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62	Dmitry Sutormin	Institute for Systems Biology	Phage infection progression pathway and a phage-resistance landscape revealed with single-cell transcriptomics in Bacteroides fragilis
63	Ashley A. Wilkins	Rocky Mountain Labs, NIAID, NIH	Characterization of a putative D-Alanine-D-Alanine carboxypeptidase (BB0605) in Borrelia burgdorferi peptidoglycan remodeling
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79	Delaney Shea	OHSU	Engineering probiotic bacteria as antibiotic and anti-biofilm therapeutic delivery vehicles
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81	Björn Brindefalk	University of Idaho	Amendment of marine oil-polluted sediments with activated carbon alters microbial community structure and enhances biodegradation
82	Marshall Godsil	University of Washington	Temperate phage tolerance by the type VI-A CRISPR-Cas system in Listeria seeligeri
83	Xuhui Zheng	University of Washington	Activation of c-di-GMP and cAMP signaling during Pseudomonas aeruginosa biofilm formation
84	Tarah I. Gervais	Fred Hutchinson Cancer Center	Decoding the evolutionary tug-of-war between MxA Loop L4 and Influenza A nucleoprotein
85	Ajay Akhade	Institute for Systems Biology	A non-canonical role for Caspase-1 in controlling antimicrobial resistance of intracellular Salmonella
86	Sarina Kao	University of Washington	Investigating the interactions of HNS and phage proteins during lytic phage infection

1 Disentangling Contributions of Host Genotype and Microbiome to Host Evolution

Ellie Andrews Washington State University Vancouver

There is growing recognition that microbiomes play an important role in host health and wellness. There is evidence that host-associated microbiomes strongly shape host phenotypes, and this has led to a prevailing hypothesis that microbiome evolution could allow organisms to cope with novel stressors. Observations have noted patterns of concordance between microbiome composition and host population differentiation, but few studies have directly tested whether the microbiome contributes to host adaptation. Here, we conducted a replicated, seasonal field experiment to assess the contribution of microbiome evolution to Drosophila adaptation in response to Spinosyn, a commonly applied organic insecticide. We found widespread evidence of rapid adaptation of Drosophila genomes and microbiomes, especially in populations exposed to Spinosyn that had evolved insecticide resistance. We used common garden rearing and axenic manipulations to determine whether microbiome evolution contributed to the evolution of insecticide resistance. We found microbiome evolution did not contribute to differences in host survival. Microbiome evolution did shape host body mass, but it reduced signatures of host adaptation to Spinosyn. Finally, we transplanted microbiota from populations that had evolved insecticide resistance into a novel host genetic background and found no significant difference in insecticide tolerance or body mass. These results demonstrate that host populations evolve rapidly and repeatedly in response to a novel environmental stressor, and suggest microbiomes may contribute comparatively little to host rapid adaptation to novel environments.

2 The investigation of the killer toxin binding interface to the plasma membrane receptor

Dayna Buitron University of Idaho

Fungal infections pose risks to human health, ranging from minor infections to severe invasive diseases and death. Available treatments do not always eradicate these infections, and disease recurrence can be common. Antifungal killer toxins produced by the baker's yeast Saccharomyces cerevisiae provide a potential pathway toward more effective antifungals. These killer toxins assist yeast in niche competition by targeting and killing nearby competing fungi. The killer toxin K1 displays potent killing activity towards an important human pathogen named Nakaseomyces glabratus. Elucidating the mechanism of K1 toxicity is key to understanding approaches toward curing fungal infections. K1 is known to bind to the Kre1 cell wall and membrane receptor; however, the binding mechanism remains largely unexplored. Experimental data suggests that a 112 amino acid region of the carboxyl terminus of Kre1 is required for K1 intoxication. Kre1 truncations of varying carboxyl terminus lengths have been constructed to test for K1 susceptibility, revealing that the minimal binding site of K1 is only 35 amino acids in length. Additionally, cell health assays on the truncated strains revealed that deletions within the K1 binding site grew considerably slower than wild-types. The Kre1, with a truncation of 35 amino acids, displayed only minor growth defects compared to the wild-type. This work will help us to better understand how K1 targets and kills fungal cells. Understanding the specific binding domain is essential for developing effective drugs to combat emerging fungal pathogens like Nakaseomyces glabratus.

3 Investigating crystal structures of novel PETase EstB and putative MHETase NlhH

Cass Biles Reed College

Plastic waste is one of the leading environmental issues of our time. With an increasing demand for plastic worldwide, carbon emissions and waste products are projected to skyrocket, leading to accumulation in landfills and oceans. Despite extensive research into recycling, current methods have limitations and largely cause negative impacts on the environment. The emergence of biological mechanisms capable of degrading plastic waste into reusable monomers has paved a new path for recycling, and may provide a remedy to the plastic waste problem. The Mellies lab has identified novel esterase, EstB, from Pseudomonas sp. B10, which has displayed activity against polyethylene terephthalate(PET), one of the most abundant thermoplastic polymers. EstB has been shown to be capable of digesting PET into mono(2-hydroxyethyl) terephthalate (MHET) and bis(2-hydroxyethyl) terephthalate (BHET). Additionally, whole genome sequencing and alignment has identified a second gene, nlhH, as a putative MHETase capable of freeing terephthalic acid (TPA) and ethylene glycol(EG) from MHET. In this project, I purified over 130mg of EstB, showed esterase activity through 4-nitrophenyl butyrate absorbance assays, and began crystallization condition trials. In addition, I began work towards an nlhH vector by amplifying nlhH from consortia 9.2.

4 Characterization of the Hypothetical Protein bb0656 in Borrelia burgdorferi: Insights into Iron-Independent Adaptations and Infection Dynamics

Kamryn Cregger Rocky Mountain Laboratory / NIAID / NIH

As the leading cause of tick-borne disease in the United States, approximately 300,000 cases of Lyme disease are diagnosed each year. The Lyme disease spirochete, Borrelia burgdorferi, is transmitted through the blood meal of infected Ixodes ticks. This bacterium has a unique enzootic life cycle that involves both tick vectors and mammalian hosts. Borrelia spirochetes are characterized by unique physiological adaptations to survive their specialized niches. For example, B. burgdorferi has evolved to evade host defenses by relying on manganese instead of iron. Specifically, the gene bb0656 is predicted to encode a HemN-related non-iron pseudo-S-adenosyl-l-methionine (SAM) enzyme. This family of enzymes appears unique to Borrelia, suggesting evolution of the enzyme to function in iron-deficient environments. Radical SAM enzymes are known to catalyze various reactions such as methylation, methylthiolation, sulfur insertion, isomerization, carbon-carbon bond formation, and oxidation by utilizing SAM. To explore the role of bb0656 in B. burgdorferi, the only encoded SAM enzyme that has been identified, we compared a wild-type strain with a bb0656 transposon mutant. This mutant displayed a modest phenotype during in vitro growth, but absence of this gene did not seem to impact murine infectivity or tick colonization. Radical SAM enzymes constitute one of the largest super families of enzymes to date with wide-ranging effects not limited to cofactor biosynthesis, enzyme activation, protein modifications, and tRNA modifications. Given the lack of in vivo phenotype, and Borrelia's unique adaptation to not require iron, we will evaluate in vitro stress conditions to identify potential functions for bb0656.

