did not show any changes among the strains, ∆*aur* and ∆*cntL* had drastic reductions in their cytotoxicity levels as compared to the wild-type in the same cells. The intracellular replication had a differential phenotype for all the mutants in MIO-M1 and THP1 cells. All the mutants exhibited significant replication defect with no changes in cytotoxicity for ∆*aur* in MIO-M1. The other mutants showed a slight reduction in their LDH levels as compared to wild type. Further, the inflammatory mediators in MIO-M1 and ARPE-19 cells exhibited significantly reduced expressions in the mutant infected cells. In vivo studies indicated reduced corneal haze in the mice eyes infected with ∆*aur* and ∆*cntL,*

with significant reduction in bacterial burden for ∆*cntL*. However, the whole eye lysates had reduced levels of cytokines expressions in both the mutant infected eyes as compared to the control.

Conclusion: Our study revealed that metallophores in *S. aureus* are essential for its virulence and help in causing endophthalmitis. These data could be explored further to develop therapeutic targets to treat bacterial ocular infections.

Immune regulatory gene-1 (IRG-1) deficiency exacerbate autoimmune uveitis by promoting Th1 and Th17 immune response

Authors: Sukhvinder Singh[1] and Ashok Kumar[1]

[1]Department of Ophthalmology, Visual and Anatomical Sciences, Kresge Eye Institute, Wayne State University School of Medicine, Detroit, MI, USA.

Purpose: We recently reported the activation of the IRG1/itaconate axis in promoting inflammation resolution during endophthalmitis. This study aims to determine the immunomodulatory role of IRG1 in the pathogenesis of autoimmune uveitis (EAU), the most common cause of intraocular inflammation in patients with autoimmune conditions.

Methods: EAU was induced in wild-type (C57BL/6J) or *Irg1*$^{-/-}$ mice by immunization with interphotoreceptor retinoid-binding protein (IRBP). Disease severity was assessed based on fundus examination and retinal function (ERG) testing. Ocular tissues (retina and cornea) and cervical lymph nodes were harvested at 21 days post-immunization. Flow cytometry was used to determine the frequencies of myeloid (macrophages, monocyte, and neutrophils) and lymphoid (Th1, Th17, and Tregs) cells. In vitro, studies were performed using mice splenocytes and challenging them with IRBP in the presence or absence of itaconate, a metabolite produced by IRG1 activity. ELISA and qPCR assays were performed to measure inflammatory cytokines/chemokines.

Results: Our data show that *Irg1*$^{-/-}$ mice developed severe EAU compared to wild-type mice. The exacerbated EAU in *Irg1*$^{-/-}$ mice is accompanied by increased frequencies of myeloid cells, specifically macrophages, as well as effector T cells (Th1 and Th17) and decreased levels of regulatory T cells (Tregs). In vitro experiments using splenocytes revealed activated effector T cell phenotypes were enhanced in *Irg1*$^{-/-}$ mice. However, in splenocytes treated with a 4-OI, the activated T cell phenotypes and production of inflammatory mediators were significantly suppressed.

Conclusion: Our study demonstrates that IRG1 regulates innate and adaptive responses during EAU. Itaconate supplementation exerts anti-inflammatory effects, indicating its therapeutic potential in the treatment of EAU.

Characterization of a novel C-terminal cell surface targeting motif in *Vibrio cholerae*

Cameron Roberts[1], Austin Shannon[1], Kwame Kannatey-Asibu[1], Konstantin Korotov[2], and Maria Sandkvist[1]

[1]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

[2]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA

The Type II Secretion System (T2SS) is responsible for the transport of pre-folded effector molecules through the outer membrane of select gram-negative bacteria. Effector molecules include hydrolytic enzymes and toxins, such as cholera toxin, the primary virulence agent of *Vibrio cholerae*, which are freely released to the extracellular space following transport. Still other T2SS effectors that are expressed with a C-terminal tripartite tag termed GlyGly-CTERM are localized to the cell surface and partially released with outer membrane vesicles (OMVs). These effector molecules, including the *V. cholerae* serine protease VesB, are largely unrelated apart from the C-terminal tag. Bacteria that express proteins with a GlyGly-CTERM are also found to encode a unique rhomboid protease, rhombosortase (RssP). Surface and OMV localization of VesB requires both processing by RssP and transport by the T2SS. To test the ability of the GlyGly-CTERM to target proteins normally lacking a GlyGly-CTERM to the cell surface and subsequently outer membrane vesicles, hemagglutinin protease (HAP) was tagged with a GlyGly-CTERM. Addition of a GlyGly-CTERM to HAP, which is a T2SS dependent effector normally released to the extracellular space, results in partial surface localization, a fraction of which is released in association with OMVs. Similarly to VesB, surface localization of the active HAP chimera requires both RssP and a functioning T2SS. Conversely, preliminary results indicate the GlyGly-CTERM did not allow the normally periplasmic protein β-lactamase to be secreted indicating that the GlyGly-CTERM functions in tandem with additional secretion signals for surface localization. Additional chimeras consisting of VesB and the GlyGly-CTERM from additional proteins reveal that this domain functions independently of its passenger protein as a molecular ZIP code to retain T2SS effectors to the cell surface. Study of this system could be leveraged towards the development of new technologies and will also aid in the understanding of *V. cholerae* pathogenesis as the GlyGly-CTERM protein VesB has been shown to cleave the A subunit of cholera toxin, possibly resulting in its activation.

