LIGHTNING TALKS

Recovery of *Salmonella* in Environmental Waters using Backflush Solutions and Dead-End Ultrafiltration Filters

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Introduction: Dead-End Ultrafiltration (DEUF) is used to recover pathogenic bacteria like *Salmonella* from irrigation and surface waters. Bacteria are eluted from the DEUF filters using a backflush (BF) solution. Understanding survivability on/in the filters and BF is important to ensure accurate detection of live pathogens. **Purpose:** To assess *Salmonella* survival in BF solutions and DEUF filters stored at 4°C over time, focusing on solution composition, natural contamination, and filter effects. **Methods:** Daily plate counts on modified tryptic soy agar-yeast extract (mTSA-YE) were performed. Five BF solutions (complete BF, Tween80, NaPP, Y-30, and DI-H2O) were inoculated with *Salmonella* Gaminara GFP 5695 and survival was assessed daily. BF eluates from surface water DEUFs (n=3) and one control (DI-H_2O) were inoculated with GFP *Salmonella* and stored at 4°C. DEUFs (n=30) filtering surface water were inoculated (10^6 CFU/mL) and stored at 4°C. Filters were backflushed daily (three positive, one control), and eluate plating was conducted over six days. **Results:** No significant effect of BF composition was observed on *Salmonella* survival (p=0.907). Inoculated BF showed a significant time effect (p=0.014), with log CFU/mL reduction from Day 2 (5.31) to Day 8 (3.70). Background microflora counts declined significantly from Day 2 (5.61) to Day 8 (4.73). Filter storage showed consistent Gaminara levels (average=3.74), while background flora declined over time (average=3.14). **Significance:** These findings demonstrate *Salmonella*'s stability during storage at 4°C on DEUF filters and in all tested BF solutions, unlike the background microflora, giving more flexibility of storage time before analysis for live pathogen detection.

Rapid Detection of Active Coronavirus Infection by Lateral Flow Test Strips: A New Approach to Distinguish Replicating Viruses from Non-Replicating Viruses

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As the Coronavirus Disease 2019 (COVID-19) pandemic continues to challenge global health systems, the emergence of immune-evasive variants has led to complicated pharmaceutical and diagnostic interventions. This underscores the urgent need for innovative diagnostic tools that can distinguish between active and dormant viral particles. Our research focuses on developing a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas13a-based Lateral Flow Assay (LFA) targeting the antigenome of mouse hepatitis virus A59 (MHV-A59), a surrogate model for coronaviruses, such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). By detecting active viral replication through CRISPR-LFA, this method offers significantly improved accuracy in infection detection compared with traditional positive RNA-based systems. Furthermore, this research addresses critical biological questions, including the processing of viral RNA segments, such as the nucleocapsid and spike protein genes, during replication, as well as the influence of host immune responses on these processes. This tool enables the real-time monitoring of viral dynamics, providing valuable insights into viral life cycles. From an epidemiological perspective, developing this diagnostic tool could significantly enhance public health by enabling earlier and more accurate detection of infections, facilitating timely intervention strategies, and improving outbreak management and control. These advancements are essential for a better understanding of viral behavior and for controlling future pandemics.

Exploring The Role of MbcS-Dependent Branched-Chain Fatty Acid Synthesis in *Staphylococcus aureus*

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Staphylococcus aureus is a major contributor to polymicrobial infections, such as those in chronic wounds, where it is commonly found with *Enterococcus faecalis* and *Pseudomonas aeruginosa*. Antibiotic resistance often results in treatment failure. As the cytoplasmic membrane has emerged as a target for antibiotic therapy, a greater understanding of the mechanisms involved in its biogenesis is critical to managing these infections. We know that *S. aureus* is highly reliant on branched-chain fatty acids (BCFAs) for membrane biogenesis during infection, and that the branched-chain a-keto acid dehydrogenase (BKDH) complex synthesizes the primers for fatty acid synthesis *in vitro.* We recently discovered a second route to BCFA synthesis that depends on a branched-chain acyl-CoA synthetase named MbcS, which mediates salvaging of exogenous precursors. To understand the conservation of MbcS across species, we performed bioinformatics analysis and found that *mbcS* is only present in *S. aureus* and a few other human-associated staphylococci. Interestingly, our analysis revealed that the presence of *mbcS* is anticorrelated with two genes annotated to encode butyrate kinase (*buk*) and phosphotransbutyrylase (*ptb*), which can salvage precursors for BCFAs synthesis in other species. We are currently testing the *hypotheses that MbcS is a high affinity pathway that replaced Ptb and Buk to compete for BCFAs precursors in the polymicrobial environment*. Our findings will be discussed. Overall, this research will provide insights into mechanisms underlying microbial synergy in the polymicrobial environment and is an important first step toward developing novel therapeutics to combat these complex infections.