5 Defining the domains of K1 killer toxin homologs in Saccharomyces yeasts

Sarah Coss University of Idaho

Antifungal toxins produced by Saccharomyces yeasts have been shown to inhibit the growth or kill certain strains and species of yeast. The killer toxin K1 is particularly effective at controlling the growth of drug-resistant opportunistic pathogen Nakaseomyces glabratus. Homologs of K1 were identified by sequence homology in diverse yeasts, and three have been identified as antifungal toxins despite their low sequence identity to K1 (~20%). The immature K1 toxin contains four domains (delta, alpha, gamma, and beta) that are cleaved by carboxypeptidases Kex1 and Kex2 to produce the mature toxin. However, the domain organization and functions of K1 homologs are unknown. We show the conservation of putative Kex1 and Kex2 cleavage sites in the K1 homologs that likely define delta, alpha, gamma, and beta domains. The biological relevance for proteolytic processing of these putative cleavage sites is supported through structure modeling of the homologs, which position these cleavage sites on flexible, solvent-exposed loops. Functional assays reveal that, like K1, the homologs contain cytotoxic alpha domains, suggesting that alpha domain toxicity is conserved across the K1 homologs. Moreover, this indicates that most K1-homologs are likely active antifungal toxins and that variations in toxin activity are due to the structure and sequence diversity of the other domains. The combined multidisciplinary approach has enabled the characterization of novel K1-homologs, confirming their potent antifungal activities. These findings provide critical insights into the structure and function of these toxins, which will enhance our understanding of their potential applications in combating fungal diseases.

6 Salmonella and the Microbiome: How Metabolism plays a role in competition in a Healthy Gut

Samira Diaz De Leon Washington State University

Salmonella enterica causes 1.35 million infections in the United States each year. Symptoms consist of abdominal pain, diarrhea, and nausea, which typically can self-resolve. When S. enterica infects the gastrointestinal (GI) tract it has to compete for nutrients against the native microbiome. This project focuses on how Salmonella's growth is affected when competing against diverse microbiota with different metabolisms. We isolated 11 microbial isolates from swine fecal matter to establish a "nanobiome" representing the gastric microbial community. Species isolated under hypoxic (1% oxygen) conditions are presumed anaerobe while species grown in normoxic (20% oxygen) conditions are presumed aerobic. We studied the growth dynamics when competing with S. enterica against our nanobiome. We attempted to replicate the intestinal environment by culturing cells in fecal-enriched media and maintaining 37° C in a hypoxic atmosphere. Competitions were organized with a mixture of the microbial isolates, S. enterica, and the fecal-enriched media as the nutrients that allow for the growth of the isolates and Salmonella. Absorbance and fluorescence were measured over 18 hours to determine the effects of the microbial isolates on S. enterica. Using a fluorescence S. enterica, a thorough analysis of cell growth was averaged theoretically and graphed to distinguish the activity of S. enterica competing with the microbiome. Based on results we believe the microbiome plays a significant role in reducing S. enterica growth. Overall, these experiments will give us an insight into how the microbiome plays a role in preventing infection.

7 Characterization of Cytotoxic Aerolysin Homolog produced by Yeast

Kasen Evans University of Idaho

Fungal infections can lead to significant morbidity and mortality, with current treatments often only suppressing rather than curing infections. This study explores the killer toxin K62 from Saccharomyces paradoxus, which shares structural homologs with aerolysin toxins potent bacterial virulence factors. Preliminary data suggest that similar toxins are produced by several human fungal pathogens, highlighting K62's potential significance. This research aims to deepen our understanding of K62's function in fungal virulence and contribute to developing new antifungal strategies. We aim to investigate K62's role in fungal pathogenicity through a series of assays. First, we examined the relationship between K62 production and double-strand RNA (dsRNA) viruses. Curing S. paradoxus of dsRNAs using cycloheximide eliminated K62 production, indicating that dsRNA elements encode the K62 gene. Injection assays of cured strains of S. paradoxus into Galleria mellonella larvae did not significantly impact larval survival, suggesting that K62 is not involved in fungal pathogenesis. Phagocytosis assays with Dictyostelium discoideum revealed no significant effects on the survival of the amoeba with or without K62 expression, suggesting that K62 does not influence the survival of yeast after engulfment. These findings indicate that while K62 displays cytotoxic activity against fungi, a role in the pathogenicity of animals and amoeba is unlikely.

8 Exploring the adaptive landscape of alphavirus nsP3 and the role of a novel nuclear localization signal using deep mutational scanning

Tiia Freeman Fred Hutchinson Cancer Research Center

Alphavirus-encoded non-structural protein 3 (nsP3) is implicated in controlling host-specificity. Notably, mosquito-transmitted alphaviruses use short linear motifs (SLiMs) within the hypervariable domain (HVD) of nsP3 to interact with distinct host factors in mosquito vector and vertebrate host cells. Existing proteomic studies have uncovered over 100 host factors that interact with the nsP3 HVD. However, only three of these proteins have been mapped to specific HVD SLiMs. Identifying and characterizing nsP3-host factor interactions is essential for deciphering host-specific virus-host dynamics, potentially improving our understanding of host specificity and viral replication mechanisms across alphaviral species. The prototype alphavirus, Sindbis (SINV), serves as an effective model for studying alphavirus-host interactions due to its tractable, lab-safe plasmid construct. In this study, we used deep mutational scanning (DMS) to determine how variations in the SINV nsP3 HVD impact host-specific interactions. Our DMS screens identify single-residue variants that confer host-specific fitness benefits in human (HEK-293T) or mosquito (C6/36) cells. Additionally, prompted by the recent identification of an NLS in Barmah Forest virus nsP3, we performed computational analyses using three programs—NLStradamus, PSORT II, and NucPred—to cross-validate the presence of NLS regions in the HVD of other alphaviruses. Our analysis uncovered putative NLS sequences across several alphaviruses, including a notably strong NLS at the C-terminus of Sindbis virus's HVD, potentially indicating partial nuclear localization. Further validation of these NLS functions and the effects of specific single-residue changes on viral replication will deepen our understanding of alphavirus-host interactions and inform therapeutic strategies targeting host-specific viral factors.

9 Decoding the Evolutionary Tug-of-War Between MxA Loop L4 and Influenza A Nucleoprotein

Tarah I. Gervais Fred Hutch Cancer Center

Myxovirus resistance protein 1 (MxA) restricts influenza A viruses (IAV) by binding the IAV nucleoprotein (NP). Evolution-guided functional studies have suggested that the unstructured Loop L4 of MxA interacts with IAV NP. However, because Loop L4 is intrinsically disordered, the interaction between NP and MxA is poorly understood. Using protein modeling and molecular dynamics, we identified a hydrophobic pocket in NP that might represent a critical site for interaction with Loop L4. Based on this prediction, we hypothesize that mutating interaction residues at or proximal to this hydrophobic pocket in NP will weaken or disrupt its interaction with MxA and affect MxA restriction of IAV. To test this hypothesis, we are mutating NP residues. We will test these NP variants using a minireplicon system to investigate how these changes affect NP function in the absence of MxA, and how they affect escape from MxA restriction. Reduced MxA restriction of influenza's replication machinery would indicate that the mutated NP residues are critical for MxA's ability to bind and restrict NP, supporting the hypothesis that this hydrophobic pocket and Loop L4 are at the interface of this interaction. These findings will enhance our understanding of the MxA-NP interaction and may inform future strategies for targeting viral resistance mechanisms in IAVs.