Reconstitution of an ATP-dependent positioning system for synthetic condensates in *Escherichia coli*

Bacteria can spatially organize their biochemical reactions via the use of biomolecular condensates – membrane-less organelles that consist of a dense phase with material properties that differ from the surrounding cytoplasm. Given the importance of biocondenates in providing reaction isolation for a diversity of biological processes across all cell types, understanding and controlling the subcellular organization of biocondensates has become a topic of great biotechnological interest. However, condensates are only recently becoming appreciated in bacteria and much of their regulation is unclear. One of these being the spatial regulation of condensates within the cell. To date, the only known system that spatially regulates condensates is the carboxysome positioning system. Carboxysomes are responsible for ~35% of our atmospheric carbon fixation. In cyanobacteria and some chemoautotrophs, carboxysomes are evenly distributed on the nucleoid. The carboxysome positioning system (McdAB) is a minimal and self-organizing system, composed of two proteins: an ATpase called McdA, and its partner protein, McdB. In its native host, McdA non-specifically binds to the nucleoid and oscillates along the cell length to position carboxysomes. Meanwhile McdB associates with both the carboxysome and McdA which stimulates McdA's ATPase activity. This results in a system that can constantly position the McdB-bound carboxysomes by following the oscillating McdA gradient. Our goal is to reconstitute the McdAB system to position synthetic condensates in *E. coli* to provide a model system for the study and control of spatially regulating biocondensates in bacteria as a biotechnological tool. Here, we created a fluorescent fusion of McdA and showed that McdA binds the *E.coli* nucleoid. We also find that McdB can form condensates in *E. coli*. When these proteins are coexpressed in *E. coli*, strikingly, McdA is able to distribute multiple McdB condensates along the length of the nucleoid. These experiments provide a foundation for the development of a minimal and self-organizing condensate positioning system that allows for the distribution and faithful inheritance of condensates following cell division.

Alveolar Neutrophilia is Temporally Correlated with Staphylococcal Enrichment, not Pulmonary Injury, Following Hyperoxia Exposure

Brody R. Mayoras[1]*, Brooke M. Rosenthal[1], Shanna L. Ashley[1], Jenna E. Luth[1], Daniel J. Fergle[1], Nicole R. Falkowski[1], Christopher A. Brown[1], Gary B. Huffnagle[1], Robert P. Dickson[1], and Jennifer M. Baker[1]

[1] University of Michigan, Ann Arbor, MI, 48104

A modified version of this abstract was presented at the 2023 American Thoracic Society conference.

Patients with respiratory conditions are commonly prescribed supplemental oxygen. Our lab has previously observed dominance of *Staphylococcus* spp. in the lungs of ventilated patients and oxygen-exposed mice, but the consequences of this staphylococcal enrichment on lung injury and inflammation are unknown. C57BL/6 mice (n=8 per timepoint) were exposed to hyperoxia (95% O_2) for 72 hours and subsequently returned to room air (21% O_2) for 2, 3, 4, 5, 10 or 14 days. Either bronchoalveolar lavage fluid (BALF) or whole lung tissue was collected on day 0 (healthy controls), day 3 (oxygen-exposed), and at given recovery timepoints. We quantified lung injury via IgM ELISA and inflammation using hemocytometry, differential leukocyte counts, and IL-1α ELISA. We measured *ex vivo Staphylococcus xylosus* growth in BALF via spectrophotometry and characterized lung microbiota via 16S rRNA gene amplicon sequencing and droplet digital PCR. We found that lung injury and IL-1α peaked at 3 days of hyperoxia exposure and decreased during recovery. Short-term changes in lung microbiota occurred, as *Staphylococcus* spp. relative abundance increased during hyperoxia and through recovery day 4. However, the 16S rRNA gene copy number and *Staphylococcus xylosus* growth in BALF were not significantly different across timepoints. Notably, the decrease in *Staphylococcus* spp. relative abundance at recovery day 5 was concurrent with alveolar neutrophilia. These data indicate that relative staphylococcal dominance, rather than outgrowth, following hyperoxia may perpetuate alveolar inflammation via neutrophilia. While hyperoxia and oxygen-induced lung injury both appear to influence staphylococcal dominance, these changes in the lung microbiome are not permanent. Future work will focus on comparative analyses of *Staphylococcus aureus* in this context.