A Comprehensive and Synergistic Approach to Killing *Serratia marcescens*

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Background: *Serratia marcescens* is a gram-negative, anaerobic bacterium responsible for hospital-associated infections, particularly in elderly, and neonatal patients. Its ability to form biofilms on medical devices and acquire antibiotic resistance significantly complicates treatment. This study explores a comprehensive approach combining antibiotics, bacteriophages, and antimicrobial peptides to combat *S. marcescens* in both planktonic and biofilm forms. **Methods:** Eleven *Serratia marcescens* strains were cultured in THB media. Planktonic cultures were prepared by inoculating single colonies into THB and incubating at 37°C with shaking for 24 hours until an OD600 of ~108 CFU/mL was reached. For biofilm formation, diluted cultures were added to well plates and incubated statically at 37°C for 24 hours. Biofilms were fixed, stained, and dissolved for quantification at 490 nm. Growth was assessed via OD600 for planktonic cultures and crystal violet staining for biofilms. **Results:** Effective antibiotics include pen-strep, kanamycin, chloramphenicol, with combination treatments significantly reducing biofilm formation ($p < 0.0001$). Incorporating bacteriophage with antibiotics achieved the most substantial reduction in both planktonic and biofilm cultures ($p < 0.0001$). The addition of antimicrobial peptides further enhanced efficacy, resulting in approximately 98% planktonic and 95% biofilm reduction across all tested strains, including CDC-resistant strains. Growth curves and heat maps demonstrated resistance patterns. **Discussion**: This study underscores the potential of combining antibiotics, bacteriophages, and peptides as a robust therapeutic approach against *S. marcescens*, particularly in eradicating resistant biofilms. Future research will focus on evaluating the efficacy of these treatments in vivo to assess their potential for clinical application.

Identification of the Essential Proteins for *N*-acetylglucosamine Transport and Metabolism by the Gram-negative Saprophyte *Cellvibrio japonicus*

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Understanding microbial degradation of recalcitrant polysaccharides is crucial for a variety of biological processes, including nutritional contributions of the gut microbiome, global carbon cycling, and the production of renewable fuels and chemicals. Chitin is the second most abundant polysaccharide found in nature, and consequently its utilization by microbes plays a significant role in carbon and nitrogen cycling. Chitin derivatives are also increasingly being used for biotechnological applications, specifically its monomeric subunit *N*-acetylglucosamine (GlcNAc). Chito-oligosaccharides are found in formulations for joint supplements and soil amendments, making chitin degradation products key metabolites in both biomedical and agricultural industries. To study the degradation and metabolism of chitin, our laboratory uses the saprophytic Gram negative soil bacterium *Cellvibrio japonicus,* which has emerged as a powerful model microbe due to its ability to degrade numerous insoluble polysaccharide substrates. While the early stages of chitin depolymerization by *C. japonicus* have been characterized, the mechanisms for the latter stages of chitin utilization, particularly the transport and metabolism of GlcNAc, are currently unknown. Using a systems biology approach that included transcriptomics and mutational analysis, our lab has identified several key genes the encode proteins for the transport and metabolism of GlcNAc. On-going work will characterize every gene in the operon to assign each a physiological function. We anticipate that by understanding the mechanisms by which *C. japonicus* transports and metabolizes GlcNAc we will gain insight in the final stages of chitin bioconversion, and the application of this knowledge will help enable biomedical and/or agricultural applications.