10 Exploring the Potential of the Novel Killer Toxin K21L in S. kudriavzevii

Lily Givens University of Idaho

Fungal infections, particularly those caused by Candida species, pose a significant threat to global health. A potential alternative to traditional antifungal drugs lies in the application of "killer toxins" produced by certain yeast strains. Yeast exhibiting the "killer" phenotype are capable of secreting toxins that are harmful to neighboring susceptible yeasts while maintaining immunity themselves. A survey of killer toxin-producing yeasts has discovered a novel killer toxin found in Saccharomyces kudriavzevii. This killer toxin is a homolog of the K21 toxin found in Saccharomyces paradoxus and has been named K21-like (K21L). K21L is strikingly similar to K21, with 88.15% similarity and 81.21% identity at the amino acid level. In addition, secondary and tertiary structure predictions of both toxins show many conserved features, indicating K21 and K21L may share similar mechanisms of antifungal activity. This project aims to confirm the presence of the K21L gene and killer toxin expression by S. kudriavzevii and to characterize the function of these killer toxins. Preliminary data shows differences in killer toxin spectrums of activity and immunity and similar optimal toxin production conditions. The homology of K21L and K21 at both the sequence and structural level allows for the development of chimeric toxins designed using molecular modeling to determine exact mode of function of both toxins. The successful creation of chimeras with altered spectrums of activity and immunity will provide insight into mechanisms that dictate killer toxin specificity, which is essential for the potential use of these toxins to target human pathogens.

11 The influence of growth conditions on protein expression and growth between B. burgdorferi and B. hermsii

Josh Gold Rocky Mountain National Laboratories (NIAID/NIH)

Borrelia burgdorferi (Bb) is the spirochetal causative agent of Lyme disease (LD), the most common vector-borne illness in the northern hemisphere . With a strikingly similar genome (maybe add percentage), Borrelia hermsii (Bh) is the spirochetal causative agent of Tick-borne relapsing fever. Additionally, each organism is grown at different optimal conditions in vitro, and many groups have different conditions that they use. Many of these differences have also not been compared between the two spirochetes. As a result, it is very difficult to identify growth conditions for comparative studies between the two spirochetes. Therefore, we are evaluating standard growth conditions commonly used for the two spirochetes to identify a single condition which also allows for differential gene expression commonly seen in Bb (i.e., OspC). To test this, we set up growth curves under different conditions (temperature, oxygen, and serum levels) for both spirochetes, and looked for changes in growth patterns and gene expression. We found that both spirochetes grow well at different temperatures (35C vs 37C), Bb was more tolerant of higher oxygen levels (5% CO_2) while Bh required more restrictive conditions (3% O_2). As a preliminary analysis, we evaluated gene expression for Bh using the standard culture conditions (3% O_2, 35C, 12% RS) compared to that of 25C to look at differential gene expression as compared to Bb.

12 RNA-mediated CRISPR-Cas13 inhibition via crRNA structural mimicry

Victoria Hayes University of Washington

Bacteria and their viral contenders compete in an evolutionary arms race. In response, bacteria have evolved CRISPR-Cas adaptive immune systems that are implemented to defend against bacteriophage viruses (phages). CRISPR-Cas systems are massively diverse and complex, which has pressured phage to evolve anti-CRISPR (Acr) mechanisms to counteract bacterial immunity. Acrs are proteins that target a respective CRISPR type to inhibit its function, allowing for phage infections to transpire. We have recently discovered an Acr mechanism, where a noncoding RNA located upstream of a protein Acr inhibits type VI CRISPR-Cas13 function. When initially characterizing this RNA-Acr (rAcrVIA1), we performed sRNA-Seq to confirm transcription alongside structural analysis using RNA-folding prediction software, which indicated a secondary structure consisting of two stem loops. We also mutated individual nucleotides in each predicted stem loop and subject rAcrVIA1 to a series of plasmid targeting assays which showed that inhibition of Cas13 immunity is abolished when the structure of rAcrVIAI is altered. When investigating rAcrVIA1's inhibitory mechanism, we performed affinity chromatography to purify Cas13 in the presence of rAcrVIA1 and detected an associated 59nt RNA. Our collaborator Ning Jia (SUSTech) solved the Cryo-EM structure of this complex, detecting a sequence-matched mature form of rAcrVIA1, including its two predicted stem loops. Future work will be done to characterize the regulation of rAcrVIA1 and its relative binding affinity to Cas13 compared to its usual associated guide RNA. Overall, we want to further elucidate prokaryotic immune antagonism and the implications that Acrs and structural mimicry have for CRISPR gene editing therapies.

13 Slippery slope: Sebum shapes microbial physiology and antibiotic response

Dante James University of Oregon

Human skin contains up to 6,000 sebaceous glands per square inch. These glands produce sebum comprised of lipids, antimicrobials, and proteases. Sebum plays instrumental roles in skin barrier and defense; however, how sebum influences bacterial growth and antimicrobial susceptibility remains unknown. Sebum composition depends on host factors including age, gender, and microbiota. However, synthetic sebum (SS) formulations have recently become available. SS recapitulates key lipids of human sebum providing a reproducible alternative to study the influence of sebum on microbial physiology. Staphylococcus aureus is a skin pathogen, causing a range of diseases from boils to eczema. While antibiotic resistance is a major concern, even clinically susceptible S. aureus strains can survive antibiotic treatment. This process, termed antimicrobial tolerance, results from the environment and physiological state of the bacteria. We hypothesized that sebum impacts S. aureus tolerance to antimicrobials. We screened 100+ antimicrobials against S. aureus in the presence and absence of SS. The addition of SS increased the efficacy of 16 compounds and decreased the efficacy of 7. Several hits are treatments for skin infections. As proof of principle, we focused on investigating the ability of SS to protect S. aureus from membrane depolarizing compounds. Fatty acids alone are insufficient for this protection, and S. aureus's ability to incorporate fatty acids into its membrane does not contribute to its survival. Future work is focused on dissecting the mechanisms by which SS impacts S. aureus antimicrobial susceptibility. This project may advance therapeutics against pathogens by utilizing host secretions in conjunction with antimicrobials.

14 Mapping alpha-defensin interactions with viral capsid proteins using proximity labeling

Hannah H. Kim University of Washington

Alpha-defensins are innate immune peptides that exhibit broad antibacterial, antiviral, and antifungal activity. Infectivity of many non-enveloped viruses is neutralized by α-defensins, including human adenoviruses (HAdVs), rotaviruses, and picornaviruses. Evidence suggests that α-defensins bind directly to viral capsids to neutralize infection, but the molecular details of these interactions remain poorly understood. To further investigate, I developed a method utilizing a biotin crosslinker called sulfo-SBED to map α-defensin binding sites on the viral capsid. Sulfo-SBED is a photoactivated crosslinking reagent capable of covalently transferring a biotin label from one protein to its specific interactors. Using this crosslinker, I first studied the interaction between human α-defensin 5 (HD5) and HAdV as proof of concept, utilizing identified binding sites from structural and biochemical studies as benchmarks. I optimized and scaled up the reaction conditions to conjugate sulfo-SBED to the N-terminus of HD5. When tested against HAdV-5, the modified HD5 (HD5-SBED) still neutralized at a concentration comparable to normal HD5, suggesting that the conjugation reaction did not affect HD5 function. To test the ability of HD5-SBED to label HAdV capsid proteins that interact with HD5, I incubated HAdV with a neutralizing concentration of HD5-SBED, resolved the viral proteins by SDS-PAGE, and probed for biotin with streptavidin. I observed specific biotinylation, as the three major capsid proteins known to interact with HD5 showed dose-responsive labeling, and photoactivation was required for biotinylation. These results indicate that this approach can identify previously uncharacterized interactions between defensins and neutralized viruses, making this a powerful tool for discovery.