Sara MI-ASM poster abstract 197 words, 1,412 characters (w/spaces)

Tetracycline Inducible System for Gene Expression in *Treponema denticola*

Sara Agolli1 J. Christopher Fenno and Paula Goetting-Minesky
Department of Biologic and Materials Sciences and Prosthodontics, University of Michigan School of Dentistry, Ann Arbor, Michigan

This project focuses on developing a tetracycline-inducible expression system (iTet) for the oral pathogen *Treponema denticola*, a spirochete implicated in periodontal disease. The system constitutively expresses TetR, which controls expression of a gene of interest downstream of a promoter that includes the *tetO* binding site for TetR. This enables controlled expression levels proportional to the amount of anhydrotetracycline (aTc) added. The project aims to create a shuttle plasmid construct with the iTet-gene cassette that can express in both *E. coli* and *T. denticola*. Initially, the construct targeted the *msp* gene encoding a cytotoxic pore-forming protein. However, leaky expression prevented obtaining *E. coli* clones. To circumvent this, the *fhbB* gene mediating complement resistance was used instead. Successful aTc-induced expression of FhbB was demonstrated in *E. coli* via Western blotting. Moving forward, the iTet-*fhbB* construct will be transformed into *T. denticola* to compare levels of complement resistance between the wild-type, *fhbB* knockout, and the complemented knockout harboring the inducible system. Expression calibration will optimize FhbB levels to mimic the wild-type, enabling functional studies of this virulence factor's role in serum resistance and periodontal pathogenesis. This inducible system provides a valuable tool for controlled gene expression analysis in *T. denticola*.

Abstract:

Manal Chishty
Dr. Paul Price
Poster
mchishty@emich.edu

Effect of EDTA on Production of Antimicrobial Agents in Bacterial Strains.

Antibiotic resistance is dangerously increasing at a rapid rate. To counter this, researchers all over the world are searching for novel antibiotics and novel methods to induce antibiotic production from natural sources. We hypothesized that restricting divalent cations like iron by growing multiple bacteria together with EDTA, ethylenediaminetetraacetic acid, could induce a stronger production of bacterial secondary metabolites with antimicrobial properties. We found that co-culturing multiple bacteria in the presence of EDTA in nutrient-limiting media leads to a general increase in antibiotic production.

Effectiveness of TheraBreath™ Oral Rinses Against *Streptococcus mutans*

Zaid Aboona[1], Laura A. Young[2], and Joshua J. Thomson[2]
[1]University of Detroit Mercy, Department of Biology
[2]University of Detroit Mercy, School of Dentistry, Division of Integrated Biomedical Sciences

Streptococcus mutans is a Gram-positive coccus that has the ability to metabolize dietary sugars and produce lactic acid as a byproduct. The acid in the local environment of a tooth surface can cause demineralization of tooth enamel leading to caries (tooth decay). *S. mutans* also encodes glucosyltransferase enzymes that convert sucrose into insoluble extracellular polysaccharides, which are a vital component for biofilm formation of *S. mutans* and provide binding sites for other oral microorganisms, ultimately aiding in the development of dental caries. Prevention of dental caries requires routine mechanical removal of plaque biofilm to reduce the number of acid-producing bacteria, including *S. mutans*, near tooth surfaces. Prevention may also include the use of antiseptic or therapeutic mouthrinses to kill or remove bacteria that are difficult to reach mechanically. The goal of this study was to determine the antibacterial and antibiofilm activity of various TheraBreath™ oral rinses compared to other commercial mouthrinses, using *S. mutans* as a model oral pathogen. In vitro biofilms of a type-strain of *S. mutans* were grown for 24 hours in the presence of sucrose. Oral rinses were added to wells and rotated at 100 rpm for 15 minutes. Six separate experiments were conducted, each with duplicate treatment wells. Following treatment, biofilms were assessed using a Live/Dead vitality stain to assess antibacterial activity and crystal violet stain to measure total biofilm remaining after treatment. Additionally, liquid killing assays were performed to determine bactericidal activity. Our findings suggest that TheraBreath™ Whitening Fresh Breath and TheraBreath™ Healthy Gums exhibit antibacterial properties against in vitro biofilms and planktonic *S. mutans*. TheraBreath™ Healthy Gums was the only formulation tested that had comparable bactericidal activity to other commercial brands of oral rinses.

The antibiotic resistance crisis poses a serious threat to modern medicine through an increased rate of deadly microbial infections. New antimicrobial compounds can be elicited from soil microbes when grown in competition with other microbes while also varying carbon sources. Antibiotic-producing bacterial strains were grown in co-culture on agar media plates and then inoculated into liquid media containing one of twenty polysaccharide carbon sources. Products were extracted and tested against safe relatives to pathogenic strains. We found that changing both co-cultured microbes and the polysaccharide were important in eliciting the production of antimicrobial secondary metabolites.