CRISPR Engineering of Bacteriophage T4 Nanoparticle as a Next-Generation Flavivirus Vaccine Design Platform

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Dengue virus (DENV) and Zika virus (ZIKV), both members of the Flavivirus genus, pose significant global public health threats. The currently licensed live-attenuated dengue vaccine has limited efficacy, and there's no approved Zika vaccine. T4 bacteriophage nanoparticles show promise for next-generation vaccine development. Utilizing CRISPR-based genome engineering, we repurposed the T4 bacteriophage as a vaccine delivery system by genetically incorporating antigenic domains onto its capsid surface. As the immunogen, we selected domain III (DIII) of the viral envelope (E) protein, known for its independent folding and capacity to elicit a potent neutralizing antibody response. DIII was fused to the small outer capsid (Soc) protein of T4 and inserted into the phage genome using Cas12a-mediated genome editing. Each engineered phage particle displayed approximately 50 copies of Soc-DIII. To enhance antigen display, we optimized regulatory elements controlling Soc-DIII expression. Codon optimization using a T4–*E. coli* balanced codon set resulted in an 8.9-fold increase in expression compared to unoptimized sequences. Also, dual optimization—balancing codon usage between T4 and *E. coli*—led to a 2.17-fold improvement over E. coli optimization. Doubling the T4 late promoter in combination with balanced codons further enhanced expression by 4.47-fold relative to the single-promoter construct. We developed seven vaccine constructs using the optimized system, e.g., monovalent, divalent, and tetravalent formulations targeting all four DENV serotypes and a ZIKV-specific candidate. Immunogenicity assessments of phage-based vaccines are ongoing. This platform offers a scalable, safe, and cost-effective strategy for generating strong immune responses against flaviviruses and other emerging viruses.

Switchgrass Deconstruction by *Cellvibrio japonicus* Utilizes two Carbohydrate-Binding Proteins

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The plant cell walls are the source of the most abundant polysaccharides on the planet, which include cellulose, hemicellulose, and pectin. Plant polysaccharides have increased interest in biotechnological applications for their conversion into renewable fuels and chemicals. One plant identified as a source for industrial use is the perennial switchgrass because it does not compete with food availability like many current cellulosic biofuel sources. Previous research on lignocellulose bioconversion by the Gram-negative saprophyte *Cellvibrio japonicus* suggested that switchgrass utilization by *C. japonicus* relies on secreted Carbohydrate-Active enZymes (CAZymes) to degrade the plant polysaccharides. We have further characterized *C. japonicus* switchgrass utilization via a systems biology approach that includes transcriptomic analysis and functional mutational studies to identify the physiologically-relevant CAZymes that are required. The transcriptomic analysis found up-regulation of a diverse set of CAZyme-encoding genes that included many genes predicted to be important for plant cell wall degradation, as well as genes important to the decomposition of other non-plant polysaccharides. Interestingly, our functional mutational analysis suggested that the physiologically important CAZymes also included two carbohydrate-binding proteins belonging to CBM family 2 that do not contain active sites capable of polysaccharide cleavage. We hypothesize that these proteins play roles in polysaccharide separation that improve substrate access for catalytic CAZymes. These results provide new avenues for polysaccharide deconstruction by elucidating the importance of ancillary non-cleaving proteins to increase the efficiency of plant biomass degradation.

New and Unexpected Findings in the Metabolism of Inositol Pyrophosphate in *Candida albicans*