15 Phishing for Phage in the Little Spokane: Isolation and Characterization of Aeromonas Bacteriophages

KaiLi Knapp Gonzaga University

Aeromonas salmonicida are water-borne bacteria that primarily infect fish but can also be pathogenic to humans. While antibiotics are an option to treat infection, A. salmonicida have developed resistance to multiple drugs. Phage therapy approaches are in use or development to prevent Aeromonas infection in aquaculture and farmed fish. This strategy would involve releasing phage targeting Aeromonas into the environment, yet little is known about the existing Aeromonas phage community in aquatic systems. Our project aims to assess the composition of Aeromonas phage present in the water surrounding a local hatchery. Water samples were collected seasonally from hatchery effluent and sites along the Little Spokane River. Phages were isolated using an enrichment approach with A. salmonicida type strain as the host. We have isolated and characterized 21 phages based on plaque size and morphology, bacterial host range, and impact on Aeromonas bacterial growth kinetics. Preliminary findings have shown an association of host range with plaque phenotype and season, which provides insight into the diverse factors that could influence the effectiveness of phage therapy in aquaculture.

16 Involvement of the posttranscriptional regulatory protein RsmA in phenotypes controlled via a plant-responsive transcription factor in the Populus endophyte Pseudomonas GM79

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A number of plant-associated proteobacteria, including members of the Populus microbiome, have plant-responsive transcriptional regulatory proteins called PipR. PipR proteins play roles in interactions between bacteria and their plant hosts. Previously, we identified a plant-derived compound, N-(2-hydroxyethyl)-2-(2-hydroxyethylamino) acetamide (HEHEAA), as a potent effector of PipR-mediated gene regulation in the Populus root endophyte Pseudomonas GM79. RNA-seq and phenotypic analyses revealed that hydrogen cyanide production is repressed by PipR-HEHEAA. This makes sense because hydrogen cyanide is toxic in the plant host environment where HEHEAA is made. A puzzling finding was that a peptidase named PipA that is induced by PipR-HEHEAA needs to be active in strain GM79 to see repression of hydrogen cyanide production. To identify proteins degraded by PipA peptidase, we compared proteome profiles of the cell lysates of strain GM79 with or without treatment with purified PipA peptidase. We found that RNA-binding RsmA regulatory proteins, of which strain GM79 encodes three (RsmA1, 2, and 3), were among the top five proteins degraded by PipA peptidase. When we constructed three individual rsmA mutants, we found that the rsmA3 mutant exhibited phenotypes in hydrogen cyanide production like those exhibited by the pipA mutant. These results suggest that an RNA binding protein is involved in PipR-HEHEAA regulatory pathways in Pseudomonas GM79.

17 Observing paradoxical effects and heteroresistance in Enterococcus faecalis populations centered around the gut microbiome

Audrey Morris University of Idaho

Enterococcus faecalis is a gram-positive bacterium present in the gastrointestinal tract, responsible for metabolizing nutrients and maintaining a healthy gut microbiota. In states of dysbiosis, E. faecalis can cause infection through pathogenic states and biofilm production. Antibiotics, such as Vancomycin, are unable to combat the increased number of E. faecalis populations due to its observed resistance against common antimicrobials. Deriving from this, our focus is heteroresistance, a phenomenon where a bacterial strain exhibits two subpopulations, one expressing resistance to a certain antibiotic, and the other expressing susceptibility. Thus far, E. faecalis has demonstrated susceptibility to Ciprofloxacin, a DNA gyrase inhibitor and displayed a paradoxical effect. This paradoxical effect can be described as a counterintuitive bacterial response to increasing antibiotic concentrations, implying that a significant dose beyond the observed MIC may have no effect on the population at all. Future research will continue to focus on mapping interactions with varying antibiotics, in terms of this paradoxical effect and heteroresistance. The key focus being pharmacokinetic modeling and genomic analysis to further understand the behavior of Enterococcus faecalis in relation to antibiotics

18 Optimizing Transformation Efficiency of Salmonella enterica serovar Typhimurium for the Identification of PFOA Resistance Genes

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Per- and poly-fluoroalkyl substances (PFAS) are highly stable synthetic chemicals used in consumer goods and industry. PFAS, also known as forever chemicals, are bio accumulative and persist in the environment. Epidemiological studies suggest significant risks to human health and ecosystems. Our research focuses on identifying cellular and molecular targets of PFAS toxicity. We have isolated Salmonella enterica serovar Typhimurium (Ames strain) mutants that have increased resistance to the PFAS chemical perfluorooctanoic acid (PFOA). Our aim is to identify which gene or genes are altered in these mutants and are therefore responsible for increased resistance to PFOA. This work describes a study to optimize the transformation efficiency of S. Typhimurium in preparation for screening a genomic library constructed from our mutants. Transformation efficiencies were compared using chemically competent cells vs. electroporation. Compared to transformations using chemically competent cells, higher numbers of plasmid transformants were obtained using electroporation. Optimal electroporation parameters using an UECM 830 square wave electroporator were 2000 V and 3 pulses of 100 us, using 2 mm gap cuvettes and 5 ng of plasmid DNA. Highest efficiencies were obtained using 40 ul of early log-mid log phase bacterial cultures (OD600 of 0.4 nm). An indispensable requirement for being able to screen genomic libraries is high efficiency transformation. Next steps in this study are to construct the genomic library from DNA isolated from PFOA-resistant bacteria and transform the library into wild type S. Typhimurium grown on PFOA containing medium to identify plasmids containing resistance genes.

19 Deep analysis of 3D T cell migration using Migrate3D reveals distinct motility patterns for discrete subsets of HIV-1-infected cells

Emily Mynar University of Vermont

Recent intravital imaging studies revealed the presence of small T cell syncytia in HIV-1-infected mice (reviewed in PMID: 31283440). Though they represent only a small fraction of infected entities, these syncytia are present within the first 24-48 hours of infection by fully replicative transmitted/founder virus. Our initial characterization of these syncytia (PMID: 26703714) documented that they are highly motile, make frequent transient contacts with uninfected cells, and can transfer virus, strongly suggesting that they function as mobile virus spreaders. Further quantitative fluorescence microscopy analyses (PMID: 31757023) and flow cytometry (unpublished) revealed that syncytia constitute a discrete subpopulation of infected cells.
To explore how syncytia differ functionally from mononucleated infected cells, we have begun analyzing their migratory behavior in 3D collagen hydrogels using light sheet fluorescence microscopy, followed by segmentation and tracking. To extract meaningful biological information from cell tracking data, we developed Migrate3D, a Python software package (preprint PMID: 36711888) to process cell tracking data from other image acquisition tools. Migrate3D offers step-based calculations, single-cell summary statistics, mean squared displacement, and machine learning-based analysis of cell subpopulations. The software supports flexible parameter adjustments for customized analysis of diverse motility patterns across a range of cell types, both in 2D and 3D time-lapse datasets.
Migrate3D analyses reveal unique migration patterns that distinguish HIV-infected from uninfected cells and syncytia from infected mononucleated cells. These insights point to candidate molecular mechanisms which may underlie these differences and guide further investigations into how mobile T cell syncytia contribute to early HIV-1 spread.