Identifying novel phage defense systems in *Vibrio cholerae*

Elise N. Trost, Jasper B. Gomez, Chris M. Waters

The rise of antibiotic resistance is one of the greatest public health threats today. Thus, it is important to find alternate methods to combat multi-drug resistant bacterial pathogens. One alternative is phage therapy, which has been used for almost a century and has renewed interest due to its ability to target and lyse specific bacteria without disrupting the microbiome. Bacteriophages, however, have evolved a myriad of defense mechanisms to protect against phage infection. Thus, it is important we uncover new phage defense systems and study their underlying mechanisms. To identify new phage systems, we study the bacterial pathogen *Vibrio cholerae* which is intimately linked to phage predation both during environmental persistence and during infection in humans. Additionally, three novel phage defense systems have been recently discovered in this bacterium. I hypothesized that *V. cholerae* still harbors undiscovered phage defense systems. A previous screen of a *V. cholerae* cosmid library encoding a 25kb fragment of *V. cholerae* genome within *Escherichia coli* confers protection against T2 coliphage. Further analysis of this 25kb fragment showed multiple genes identified as domains of unknown function. Using molecular techniques, I have identified one candidate gene, *IT1766*, which could be involved in phage defense, and has not been shown to be involved in any previously established phage defense systems. Currently, I am using molecular techniques to confirm that this gene confers phage defense and investigate the mechanism of defense. My studies will uncover the molecular mechanisms by which these systems protect bacteria from phage infection, improving our understanding of how bacteria can resist phage therapy in order to create more effective phage therapeutics.

The Effects of CO2, Temperature and Nutrients on Microcystis Growth and Toxin Production

MacKay, Marlayna[1] Nitschky, H.[1,2] Kramer, B.[2] May, O.[1] Boyce, J.[1] Errera, R.[2] Dick, G.[1,2]

[1] *Department of Earth and Environmental Science, University of Michigan*
[2] *National Oceanic and Atmospheric Administration (NOAA), Great Lakes Environmental Research Laboratory (GLERL), Cooperative Institute for Great Lake Research (CIGLR)*
marlaynm@umich.edu

The harmful bloom-forming cyanobacteria species, *Microcystis,* is expected to become more globally abundant in the future due to climate and anthropogenic changes. Such change includes: increased runoff of nutrients, water stratification of lakes, warming waters and acidification from increased atmospheric CO_2 levels. *Microcystis* prefer warmer environments, can produce different strains quickly, and can control their buoyancy to reach different depths within the water column. These adaptations make *Microcystis* resilient to change in the environment. The resiliency of Microcystis is a problem because ecosystem and human health is negatively impacted by the ability of certain strains to produce the hepatotoxin microcystin, among other toxins. The cleanup of *Microcystis* can be economically costly and such disasters can reduce quality of life for residents living near cyanobacteria harmful algal blooms (cHABs). Thus, it is concerning that the predicted changes are expected to benefit *Microcystis* because it may lead to the proliferation of more toxic strains that pose risk to both the local ecosystem and humans. In addition to warming temperatures, acidification is becoming a larger problem, due to global warming and CO_2 production. The atmospheric CO_2 that is absorbed into water forms carbonic acid (HCO_3^-), which causes reactions that lower the water's pH. In order to understand how different strains of *Microcystis* respond to varying levels of acidification, three strains were isolated from western Lake Erie. We used several strains of *Microcystis* isolated from Lake Erie (LE18-22.4, LE19-59.1, LE19-84.1) and monitored their growth under differing conditions, for a period of 15 days. Conditions tested include changes in CO_2 concentration (200, 400, 1000 ppm), nutrients (Urea, NO_3, NH_4) and temperature (18° C, 24° C), respectively. *Microcystis* cell density was calculated based on both manual and flow cam cell counts. Flow cam counts were used to estimate the *Microcystis* counts, while manual microscope counts were used as a ground truth to confirm calculations. Concentration of *Microcystis* was used to model growth rates, based on counts obtained by both methods. Pending results will help us better understand how *Microcystis* growth is affected by acidification and other climate change effects. Understanding how these factors will affect *Microcystis* and other parts of the microbial ecosystem can help us find ways to curb harmful impacts of *Microcystis* on human health and the aquatic ecosystem.