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Inositol pyrophosphates (PP -InsPs) are a class of highly conserved signaling molecules that regulate a wide array of cellular processes across eukaryotes. These processes include phosphate homeostasis, stress responses, transcription, chromatin remodeling, and processes related to pathogenesis, including macrophage damage in the opportunistic fungal pathogen *Candida albicans*. The kinases and phosphatases governing PP-InsPs synthesis and degradation have been studied extensively. PP-InsPs synthesis begins with cleavage of phosphatidylinositol-4,5-bisphosphate (PIP_2) by phospholipase C to liberate soluble IP_3, which is a substrate for phosphorylation by Ipk2 and Ipk1 to generate IP_6. IP_6 is further pyrophosphorylated by Kcs1 on the 5C to make 5-IP_7; 5-IP_7 is pyrophosphorylated on the 1C by Vip1 to make 1,5-IP_8. Comparing the genes that regulate this pathway in the ascomycetes, *S. cerevisiae* and *C. albicans* reveal intriguing differences: the kinase genes *IPK1 a*nd *IPK2* are non-essential in *S. cerevisiae* and essential in *C. albicans*. This distinction points to a potentially unique and critical role for these gene products in *C. albicans*, offering a promising avenue for therapeutic targeting against this pathogen. Secondly, our metabolomic investigations identified new inositol pyrophosphate isoforms in *C. albicans*, including 2-IP_7, 4/6-IP_7, 4/6,5-IP_8, and an unidentified IP8 isoform, not previously observed in other yeast species. Our main questions now focus on understanding which kinases synthesize these isoforms, which phosphatases regulate their cellular levels, and whether the enzymatic activity involved in producing these metabolites plays a role in the pathogen's virulence.

IS*1* Mediated Chromosomal Amplifications As An Adaptive Strategy In *E. coli* B: Polymyxin B Heteroresistance And Beyond

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Antimicrobial resistance (AMR) is a growing cause of concern across the globe and has resulted in the need for an improved understanding of AMR mechanisms. Currently, polymyxins (colistin) are the last line of defense against Gram-negative infections. Polymyxins are positively charged lipopeptides that interact with the negatively charged lipopolysaccharides (LPS) on the outer membrane of Gram-negative bacteria, disrupting the permeability barrier and resulting in bacterial cell death. However, Gram-negative bacteria modify the lipid A of LPS by the addition of amine containing moieties such as aminoarabinose (L-Ara4N) sugar via the *arn* operon, masking the overall negative charge, resulting in the polymyxin resistance. AMR is often accompanied by heteroresistance, where a subset of an otherwise susceptible bacterial population is transiently resistant to the drug. In this study, we employed MIC testing, frequency of resistance assays, and directed evolution to determine the role of LPS modifications in heteroresistance in our lab strain of *E. coli* BL21(D3), which seems to be inherently heteroresistant to PMB, as observed by a population analysis profile assay. We show a role for the L-Ara4N modified LPS in heteroresistance, occurring through a large, tandem chromosomal amplification of the *arn* operon and driven by IS*1* elements, located ~100kb away. ESI-MS and TLC analysis of P-32 labeled LPS show increased abundance of L-Ara4N lipid A in the HR strains. Future studies will investigate the impact of these amplifications on heterogeneity in gene expression as a survival advantage to these bacterial populations in various stressful conditions, such as antibiotic challenge.

Bacterial Lipoprotein Remodeling: Mediator of Copper Resistance and Immune Evasion

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Bacteria possess an ever-evolving ability to evade host immune responses, resulting in a continuous interplay of action and counteraction between bacterial mechanisms and the host immune system. Bacterial lipoproteins are vital membrane components ubiquitous in both Gram-positive and Gram negative bacteria, serving a crucial role in the immune system's ability to recognize and respond to bacterial presence. Their abundance and conservation make them ideal microbe-associated molecular patterns (MAMPs), recognized by Toll-like receptor 2 (TLR2) complexes on immune cells. However, bacterial lipoproteins undergo N-terminal modifications, creating diverse chemotypes. Studies from our lab uncovered lipoprotein-modifying enzymes (Lit, LnsAB, Lhat) and are focused on investigating their role in immune evasion. Evidence suggests that these chemotypes differentially influence TLR2-mediated immune responses, with certain variants, such as lyso-lipoproteins created by the Lit enzyme, being immune silent. Additionally, we believe chemotype conversion might enhance bacterial resistance to copper, a potent antimicrobial agent deployed by macrophages to kill intracellular bacteria. We identified Lit gene paralogs (Lit2) on plasmids in specific *Listeria monocytogenes* isolates, co-localized with genes related to copper resistance. A two-component regulatory system (CopRS) on the same plasmid was characterized, demonstrating co-regulation of copper resistance genes and lipoprotein-modifying genes. These findings represent the first evidence of a functional link between copper resistance and lipoprotein modification. Furthermore, our results provide insight into how the CopRS system integrates environmental cues, such as oxygen-controlled electron transport chain activity, to regulate lipoprotein remodeling. This regulatory mechanism highlights its significance in bacterial virulence and survival under host-imposed stress conditions.