20 Activity-based protein profiling for high-throughput functional annotation of uncharacterized proteins

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Raya McAnany

Advances in DNA sequencing have led to the discovery of numerous Proteins of Unknown Function (PUFs) that lack sufficient sequence similarity to allow for function prediction through traditional computational homology. Many of these PUFs are conserved across taxa, suggesting their functional importance. Functional annotation of these proteins could provide key information about microbial physiology and help identify new enzymes for biotechnology applications. Traditional approaches to experimentally-driven annotation, however, are time-intensive and cannot keep pace with the rapid discoveries of sequencing. To address this problem, we applied Activity-Based Protein Profiling (ABPP) to experimentally determine the functions of PUFs by targeting enzyme activity with small-molecule chemical probes. Probes specific to reactive cysteines (Iodoacetamide, IAA), serine hydrolases (Fluorophosphate, FP2), and enzymes that hydrolyze ATP (ATP-ABP) labeled proteins in four organisms: Escherichia coli Lactobacillus iners, Chlamydomonas reinhardtii , and Akkermansia muciniphila Fluorescent tagging allowed visualization on protein gels, revealing active proteins. These active proteins were enriched by click chemistry-based biotinylation and identified using LC-MS/MS proteomics. Proteome multiplexing reduced labeling efficacy, but increased throughput and still enabled detection of unique and uncharacterized proteins from each organism. Selected proteins identified through ABPP were recombinantly expressed, and reporter assays were utilized to investigate if the proteins display the predicted enzymatic activity. This research emphasizes ABPP's potential to advance functional annotation across multiple taxa, thereby enhancing genomic data accuracy and understanding of non-model microbes, such as those critical to human health. Future studies will focus on improving multiplexing efficiency to allow high-throughput annotation of PUFs across diverse species.

21 Threatening Heteroresistant Klebsiella pneumoniae in Human Gut Microbiome

Sydney Oakley University of Idaho

Klebsiella pneumoniae is a gram-negative, facultative anaerobe, primarily associated with causing pneumonia. However, it is also linked to causing disruption in the gastrointestinal tract. The evolution of new antibiotic-resistant strains of K. pneumoniae threatens the ability to diagnose and treat these infections. Heteroresistant K. pneumoniae occurs when a subpopulation of cells is resistant to a higher antibiotic concentration than the minimum inhibitory concentration, or MIC, of the wild-type population. The methods and consequences of this heteroresistance are still largely unknown. To better understand the heteroresistance to various antibiotics, a population analysis profile (PAP) assay is to be utilized. This process involves spreading the bacteria on agar plates that have an increasing concentration of an antibiotic. Concentrations begin below the MIC and increase to an 8-fold MIC concentration. Colonies growing at ≥8 × MIC are considered heteroresistant. These resistant subpopulations revert back to the susceptible wild type when removed from the high antibiotic concentration. Research into the heteroresistance of Klebsiella pneumoniae will broaden our understanding of the pathogenesis in aerobic and anaerobic human gut environments.

22 Understanding the Synergy of PET-degrading Microbial Consortia

Patricio Palomino Reed College

Plastic pollution is a worldwide problem that is managed with flawed approaches such as incineration or recycling of plastics. The poor efficiency of these mitigation techniques has sparked the search for more sustainable solutions. Microbial degradation of Polyethylene terephthalate (PET) offers a new approach to the problem. In 2023, a mix of three consortiums of Pseudomonas and Bacillus species were found to degrade 17% of PET in a two week period. Combining consortiums together leads to increased plastic degradation as the diversity of enzymatic activity is greater and bacteria are able to form stronger biofilms on the surface of the plastic. This means that understanding the population dynamics of the bacterial species is crucial to improving the efficiency of PET degradation. It's been noted that after two weeks of incubation, the Bacillus species completely dies off. We tested whether Pseudomonas induces the sporulation of Bacillus due to an increasing pressure to compete for limited resources. We created a Pseudomonas pre-conditioned media, which statistically significantly increased the sporulation of Bacillus, indicating that effector proteins secreted by Pseudomonas present in the pre-conditioned media are being used to kill off Bacillus. Moreover, an analysis of the genes that are being up and down regulated by the consortia indicated an increase in sporulation of Bacillus after two weeks of incubation. Finally, growing the cultures in low nutrient plates showed a significant zone of inhibition of Bacillus, further aligning with the hypothesis that Pseudomonas is secreting effector proteins to outcompete Bacillus.

23 Optimization of Crystallization Conditions for Growing AhpC-Ligand Crystals

Bruce Redden Washington State University

Helicobacter pylori, a human stomach pathogen, uses alkyl hydroperoxide reductase C (AhpC) as part of its defense mechanism against the immune response of the host. AhpC is an antioxidant enzyme that protects cells against oxidative stress by reducing harmful hydroperoxides into water and alcohol. We used a structure-guided drug discovery (SGDD) approach with AhpC as a target to identify a potential inhibitor. In previous work, we have identified novel AhpC inhibitors and want to obtain the structures of the inhibitor-AhpC complexes. In a SGDD pipeline there must be an understanding of the protein-ligand complex. To understand the interaction between the target enzyme and potential inhibitors, we need a crystallized structure of this complex. Therefore, we sought to refine conditions to obtain well-diffracting AhpC protein crystals for structural studies. Following optimization of parameters XYZ using the vapor diffusion hanging drop method, the most promising condition is 23% polyethylene glycol 3350 using Tacsimate™ at pH 6. A concentration of 0.856 mg/ml of purified AhpC provides the best crystal growth. These conditions allowed the formation of pyramidal crystals ranging from 10-100 µm. Future work will aim to soak the protein crystals in inhibitor solutions and perform x-ray crystallographic studies to solve the structures of the complexes, improving our understanding of the mechanism of inhibition.

24 Organismal Acclimation of Bacillus pumilus exhibiting Ruderal tendencies

Himanshi Singh University of Idaho

The impact of stressors on gene expression, growth, and survival of Bacillus pumilus in laboratory assays is being investigated with the aim of applying these findings to natural soil environments. A variety of conifer specimens have been collected to culture and isolate strains of Bacillus pumilus and related phylogenetic strains. This process involves colony streaking, DNA extraction, amplification, and subsequent gel electrophoresis for 16S DNA testing. The results of this research will help identify genes and physiological mechanisms that enable microorganisms to withstand the complex combination of molecular stressors associated with fire and drought conditions.

25 Towards Identifying a PE-ase in a bacterial consortium

Madelyn Tarara Reed College

Plastic pollution is one of the most monumental problems facing earth's ecosystems today. In 2022, only 5% of plastic recycled in the U.S.was Low Density Polyethylene (LDPE). It is a common plastic, which is typically found in plastic bags, cling wrap, condiment bottles, and other sources. Many recycling centers do not accept flimsy plastic materials (including LDPE) due to the time and financial burden of recycling it. In 2017, the Mellies Lab identified a consortium of 5 bacteria that was shown to degrade degrade plastic, specifically Polyethylene Terephthalate (PET) plastic.1 Subsequent genomic research has also identified that this consortium of bacteria is capable of degrading plastics beyond PET, for example, Polyvinyl Alcohol (PVA) and LDPE.

To create marketable solutions to facing our worlds' plastic problem, attention needs to be brought to discover the specific molecular mechanisms that confer LDPE degradation, and the enzymes responsible. Informed by GC-MS data that indicates that chemical degradation is occurring by this community of bacteria; by generation of various hydroxylated or reduced n-alkanes, we sought to identify potential degraders of LDPE. After identifying various potential targets that have been described in the literature previously, we chose to examine 4 enzymatic targets using RT-qPCR. Our results indicate that we have two putative LDPE degraders: a carboxylesterase NlHh, and a bifunctional NADPH Cytochrome P450.

26 The investigation of killer yeast in mason bees.

Rim Tedros Tekle University of Idaho

Fungal illnesses affect millions of people and can put vulnerable populations at risk of death. Killer yeasts, like the baker's yeast Saccharomyces cerevisiae, produce toxins that exhibit new antifungal activities that could be useful in combating fungal diseases. Insects such as bees and wasps are associated with yeasts and can disperse them in the environment. Preliminary investigations have found that killer yeasts are predominantly associated with members of the Hymenoptera. This survey was done by capturing and disseminating insects; due to concerns about native pollinator health, we designed a non-lethal method of assessing yeast association using solitary bees, which may be the source of novel killer yeast strains. The lifecycle of these insects allows for the collection of empty nests that were swabbed and streaked on YPD pH 4.6 agar plates. Of the 42 yeast isolates identified, 17 were killer yeasts. Further studies will be conducted on nests built in 2024 and empty nests collected from a local nature conservancy by local high school students. We have also planted a native pollinator garden near the nest sites to attract more native bees. This study is significant because it investigates the ecological prevalence of killer yeasts and the possibility of discovering novel antifungal therapeutics without euthanizing insects.