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

Marissa K. Malleck[1], Aathmaja Anandhi Rangarajan[1], and Christopher M. Waters[1]

Department of Microbiology, Genetics, and Immunology, Michigan State University, East Lansing, MI, USA[1]

Vibrio cholerae is a bacterial pathogen responsible for the diarrheal disease cholera. The El Tor biotype of *V. cholerae* 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 predicted chemotaxis-related protein (*vc0514*) and a cyclic di-GMP phosphodiesterase (*vc0515*). Cyclic di-GMP (c-di-GMP), a signaling molecule, regulates biofilm formation and motility and contributes to bacterial infection in *V. cholerae*. Intracellular levels of c-di-GMP are regulated by diguanylate cyclases (DGCs), which synthesize cyclic di-GMP, and phosphodiesterases (PDEs), which degrade cyclic di-GMP. We observed increased motility in a Δ*zur* mutant due to the derepression of *vc0512-vc0515* genes. Using qRT-PCR, we also found that the relative fold expression of *vc0515* in *Δzur* mutants is higher than wild type, indicating that zur is a repressor of *vc0515.* Swimming motility decreased and biofilm formation increased in a Δ*vc0515* mutant due to increased c-di-GMP concentrations. The upstream region of *vc0515* also includes a predicted *hapR* binding site that represses the promoter of *vc0515*. HapR, the central regulator of quorum sensing in *V. cholerae,* is induced at high-cell density. We observed the expression of *vc0515* was induced at low-cell density and repressed at high-cell density, suggesting HapR directly represses *vc0515*. Genome analysis of 7th pandemic strains isolated from various geographic regions 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. Our results suggest that horizontal acquisition of a c-di-GMP signaling network contributes to the emergence and persistence of the 7th *V. cholerae* pandemic.

Fatimah Rezk

Microbiology Reseach

Dr. Johnathan Finkel

Abstract

Bacteriophages are viruses that target bacteria. The demand for studying bacteriophages has risen by the importance of therapeutic phages. Because of bacteriophages' high specificity for their target bacteria, it can help us develop more targeted and 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. Our goal as a group is to identify the function of genes in the phage Xeno. Xeno is a siphovirdae bacteriophage that targets Mycobacterium smegmatis. Its genome is 42395 nucleotides long and was calculated to contain 69 different genes. Only 27 of the 67 proposed functions. The purpose is to complete the cytotoxicity and defense assays and begin the cloning required for the hybrid experiments.

Alexandra Walsh
Paul Price, faculty mentor
New antibiotics are needed to combat the antimicrobial crisis. Co-cultured bacterial strains when grown in various polysaccharides supplemented with known antibiotics can lead to the production of novel antimicrobial metabolites from otherwise genetically silent biosynthetic gene clusters. We tested four *Streptomyces* strains and one non-actinomycete strain by co-culturing them with three different auxotroph strains (*M. smegmatis, E. coli,* and *B. subtilis*) on media supplemented with known antibiotics. These strains were then co-cultured with various polysaccharides to look for new antibiotic production. We observed a general increase in antibiotic production under these conditions.

Effect of short-term fluoride exposure on *S. mutans* acidogenesis: an *in vitro* biofilm study

Yoshino N, Calderon SC, Kashima M, Almalki J, Capalbo LC, Tenuta LMA

Fluoride is the main anticaries agent, and it works mainly by affecting the dynamics of tooth mineral demineralization/remineralization. However, fluoride can also affect microbial metabolism, which is considered of less importance for caries control, because of the higher fluoride concentrations needed. In this study, we investigated if short-term exposure of cariogenic biofilms to fluoride (concentrations ranging from that in over-the-counter (OTC) mouthrinses to professional use products) would affect bacterial acid production. *S. mutans* biofilms (3 independent experiments) were formed for 4 days on 4 x 7 mm bovine enamel slabs suspended in tryptic soy broth with either 1% sucrose for 8h/day (feast period), or with 0.1 mM glucose for 16h/day (famine period). Different fluoride treatments, with increasing fluoride concentrations (0, 226 ppm F (fluoride concentration in OTC mouthrinses), or 2,260 ppm F (10 times higher)) were applied for 1 min before and after each feast period (n=6 biofilms/experiment). Culture media was changed at each feast-famine period and its fluoride concentration and pH (indicator of biofilm acidogenicity) were determined. Fluoride in the media and pH values after the last feast challenge were compared by ANOVA ($\alpha=5\%$). In all experiments, after the last feast challenge, fluoride concentration in culture media was increasingly higher ($p<0.05$) as the F in treatments increased (0.1 ± 0.03, 0.5 ± 0.04 and 2.7 ± 0.4 ppm F for groups 0, 116 and 2,260 ppm F, respectively). Likewise, pH was significantly higher ($p<0.05$) for the 2,260 ppm F group when compared with the other two (4.5 ± 0.3, 4.6 ± 0.4 and 5.1 ± 0.4, respectively). The results confirmed the inhibition of acidogenesis by fluoride, but only at concentrations higher than those achieved with daily use of fluoride mouthrinses.