Unraveling the Phosphorylation Dynamics of RssB: Implications for RpoS Regulation and Bacterial General Stress Response in *Escherichia coli*

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Bacteria use various regulatory mechanisms to adapt to environmental changes, one of which is the two-component signal transduction system (TCS). A typical TCS consists of a histidine kinase (HK) that activates a response regulator (RR) through phosphorylation of a conserved aspartate. In *E. coli* K-12, RssB is an atypical RR. The N-terminal domain contains a phosphorylatable aspartate (D58), while the C-terminal domain is an inactive PP2C-type phosphatase. RssB regulates the degradation of the stress response sigma factor RpoS by delivering it to the ClpXP protease. Phosphorylation of RssB increases its affinity for RpoS, promoting degradation in vitro. However, the physiological relevance and regulatory mechanisms of RssB phosphorylation remain undefined, as RssB lacks a cognate HK and may be phosphorylated by other sources, such as acetyl phosphate or promiscuous HKs. We aim to better understand the mechanisms of RssB phosphorylation and dephosphorylation by using a combination of methods, including reporter fusions to measure RpoS and RssB activities and in vivo phosphorylation assays. We compared RpoS activity and levels in single-residue mutations of the RssB phosphorylation site, including D58E as a phosphomimic and D58A and D58P as non-phosphorylatable mutants, to wild-type RssB under different growth conditions. In nutrient-rich media, wild-type strains showed RpoS activity similar to D58P, suggesting low RssB phosphorylation. In glucose minimal media, wild-type strains exhibited RpoS activity similar to D58E, indicating RssB phosphorylation. These reporter strains will help identify sources of RssB phosphorylation and provide insights into the regulation of the bacterial stress response driven by RpoS.

Diagnostic Panel Evaluation and Workflow Assessment of the Respiratory Flex Assay on the Diasorin Liaison Plex®

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Background: A syndromic testing approach is an efficient method for respiratory infection diagnosis because many patients exhibit similar symptoms. This study compares workflow and performance of the Diasorin Respiratory Flex (RF) assay (Liaison Plex®), an automated sample-to-answer platform, against the NxTag® Respiratory Pathogen Panel (RPP), an open-system with separate nucleic acid extraction and amplification steps. The RF assay detects a broader range of unique pathogens (14 versus 11 for RPP). Both assays subtype several viral targets. **Methods:** A clinical correlation was analyzed by performing 18 random nasopharyngeal clinical samples previously run on the RPP versus the RF assay. Control material (Zeptometrix) with characterized results was assayed on the RF assay. Workflow studies were performed with hands-on time (HOT) and total analytical time calculated. **Results:** Positive percent agreement (PPA) was 78.6%, negative percent agreement (NPA) was 85.7%. We observed metapneumovirus false positives on RPP (n=2), a known limitation of that assay. SARS-CoV-2 was detected (n=2) on RF, which was not detectable on RPP. HOT for the RF workflow was 15 minutes, versus 145 minutes for RPP. Correlation of RF results to Zeptometrix panels was 100%. Analysis of the RPP workflow identified steps (n=5) with increased contamination risk, which was lower (n=1) with the closed workflow on the RF. Time to result was 2 hours for RF, versus 6 hours for RPP. **Conclusion:** The RF assay had good overall agreement with RPP, with broader pathogen detection, HOT savings, and reduced contamination risk. Inclusion of additional target is likely to increase diagnostic yield.