27 Alternate hazelnut shell ash catalysis and optimization of two-step chemo-microbial polyethylene terephthalate (PET) degradation

Lucas Walker Reed College

Global plastic pollution is one of the greatest issues of the modern age, and the production of single-use plastics has skyrocketed due to COVID-19. A consortium of five soil-dwelling Pseudomonas and Bacillus species can utilize polyethylene terephthalate (PET) as their sole carbon source, and two-step chemo-microbial PET degradation has been described with terephthalic acid (TPA) extraction on the gram-scale. TPA can subsequently be polymerized with ethylene glycol (EG) to form new, high-quality PET. This research investigates the use of hazelnut shell ash (HSA) compared to orange peel ash (OPA) as a biocatalyst for the glycolysis step to integrate the process with Oregon agriculture. It also explores scaling up this process, optimizing additional nutrient availability to maximize biodegradation rate and TPA accumulation, determined by culture pH as well as HPLC analysis after three weeks. HSA catalysis produces bis(2-hydroxyethyl) terephthalate (BHET) with fewer impurities than OPA catalysis but yields less product with a maximum of 76% yield compared to the 94% found previously with OPA. Additional nutrition from yeast extract results in less TPA accumulation after one week, but a significantly higher biodegradation rate and TPA accumulation is achieved after three weeks, suggesting that yeast extract is preferentially metabolized over BHET but allows the microbes to degrade BHET with higher efficiency once it is the sole carbon source available. This has promising implications for large-scale application of the process to PET recycling; further research will increase culture volumes, as well as optimize TPA extraction and characterize TPA obtained from HSA-catalyzed BHET.

28 The yEvo Mutation Browser: Enhancing student understanding of experimental evolution through interactive data visualization

Leah Anderson University of Washington

Experimental evolution is a powerful method for studying the relationship between genotype and phenotype by observing how populations genetically adapt to selective pressure. In educational settings, this approach also offers a dynamic way for students to engage with molecular genetics. One such educational effort, known as “yEvo,” introduces experimental evolution into high school classrooms, allowing students to evolve the Saccharomyces cerevisiae under various stressors and investigate the resulting genetic changes. While hands-on experiments have been successful in fostering student interest and understanding of evolution, the downstream data analysis - specifically interpreting whole-genome sequencing results of evolved yeast compared to the ancestor - remains a challenge. Students struggle to grasp the significance of their mutated genes and lack the broader context to determine which mutations are most phenotypically relevant. To address these issues, we developed the yEvo Mutation Browser, an intuitive web tool designed to assist students and researchers alike in visualizing and contextualizing genome sequencing data. Developed using R Shiny, this tool features various interactive visualizations, including a chromosome map, a breakdown of mutation types, and a gene view showing mutation sites. The app also features an option for non-yEvo-affiliated users to upload their own datasets and compare them with all yEvo data collected since 2018. The yEvo Mutation Browser streamlines data interpretation, helping students understand how organisms employ diverse genetic strategies to adapt to environmental stress. In the future, this framework could be adapted for other model organisms, offering a valuable resource for both evolutionary genetics research and education.

29 Exploring Bacterial Crosstalk in Periodontal Pathogens Using MicroSPLiT

Celine Grace F. Atkinson University of Washington

Polymicrobial synergy has been implicated in the emergence of disease, however, we still have limited knowledge of how the physical cell-to-cell interactions of pathogens truly contribute to disease progression. To better understand the role of polymicrobial synergy involved in disease progression, we are utilizing a novel tool to resolve the true positive interaction between two key oral bacteria (Fusobacterium and Porphyromonas) involved in pathogenesis of periodontitis. MicroSPLiT, a low-cost, high-resolution single-cell RNA sequencing technique for microbes, allows us to assess the gene expression of functional subpopulations that are lost in current RNA sequencing techniques. Using microSPLiT, we expect to directly advance our understanding of the regulation of genes and assess the true interaction between physically interacting microbiota involved in oral health and disease.

30 The Transcriptional Regulator Tr1 is Essential for Anaplasma phagocytophilum Adaptation to the Tick Vector

Tanner Badigian Washington State University

Anaplasma phagocytophilum (A.p.) is a vector-borne obligate intracellular bacterial pathogen and is the causal agent of Human Granulocytic Anaplasmosis (HGA). Reported HGA cases have increased >2,000% since it was first described in 1999. There are no HGA vaccines available, limiting our current prevention and treatment measures. The Ixodes scapularis tick is the primary vector involved in A.p. transmission between mammalian hosts. This process requires A.p. to adapt to the unique environments encountered within both mammalian and tick cells. To adapt between host and vector, A.p. differentially transcribes 41.5% of its genome when infecting tick cells versus human cells. Many of these genes have essential roles in the establishment and maintenance of mammal or tick infection. The gene displaying the greatest tick-specific transcription is the putative transcriptional regulator tr1. AlphaFold modeling predicts Tr1 is a homo-dimeric Helix-Turn-Helix DNA binding protein. Consistent with this model, we found Tr1 binds DNA sequences within its own promoter region. Mutation of tr1 had no effect on A.p. growth in mice but disables survival in ticks. Transcriptomic analysis during tick cell infection showed the tr1 mutant failed to transcribe many tick-specific A.p. genes. These include other transcriptional regulators, surface proteins, and secreted effector proteins. Mutation of these genes similarly led to growth defects in tick cell culture. Our findings show that Tr1 is an essential regulator of the transcriptional reprogramming A.p. undergoes to promote adaptation to the tick. Understanding how Tr1 regulates these changes may enable novel interventions that interfere with transmission prior to human infection.

31 Evolution of polyamine resistance in Staphylococcus aureus through modulation of potassium transport

Killian Campbell University of Oregon

Microbes must adapt to diverse biotic and abiotic factors encountered in host environments. Polyamines are an abundant class of aliphatic molecules that play essential roles in fundamental cellular processes across the tree of life. Surprisingly, the bacterial pathogen Staphylococcus aureus is highly sensitive to polyamines encountered during skin infections in the human host, and acquisition of a polyamine resistance locus has been implicated in spread of the epidemic USA300 methicillin-resistant S. aureus lineage. At present, alternative pathways of polyamine resistance in staphylococci are largely unknown. Here we applied experimental evolution to identify novel mechanisms and consequences of S. aureus adaptation when exposed to increasing concentrations of the polyamine spermine. Evolved populations of S. aureus exhibited striking evidence of parallel adaptation, accumulating independent mutations in the potassium transporter genes ktrA and ktrD. Mutations in either ktrA or ktrD are sufficient to confer polyamine resistance and function in an additive manner. Moreover, we find that ktr mutations provide increased resistance to multiple classes of unrelated cationic antibiotics, suggesting a common mechanism of resistance. Consistent with this hypothesis, ktr mutants exhibit alterations in cell surface charge indicative of reduced affinity and uptake of cationic molecules. Finally, we observe that laboratory-evolved ktr mutations are also present in diverse natural S. aureus isolates, suggesting these mutations may contribute to antimicrobial resistance during human infections. Collectively this study identifies a new role for potassium transport in S. aureus polyamine resistance with consequences for susceptibility to both host-derived and clinically-used antimicrobials.