Abstract

The CDC is aware of at least 25,000 candidemia which is a fungal bloodstream infection occurs each year. *Candida albicans* was the focus of this lab over the summer, it is an organism that is famously known to cause severe infection by building off of microbial surfaces known as biofilms. Biofilms form when yeast cells attach to a surface and then later fungal cells spread, beginning the change from yeast to a filamentous cell. After the final step of maturation, any cell that is not attached to the matrix are then released into the surrounding environment. This is precisely what causes the infections we see. What this summers research was focused upon was understanding the how, what, and why of the attachment and if there could be any way we could target a drug to resolve this issue. We looked for defects in the attachments by completing a virulence assay and using many strains from a mutant library, a positive and negative control was used alongside it as well. What was found of interest particularly was the Cht2 gene because of its enhanced virulence .

Title: Microbial Mysteries Deep Underfoot - In Iowa and China

Authors: Shannon P. Carraway*, Hansen Qian*, Faith J. Nhkum*, Krishen A. Patel*, James M. Tiedje, John F. Quensen, Xun Qian

Address: Michigan State University, East Lansing, Michigan, 48823

Abstract:

The Earth's terrestrial microbiome information comes from the top 30 cm of soil, yet soil can extend far beyond that. We hypothesized that these deeper soil microbes differ from those known due to their unique selective conditions, specifically carbon scarcity. Loess soils are wind-deposited over geologic time and can be 100 m deep and more easily sampled. We drilled samples from the Loess Hills of Western Iowa and the Loess Plateau of China, the deepest loess soils in the world. These soils were deposited over the last 75,000 years, and their organic carbon is minimal and stable.

We extracted DNA, and with 16S rRNA sequencing identified novel phyla and classes, the most prominent being a novel phylum, GAL-15, found in both Iowa and China. We also performed 18S, ITS, shotgun, and Nanopore long-read sequencing to characterize the community further. We attempted to grow novel taxa by incubating samples from elevated GAL-15 cores for 9 months with extremely low organic carbon to simulate their natural environment. To determine if some of the microbiome was metabolically intact, we incubated core samples with 14-C-glucose.

16S sequence analysis established a more extensive depth profile of GAL-15, which also showed high Proteobacteria and Actinobacteria. The highest GAL-15 was 14.6% at 14 m at Iowa's Hitchcock Nature Center. At this location, fungal phylum Mortierellaceae generally increased in relative abundance with depth, reaching a majority at 7 m and continuing until 10 m. The long-term incubations with cold soil extract as carbon source enriched unclassified amplicon sequence variants (ASV). Most attempts at colony isolation on low-nutrient media yielded Rhodococcus and Micrococcaceae. A few colonies are mixed taxa and appear to be incapable of independent growth, an example being Bacillus, which grew only with Micrococcaceae. At least some of the deep soil microbiome is metabolically fit as 14-CO2 was produced within 30 min. Through the characterization of these deep soil novel microbiomes, we can further understand these organisms' ecological role, physiology, and resilience in the world's predominately oligotrophic environments.

Microbial Diversity Associated with *Craspedacusta sowerbii* from Local Michigan Lakes

Montroy, Kelsie, M.*
Halcrow, Grace S.
Temar, Hallie M.
Angell, Michael
Eastern Michigan University

The freshwater ecosystems of the Great Lakes region are home to an abundance of biodiversity, including the little known non-native jellyfish species, *Craspedacusta sowerbii*. This small invertebrate is originally found in the Yangtze River Valley in China, and has been present in North America for the better part of a century. *C. sowerbii* populations are projected to grow exponentially in the coming years due to rising global temperatures, consequentially leading to a rise in their associated microbial populations. This project aims to identify and characterize specific bacterial species associated with *C. sowerbii*. Jellyfish were isolated from local freshwater lakes, Pickerel Lake and Cordley Lake. Microbial populations were homogenized through a 40µm mesh and cultured on TSA, FW70, and R2A agar media in order to support a variety of microbial species growth. Microbial isolates were characterized by morphology, biochemical assays, and 16s rRNA sequencing to later identify using the BLAST database. Of the forty-one isolates obtained, the majority belonged to genera including: *Vogesella, Brevundimonas, Pseudomonas,* and *Chryseobacterium.* Some genera found are commonly associated with fresh water bodies and are not a concerning factor. However, others exhibited concerning traits such as virulence factors and drinking water contaminants. With the projected rising populations of *C. sowerbii*, understanding the associated unique microbiome and its influence on water quality are integral to monitoring the health of local freshwater ecosystems.