Investigating the Role of Pdc12 in Regulating *THI* Gene Transcription in *Candida albicans*

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Thiamine pyrophosphate is an essential metabolite, serving as an enzymatic cofactor for several enzymes, including pyruvate decarboxylase in central carbon metabolism. However, the mechanism for genetic regulation of de novo thiamine biosynthesis (THI) has not yet been elucidated in opportunistic fungal pathogen Candida albicans. In Saccharomyces cerevisiae, a nonpathogenic yeast closely related to C. albicans, transcription of the biosynthetic THI genes is controlled by transcription factors Thi2 and Pdc2 and thiamine sensor Thi3. Through RNAseq and qRT-PCR, we found that C. albicans THI genes are regulated by extracellular thiamine and require Pdc2 for induction when thiamine is limited. C. albicans encodes the Pdc2 ortholog but it lacks obvious orthologs for Thi2 and Thi3; thus, it is unclear how Pdc2 regulates the THI genes and whether it acts alone or in conjunction with unidentified transcription factors and regulators. In S. cerevisiae and Candida glabrata, the Thi3 sensor is homologous to pyruvate decarboxylase, but it carries conserved substitutions in active site amino acids. Our research has examined a candidate gene of C. albicans, PDC12, hypothesizing that it encodes the thiamine sensor because it shows sequence homology to the THI3 gene in S. cerevisiae. To assess if Pdc12 is essential for Pdc2 to bind to DNA to promote transcription of the THI genes, we successfully knocked out both alleles of PDC12 in C. albicans through homologous recombination and used the heterozygous and homozygous PDC12 knockout strains to assess the regulation of THI6 expression and growth responses to thiamine limitation.

Impact of Polymicrobial Interactions on *Pseudomonas aeruginosa* Gene Expression

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Pseudomonas aeruginosa and *Staphylococcus aureus* are two opportunistic pathogens commonly isolated from polymicrobial communities in lung sputum of individuals with cystic fibrosis (CF). Their joint presence in the CF lung can exacerbate disease severity, highlighting the importance of studying these species together. It has been previously observed that *S. aureus* and *P. aeruginosa* exhibit a range of competitive and cooperative interactions, but these experiments were not conducted in a growth medium representative of the CF lung environment. To address this, I used a synthetic CF sputum medium (SCFM1) that approximates the biochemical composition of CF lung mucus (sputum) to more precisely characterize how *S. aureus* exoproducts affect *P. aeruginosa* gene expression in the context of polymicrobial lung infections. Using promoter-reporter assays, I found that cell-free supernatant from *S. aureus* upregulated the promoters of metal- and metabolite-related genes in *P. aeruginosa.* I also identified three novel *P. aeruginosa* promoters induced by *S. aureus* supernatant, suggesting previously uncharacterized interactions between *S. aureus* and *P. aeruginosa* in a CF-relevant context. Understanding how *S. aureus* alters the production of *P. aeruginosa* gene products in this growth environment could elucidate how co-infection dynamics alter CF patient outcomes.

Ribosome Deficiency Induces *Salmonella* Filamentation Within Host Cells

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The ribosome is the central hub for protein synthesis and is heavily targeted by antibiotics. Ribosomal mutations, antibiotic treatment, and nutrient starvation can alter translational efficiency and lead to stressed cells. Ribosome deficiency plays a critical role in stress responses and disease progression, yet how it affects bacteria-host interactions remains poorly understood. In this study, we show that a ribosome-deficient strain exhibits a surprising morphological change from rod-shape to filamentous in *Salmonella* cells growing inside host macrophages. Such filamentation depends on an acidic condition within macrophages and in a defined medium mimicking macrophage conditions. Further genetic analyses revealed that filamentation of the ribosome-deficient strain depends on overexpression of *hisH*, a gene involved in histidine biosynthesis. Transcription of the histidine biosynthesis operon is regulated by a small leader peptide HisL. Slow translation of HisL in the mutant strain activates transcription of the histidine operon and induces filamentation. In support of this model, we show that ribosome inhibitors also increase the expression of the histidine operon and cause filamentation in wild-type *Salmonella*. Bacterial filamentation has been implicated as an adaptive strategy. We show that filamentation improves the survival of *Salmonella* cells under acid stress, and filamentous cells resume normal division after the acid stress is removed. Our work thus demonstrates that ribosome deficiency caused by mutations and antibiotics induces *Salmonella* filamentation in host cells as a potential survival strategy.