32 Deciphering NLRP1-dependent inflammasome responses to double-stranded RNA

Miles Corley University of Washington

Inflammasomes are cytosolic innate immune complexes that initiate pyroptotic cell death and the release of inflammatory cytokines. Inflammasomes are critical to the host innate immune response to viral pathogens; however, aberrant inflammasome activation has been linked to increased viral pathogenesis and sterile autoinflammatory diseases. Therefore, it is imperative to understand the factors that govern inflammasome activation.

The inflammasome-forming sensor NLRP1 functions in barrier defense against a diversity of pathogens, necessitating multiple modes of pathogen recognition. For instance, NLRP1 directly senses viral infection via the detection of viral protease activity from several positive-sense RNA viruses including coronaviruses and enteroviruses. NLRP1 is also activated indirectly by the ribotoxic stress response (RSR). RSR induction by radiation or toxins causes ribosomal collisions, triggering a mitogen-activated protein kinase (MAPK) cascade involving the RSR sensor ZAKα and p38α/β, which phosphorylate NLRP1 to initiate inflammasome activation. Moreover, double-stranded RNA (dsRNA) also activates NLRP1.

NLRP1 has been proposed to directly bind dsRNA; however, ZAKα and p38 are required for dsRNA induced NLRP1 inflammasome activation. Thus, the mechanism by which dsRNA activates the NLRP1 inflammasome is unclear. To determine how dsRNA activates NLRP1 we reconstituted the NLRP1 inflammasome in inflammasome-deficient 293T cells. We found that reconstitution of the minimal NLRP1 inflammasome responds to viral proteases and other stimuli but not to dsRNA. Instead, we found that ectopic expression of the RSR kinase cascade and other host pathways restores NLRP1 sensing of dsRNA in 293T cells, suggesting that NLRP1 via an indirect event that requires upstream sensors and additional cofactors.

33 Exploring the function of cell-mediated innate immune memory in Ixodes scapularis

Cameron Coyle Washington State University

The black-legged deer tick, Ixodes scapularis, vectors at least seven pathogens of significant public health concern including the causative agents of Lyme disease (Borrelia burgdorferi) and anaplasmosis (Anaplasma phagocytophilum). The tick immune system is a significant determinant in how ticks acquire, harbor, and transmit disease (“vector competency”). We have previously shown that antigenically priming ticks results in both short- and long-term protection against bacterial colonization. One possible mechanism mediating persistent protection is innate immune memory. Innate immune memory describes enhanced innate immune responses and increased survival following previous antigenic challenges. Mounting evidence indicates that immunological memory conferred by innate immune processes exists in invertebrates, including ticks. Arthropod innate immunity can be subcategorized into humoral immunity and cellular immunity. Specialized phagocytic cells, termed “hemocytes”, are an integral component of arthropod cellular immune responses, but their role in tick innate immune memory remains unexplored. Here, we found that primary stimulation of ticks with the infection derived lipid POPG activates the expression of humoral immune genes. However, secondary stimulation promotes hemocyte gene expression. Furthermore, repeat POPG exposures increase the phagocytic ability of tick cells, as measured through fluorescence microscopy. These findings indicate that initial immune response to pathogens is mediated by activated humoral innate immunity, while subsequent pathogenic encounters are combatted with enhanced cellular innate immunity.

34 Killer toxin K62 from yeast Saccharomyces paradoxus is an aerolysin pore forming toxin

Jack Creagh University of Idaho

Aerolysin toxins are a family of pore-forming toxins best known as bacterial virulence factors that cause hemorrhagic diseases in animals. This family of toxins lacks sequence conservation and is characterized by a highly stable five beta-sheet domain. Using the protein structure prediction software AlphaFold2, we have identified K62, an antifungal produced by the yeast Saccharomyces paradoxus, exhibits the aerolysin five beta-sheet domain. Our model of K62 has high structural similarity to the bacterial aerolysin toxin parasporin-2 with an LDDT over 90 and a root mean squared deviation (RMSD) of 5.5 Å. K62 is most active on other fungi at an acidic pH and temperatures less than 30oC, which is typical of other yeast toxins that are thought to provide competitive advantage in the environment. We developed an ectopic expression system and showed K62 is sufficient to cause the antifungal phenotype. Using SDS-PAGE and recombinant K62, we show that K62 forms SDS and heat-resistant complexes like highly stable aerolysin toxin. Although K62 is an antifungal killer toxin, we have found K62 like sequences in plant pathogenic fungi such as Fusarium sp. and the emerging human pathogen Candida auris. Given the known role of aerolysins in bacterial pathogenicity, we speculate that K62-like proteins could also be relevant in fungal disease. Using computational, genetic, and biochemical techniques, we have characterized a novel aerolysin family toxin in Saccharomyces yeasts that will serve as a powerful model for the future discovery and characterization of diverse aerolysin-family pore-forming toxins.

35 The Role of the Gut Microbiome in the Colonization of ESBL-Producing E. coli in Healthy Children

Brandon Flatgard Washington State University

In 2019, antibiotic-resistant bacterial infections were implicated in approximately 4.95 million deaths worldwide, with extended-spectrum β-lactamase-producing Escherichia coli (ESBL-Ec) representing a particularly concerning threat1. These bacteria are increasingly present in the gut microbiota of individuals, particularly in low-income regions, posing a significant risk for resistant infections2. In earlier studies, we found that 74% of healthy Bangladeshi infants aged 1-12 months were colonized by ESBL-Ec3. This study aims to understand the factors behind ESBL-Ec's persistence in the gut by analyzing stool samples from Bangladeshi children with and without ESBL-Ec colonization. We hypothesize that the gut microbiota composition plays a crucial role in facilitating ESBL-Ec colonization, particularly through the expansion of resistant bacterial clones. Our initial analysis of 100 stool samples—half with high levels of ESBL-Ec (>10^3 CFU/g stool)—indicated that ESBL-Ec positive samples harbored a more diverse bacterial community, with unique microbiota composition and a notable abundance of the Bacteroidaceae family, which may support ESBL-Ec persistence. Further research will investigate specific bacterial groups, their relationship with antibiotic resistance genes, and the microbial metabolites potentially shaping gut microbiota dynamics, impacting ESBL-Ec persistence. Through comparative analyses of gut microbial networks, we aim to pinpoint microbes fostering a niche conducive to ESBL-Ec proliferation.

36 Prime Potential: Zn2+ as a secondary messenger for redox potential sensed through chemosensory zinc-binding (CZB) domains

Kailie Franco Washington State University

Chemosensory zinc-binding (CZB) protein domains control the activity of proteins using a unique 3His, 1Cys motif to bind a single Zn2+ atom. How this Zn2+-recognition mediates a useful physiological benefit for the diverse bacteria that possess CZB-regulated proteins, which include the gastrointestinal pathogen, Salmonella enterica serovar Typhimurium, remains unclear. S. Typhimurium possesses a single CZB-regulated chemoreceptor, methyl accepting chemotaxis protein A (McpA), for which neither the molecular nor biological function has been elucidated. Based on prior studies of orthologous CZB proteins, we hypothesized that McpA mediates chemotaxis responses to host-generated reactive electrophilic species (RES), such as peroxides (H2O2 and ROOH) and hypochlorous acid (HOCl). Using an injection-based microfluidics device and fluorescence live-imaging microscopy, we simultaneously viewed chemotaxis responses of wildtype and chemotaxis mutant strains to physiologically relevant concentrations of RES. We discovered that McpA indeed mediates chemoattraction to RES, although chemoattraction was not species dependent. Dissection of the sensing mechanism through via mutation of the CZB zinc-binding Cys as well as alteration of exogenous Zn2+ levels revealed that zinc-binding is required to mediate RES-sensing. Our data suggest this is due to the direct oxidation of Zn2+ to zinc oxides, which are not recognized by McpA. While chemoattraction to RES might seem counter-intuitive, our work suggests that S. Typhimurium is indeed attracted to RES. We propose that the underlying molecular function of CZB domains is to perceive exogenous redox potentials based on the relative abundance of Zn2+ (low potential) versus ZnO (high potential).