Incorporation of Microbiology-focused Activities During Oral Hygiene Instructions to Enhance Oral Health Knowledge

Mohammed Almawiri[1] and Joshua J. Thomson[2]

[1]University of Detroit Mercy, Department of Psychology, Detroit, MI 48221
[2]University of Detroit Mercy, School of Dentistry, Division of Integrated Biomedical Sciences, Detroit, MI 48208

ABSTRACT

Background: Oral diseases continue to be a major public health concern globally, necessitating the development of comprehensive oral health initiatives. Comprehensive care includes health education strategies that highlight the importance of disease prevention. Bacteria are central to most oral diseases, yet few studies have explored the potential impact of including specifics of microbial pathogenesis on oral health education. Therefore, the purpose of this study was to determine if integration of microbiology-focused instructions and activities improve oral health knowledge, beliefs, and behaviors.

Methods: A pre-test/post-test experimental study was conducted among undergraduate life-science students. Upon randomization, students were allocated to the control group (n=35) who received Standard Oral Hygiene Instructions (SD-OHI) and to the experimental group (n=40) who received Microbiology-focused Oral Hygiene Instructions (MICRO-OHI). Questionnaires were given to each group before and after the educational intervention for measurement of changes in oral health behaviors, beliefs, and knowledge.

Results: Descriptive statistics were performed on the pre-test/post-test responses for SD-OHI and MICRO-OHI. Both groups showed improved responses on items related to oral health behaviors, beliefs, and knowledge after educational intervention. Items related to the microbiology of dental disease had significant improvement in the MICRO-OHI group ($p<0.05$) compared to the SD-OHI group ($p=0.50$) measured using the McNemar-Bowker test. Qualitative analysis of responses to “Why should we brush our teeth?” revealed that a higher percentage of MICRO-OHI added emphasis on bacteria/biofilm removal in their responses compared to SD-OHI.

Conclusions: This study demonstrates that both methods of instruction increased health-promoting responses after intervention and that the addition of microbiology-focused instructions has the potential to uniquely augment comprehensive oral healthcare strategies.

Keywords: Oral Health Education (OHE), Oral Health Knowledge (OHK)

The CDC is aware of at least 25,000 candidemia which is a fungal bloodstream infection that occurs each year. *Candida albicans* was the focus of this lab over the summer, it is an organism that is famously known to cause severe infection by building off of microbial surfaces, known as biofilms. Biofilms form when yeast cell attach to a surface and then later fungal cells spread beginning the change from yeast to a filament cell. After the final step of maturation any cell that is not attached to the matrix or then released into the surrounding environment. This is precisely what causes the infections we see. What this summer's research was focused upon was understanding the how, what, and why of the attachment and if there could be any way, we could target a drug to resolve this issue. We looked for defects in the attachments by completing a virulence assay and using many strains from a mutant library, a positive and negative control was used alongside it as well. What was found of interest particularly was the. Cht2 because of its enhance virulence.

Evolution of Bacteriophages ICP1 and ICP3 in various Vibrio Cholera Hosts

Mehak Banga, Kaylee M. Wilburn, and Christopher M. Waters

Bacteria and bacteriophages are in a constant evolutionary arms race; with the predation of one leading to the selection of the other, all for one goal: survival. A well-known bacterial species, *Vibrio cholera*, interacts with various aquatic microorganisms and exists in numerous environmental and biological reservoirs. However, when *V. cholera* enters its infectious stage, it has the potential to cause a global pandemic. To combat the latter, specific bacteriophages that target this species were isolated. These phages are called ICP1, ICP2, and ICP3. The purpose of this study is to examine the genomes of those three bacteriophages- ICP 1, ICP2, and ICP 3- in relation to their *V. cholerae* hosts and identify the mutations that occur in the phages' genes when placed in *V. cholerae* wild type versus its mismatch repair (MMR) deficient mutant- Δ*mutS*. To accomplish this, 15 generations of the ICP phages will be grown, selected, amplified, and purified. Once the process is complete, an intensive genomic analysis of the first, fifth, tenth, and the fifteenth generation phage will be conducted. The sequencing and studying of the phage mutations over its initial, fifth, tenth, and fifteenth generation has the potential to underscore the effects a bacterial MMR deficient system has on the phage mutation rates. A greater insight into the way bacterial defense systems impact phages' genomes allows for a better foundational understanding of bacteria-bacteriophage interactions and potentially permits further exploration or utilization of that relationship.

Characterization of Phase Variation in Myxococcus xanthus Cell Fate Segregation

Jacob DeTone*, Shelby Kasto, and Penelope I. Higgs
Wayne State University, Detroit, MI 48304

Myxococcus xanthus is a gram-negative soil bacterium that is a model system for complex multicellular behavior. To obtain nutrients, swarms of *M. xanthus* predate on other microorganisms by collective secretion of antibiotics and degradative enzymes. Under starvation conditions, *M. xanthus* instead launch a developmental program resulting in at least three distinct cell fates: production of multicellular fruiting bodies filled with environmentally resistant spores (~15% of the population); a persister-like state termed peripheral rods that are arrayed outside of the fruiting bodies (~5% of the population), and programmed cell death (~80% of the population). Little is known about the regulatory mechanisms that control programmed cell death.