EARLY CAREER TALKS

Optimization of a Roche Cobas® Utility Channel Assay for *Borrelia* Species on the Applied Biosystems 7500 qPCR Instrument

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Background: Automation of laboratory developed tests (LDTs) increases throughput and decreases human error and labor costs. The Roche cobas® omni Utility Channel fully automates sample processing, but optimization guidelines are limited and cost prohibitive when performed on the cobas® instruments. We describe an off-instrument optimization procedure for a *Borrelia* species assay using the cobas® omni Optimization Kit with the Roche MagNA Pure 96 extractor and Applied Biosystems (ABI) 7500 Real-Time thermocycler. **Methods:** Quantitated bacteria was extracted on a MagNA Pure 96 and amplified on an ABI 7500 utilizing the cobas® omni Optimization Kit. Roche internal processing control was used to examine background compared to the cobas® systems. Multiple reactions were performed to evaluate probe chemistry, primer methylation, primer/probe concentration, and asymmetric primer concentration. The resulting sensitivity was determined by probit analysis and 95% CI. **Results**: The final optimized reaction utilized forward methylated primers with asymmetric primer concentrations (150 nM Fm and 200 nM R). An internally quenched probe at bp 7 was used at a concentration of 50 nM to decrease background signal. The *Borrelia* species assay had a sensitivity of 60.9 cp/ml via probit analysis on the 5800 and 62.5 via 95% CI on the 8800 system. **Conclusion:** We developed a highly sensitive *Borrelia* species assay on the cobas® Utility Channel with decreased reagent cost using an off-instrument optimization strategy. Modification of primers with methylation and internally quenched probes was beneficial for decreasing background noise. This strategy is effective for transferring assays to cobas® instruments for clinical use.

The Oral Microbiome is Not Associated With Risk of Post-Menopausal Breast Cancer in The NIH AARP Diet And Health Study

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Introduction: Breast cancer is the most common cancer among women. Studies suggest a link between periodontal disease and breast cancer risk, but whether oral microbiome differences precede or result from cancer remains unclear. We examined this prospective association in the NIH-AARP Diet and Health **Study. Methods**: The study enrolled 566,398 individuals (ages 50-71) from 1995-1996, with oral wash samples collected from a subset in 2005. We matched 348 women who developed breast cancer after oral wash collection to controls using incidence density sampling. DNA was extracted and shotgun metagenomic sequencing was performed. We used conditional logistic regression to calculate the odds ratios (OR) for the associations between alpha diversity, beta diversity, species-, and gene-level metrics, with breast cancer risk adjusting for potential confounders. **Results:** The average age of women at sample collection was 61.5 and cases developed breast cancer an average of 3.4 years after oral wash sample collection. Alpha diversity was inversely, but not statistically significantly, associated with breast cancer risk (e.g., OR Shannon=0.85; 95% CI=0.69-1.05). However, we observed no statistically significant prospective association between beta diversity measures or the presence or relative abundance of any species or genes with breast cancer risk. Additionally, neither presence (OR=1.00; 95% CI=0.72-1.38) nor relative abundance of periodontal pathogens (OR=1.03; 95% CI=0.85- 1.26) were associated with breast cancer risk.

Cell wall cue for the subcellular localization of a transmembrane protein in *Bacillus subtilis*

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Bacterial subcellular protein localization is a critical yet poorly understood process, particularly for transmembrane proteins. Several peripheral membrane proteins, for example, can detect differences in micron scale curvatures to drive their subcellular localization. This requires the protein to sample different membrane patches and eventually preferentially bind to membranes of preferred curvature. However, transmembrane proteins are unable to sample multiple membrane patches in a similar fashion, so the ultimate cues that drive their subcellular localization are less well understood. In this study, we investigated the localization mechanism of ShfA, a transmembrane protein required for sporulation in *Bacillus subtilis*. Spore formation is initiated with an asymmetric cell division, after which ShfA is exclusively produced in the larger cell and preferentially localizes to the polar septum. We demonstrate that for ShfA, a novel, highly conserved YabQ domain determines this localization. Specifically, four conserved intramembrane residues within YabQ domain are required for ShfA septum targeting. Interestingly, artificial expression of ShfA-GFP during vegetive growth revealed localization to division septum. ShfA-GFP produced in *E. coli* or *S. aureus* also localized to division septa, indicating that a universally conserved septal cue drives ShfA localization. Consistent with this model, localization of newly synthesized ShfA, was disrupted by cell wall targeting antibiotics. Additionally, ShfA interacts with at least one cell wall biosynthetic component of the divisome. We therefore propose that subcellular localization of ShfA is mediated by direct interaction with the cell division machinery and likely an unidentified physical feature of septal peptidoglycan.