M. xanthus cells undergo "yellow/tan" phase-variation. Yellow cells express the yellow pigment DKxanthene, the antibiotic myxovirescin, and are adept in predation. In contrast, tan cells are specialized for siderophore production. Under standard laboratory growth conditions, yellow cells comprise 70-80% of the population, but lack of iron increases the proportion of tan cells. Although both yellow and tan cells are required for production of mature fruiting bodies, it is not known how yellow and tan cells contribute to developmental cell lysis or production of peripheral rods. We assessed the yellow/tan ratios of mutants in genes known to be involved in developmental cell fate segregation. These analyses indicate that the majority of mutants tested exhibit perturbations of the yellow/tan ratio observed in the wild-type strain. Interestingly, extreme population bias towards either yellow or tan cells may be associated with mutants that exhibit reduced or enhanced programmed cell death, respectively. We are currently exploring whether yellow cells predominantly undergo developmental cell lysis.

Quantification of *Aspergillus sp.* in Cannabis

Jirsa, Jake R.* Hutchens, Martha A.

Lake Superior State University, Sault Sainte Marie, MI, 49783

Fungi and mycotoxins are very well-studied contaminants in the food and agriculture industry but are under-studied in the cannabis industry. The toxins produced by Fungi and their pathogenic effects, specifically *Aspergillus sp.*, are especially concerning when the main methods of consumption are inhalation of combusted plant material or consumption of edibles. Inhaling or ingesting mycotoxins can cause serious illness. Radio frequency treatment is one method of remediating microbes and their toxins in agricultural products such as Cannabis. In this study, culture plates were used to analyze various Cannabis samples for *Aspergillus sp.* and to determine the effectiveness of radio frequency treatment to remediate fungi and other microbes. It was found that many of the samples were contaminated with *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, and Aspergillus terreus* among other fungi. The sensitivity of our methods was too low to determine how effective the radio frequency treatment is, however, we detected *Aspergillus* in only one of the post-remediation samples. These findings are concerning to consumer safety but are promising regarding the effectiveness of the radio frequency treatment and give reason to study methods of remediation as a potential way to minimize this contamination.

Investigating Regulatory Mechanisms of AvcID Phage Defense Systems

Muethel, Aubree C (*), Ferrell, Micah J, Waters, Christopher M

AvcID is a novel type III toxin-antitoxin system encoded on the VSP-1 island of *Vibrio cholerae* that functions as an anti-phage defense system with homologous systems found in many medically important bacteria. AvcD (Antiviral Cytidine Deaminase) is an enzyme that deaminates deoxycytidine, effectively starving phages of nucleotides necessary for replication. There are two domains present in AvcD, a C-terminal deoxycytidylate deaminase (DCD) domain which deaminates dCTP and dCMP and an N-terminal P-loop NTPase (PLN) domain of unknown function. Purified AvcD was inhibited by high concentrations of ATP and AvcD overexpressed in bacterial cells was not active in growth inhibition until the stationary phase, when ATP concentrations decline. We hypothesize that AvcD deamination by the DCD domain is regulated by the cell's energy state via ATP binding to the PLN domain. To test this, we have evaluated mutations in a putative nucleotide binding pocket of the PLN domain for enzymatic activity, deoxycytidine starvation, and phage defense. Evaluating PLN domain mutants will elucidate how AvcID toxin-antitoxin systems function in a cellular context to confer anti-phage defense. Understanding regulation of phage defense systems like AvcID is key to future deployment of effective phage therapy as an alternative to antibiotics.

Evaluating the Repair of DNA at Broken Fragile Sites

Abstract

DNA fragile sites are difficult to replicate and insufficient quantities of DNA polymerase alpha, which causes a delay in DNA replication, result in chromosome breakage at fragile sites. Mis-repair of broken fragile sites can result in mutations and chromosome translocations. On chromosome III of yeast cells, there is a fragile site called "FS2" and it is composed of two Ty1 elements (retrotransposons). There are 25 Ty1 elements throughout the yeast genome, thus there are 25 potential sites of homology for repair of breaks at this fragile site.. The goal of this project is to examine how DNA is repaired at broken FS2 and to identify which Ty1 elements are used for repair at FS2. To identify if certain Ty1 elements are more favored for repair at FS2, an illegitimate mating assay, CHEF gel, whole genome sequencing, and statistical analysis were performed. Our preliminary data indicates that the selection of a Ty1 element for repair of breaks at FS2 is not arbitrary. These discoveries have important implications for our knowledge of how genetic material remains stable in eukaryotic organisms.