Chlamydia trachomatis Putative Chromosome Partitioning System ParABS: *In Silico* Analysis and Crispri Mediated Knockdown

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Chromosome partitioning in many bacteria occurs via a tripartite ParABS system that consists of an ATPase (ParA), and a DNA binding protein (ParB) that binds to a centromere-like site on the chromosome (*parS*) proximal to origin of replication (*OriC*). *Chlamydia trachomatis* encodes a putative ParA (CTL0845) and ParB (CTL0057), while *parS* sequence is unknown. Using consensus *parS* sequence from well-studied models, we predicted 3 putative *parS* sites on *C. trachomatis* L2/434/Bu chromosome, of which two are proximal to *OriC*. The *C. trachomatis* ParA and ParB homologs contains deviant Walker A motif (XKGGXXK[T/S]), and N-terminal arginine-rich motif (GERRxRA) / DNA binding domains, respectively. Predicted structures of Chlamydial ParA and ParB show a high degree of similarity with *Helicobacter pylori* ParA and ParB respectively. To study the role of ParA and ParB in chlamydial biology, we used targeted repression via CRISPR interference (CRISPRi). Reduced expression of ParA and ParB was confirmed by qRT-PCR and it had no apparent effect on the developmental cycle of *C. trachomatis*, as assessed by the production of infectious progeny. A ParA homolog is also encoded on the pL2 plasmid (Pgp5), and we hypothesized that it could be compensating for lower chromosomal ParA expression. ParA repression with pgp5 deletion in CTL2 with wild type plasmid as well as CTL2R (plasmid-less strain) background still did not result in significant reduction in infectious progeny production. Ongoing work is focused on performing localization studies for ParA and ParB and verification of ParB-*parS* interaction.

The Oral Microbiome and All-Cause Mortality in the US Population

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Background: Poor oral health is associated with oral microbiome changes and increased mortality risk. However, no large studies have evaluated whether the oral microbiome is directly associated with mortality.
Methods: Oral rinse specimens were collected in the National Health and Nutrition Examination Survey (NHANES) 2009-2012, a multistage probability sample of the US population. The oral microbiome was measured using 16S rRNA gene V4 region sequencing from extracted DNA. Alpha diversity, beta diversity, and genus-level data were generated using DADA2 and QIIME. Within NHANES participants 20- to 69-years-old (N=7,721, representing approximately 194 million individuals), we evaluated the association between oral microbiome metrics and all-cause mortality from the restricted-use National Death Index using Cox proportional hazards regression. **Results:** After an average of 8.8 years, 426 participants died. Continuous alpha diversity was inversely associated with all-cause mortality, but only the association for the Shannon-Weiner index was significant after adjustment for major risk factors (hazard ratio [HR] per standard deviation [SD]=0.85; 95% confidence interval [CI]=0.74-0.98). Few associations were observed at the genus-level after Bonferroni correction, but an increase in 1 SD of the relative abundance of *Granulicatella* and *Lactobacillus* were associated with a 17% (95% CI=1.11-1.24) and 11% (95% CI=1.06-1.16) increase in mortality risk, respectively. Compared to participants with no detectable *Bacteroides*, participants in the highest tertile of *Bacteroides* had decreased mortality risk (HR=0.54; 95% CI=0.40-0.74). **Conclusions:** Some oral microbiome measures were associated with all-cause mortality in this representative population cohort. Oral bacterial communities may be important contributors to health and disease.