37 The A. phagocytophilum T4SS tick effector, AteA, contains EPIYA motifs and directs tyrosine phosphorylation

Brittany Genera Washington State University

Anaplasma phagocytophilum, the second most prevalent tick-borne pathogen in North America, causes human granulocytic anaplasmosis (HGA). Transmitted by Ixodes scapularis ticks, HGA symptoms include fever, muscle aches, and nausea, which can escalate to respiratory distress and organ failure. As A. phagocytophilum transitions from tick to mammalian hosts, it must adapt to new tissues and cell types, a challenge for this obligate intracellular bacterium. Its Type IV Secretion System (T4SS) is essential for virulence, allowing effector molecules to enter host cells. Though five T4SS effectors have been identified in mammalian cells, their role in tick cells remains unexplored. We recently discovered that the effector protein AteA is crucial for A. phagocytophilum survival in tick cells, where it interacts with the actin cytoskeleton and modifies actin morphology. AteA contains two novel tandem repeat regions: the C-terminal repeat is required for actin association, while the central repeat alters actin structure. The central region also features EPLYA motifs, variants of the EPIYA motif, which are tyrosine phosphorylation sites in other bacterial effectors. Using fluorescent imaging with an anti-tyrosine phosphate antibody, we demonstrated that phosphorylated tyrosine residues are concentrated at AteA localization sites, dependent on the EPLYA-containing region. AteA is the second A. phagocytophilum T4SS effector with EPIYA motifs and the first to be tick-specific. Understanding how A. phagocytophilum uses effectors like AteA to establish infections across different hosts may offer new avenues for intervention strategies.

38 Bacterial Vampirism: mediated by chemoreceptor Tsr

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Sepsis is the leading cause of death for inflammatory bowel disease (IBD) patients, yet the mechanisms by which these bacteria enter the bloodstream are poorly understood. Using a custom microfluidics device, we simulated a gastrointestinal (GI) bleeding event and observed that femtoliter volumes of serum were sufficient to trigger bacterial localization. Specifically, we found that Escherichia coli, Citrobacter koseri, and clinical isolates of non-typhoidal Salmonella enterica serovars exhibit a rapid chemotactic response towards human serum, driven by the nutrient L-serine mediated by the chemoreceptor Tsr. This response is likely attributable to nutrient-seeking behavior, as we observed a twofold growth increase in S. enterica serovars treated with low volumes of serum compared to the untreated control. To elucidate the molecular mechanisms involved, we determined the crystal structure of S. Typhimurium Tsr (SeTsr) in complex with L-serine at a 2.2 Å resolution (PDB: 8FYV & 8VL8). This structure revealed a conserved amino acid recognition motif for L-serine shared among Tsr orthologues. Phylogenetic analysis highlights the conservation of Tsr among Enterobacteriaceae and other pathogens associated with bloodstream infections. We developed an enterohemorrhagic lesion model utilizing intestinal swine tissue to investigate the potential role of Tsr in bacterial bloodstream entry. Following exposure to the lesion, S. Typhimurium exhibited localization at the wound opening and achieved vasculature penetration exceeding 200 μm. We term these bacterial tendencies of taxis toward serum, colonization of hemorrhagic lesions, and consumption of serum nutrients as “bacterial vampirism,” which may relate to the proclivity of Enterobacteriaceae for bloodstream infections.

39 Developing narrow-spectrum antibacterial medicines against Helicobacter pylori through inhibition of redox homeostasis

Andrea Gómez Washington State University

Helicobacter pylori is a pathogen of the human stomach and causative agent of gastric cancer, a leading cause of cancer death. Multidrug-resistant strains have become prevalent, and treatment failure is common, necessitating the development of new clinical tools for managing H. pylori infections. To resist eradication, H. pylori requires alkyl hydroperoxide reductase C (AhpC) to eliminate hydroperoxides (ROOH) and resist oxidative stress generated through innate immunity. During its catalytic cycle, AhpC undergoes a large conformational change from a fully folded active conformation to a locally unfolded inactive conformation. By leveraging the structural information of AhpC, a structure-guided drug discovery approach was employed. A druggable pocket was identified, and virtual ligand screening was conducted against millions of compounds to identify potential inhibitors. High-throughput screening assays were then used to validate these compounds, resulting in the identification of six promising candidates (I-VI). In vitro experiments demonstrated potent inhibition of AhpC catalysis by these compounds. Furthermore, these compounds exhibited bactericidal activity against H. pylori under oxidative stress conditions, suggesting their ability to penetrate bacterial cells and disrupt H. pylori survival. Compound IV showed promising activity with an effective concentration (EC50) of 33 μM and a minimal inhibitory concentration (MIC) of 25 μM. Our preliminary work suggests that the action of IV should be rather specific in mediating H. pylori killing, as neither bacteria nor human cells with AhpC orthologues experience off-target toxicity. Further development and optimization of these compounds may lead to effective narrow-spectrum antibacterials as therapeutics for H. pylori infections.

40 Utilizing a synthetic soil habitat to unravel the mechanisms behind plant-associated, aerobic bacterial nitrogen fixation

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Many free-living bacterial diazotrophs have been shown to utilize the production of a protective layer of extracellular polymeric substances (EPS) to protect their nitrogenase from exposure to oxygen in aerobic conditions. Although less is known about the mechanisms of nitrogen-fixation in bacterial diazotrophic endophytes of non-nodulating plant species, it is likely that a similar phenomenon may occur. By employing a genome-wide fitness screen on one of our diazotrophic endophytes, Azospirillum palustre strain 11RA, we have identified several genes that may be involved in the production of a protective EPS layer during nitrogen-fixation in aerobic conditions. Additionally, we used confocal microscopy on a second endophyte isolated by our lab, Burkholderia vietnamiensis strain WPB in a RhizoChip, a synthetic soil habitat. This allowed us to image WPB within the roots of wild Populus and show the presence of sac-like 'nitrosome' structures that are only present when bacteria are present and nitrogenase is active. Current work includes analyzing how these identified genes impact bacterial phenotype on the RhizoChip in a nitrogen-limited environment. This work aims to identify novel mechanisms regulating endophytic nitrogen fixation and plant-microbe interactions.

41 Sphingolipid showdown: Host clearance of Mtb dependent on sphingolipids

Alec Griffith OHSU

Damage to the phagosomal membrane of a host cell represents a decisive for the intracellular pathology of Mycobacterium tuberculosis (Mtb). If this damage, primarily caused by secreted Mtb effectors, not quickly detected and repaired by the cell Mtb will begin the metabolic reprogramming of the host cell to establish its niche. If the cell, however, is able to quickly detect and respond to bacteria-induced damage it will clear the invading pathogen. We have determined that sphingolipids are an indispensable class of lipids involved in this damage repair and subsequent pathogen clearance. We see that sphingomyelin accumulates on membranes containing Mtb bacteria in a manner similar to the accumulation of sphingomyelin at sites of membrane damage. Cells infected with Mtb that are subsequently treated with sphingolipid synthesis inhibitors have increased rates of membrane damage. We show that these cells are also deficient in their ability to clear the pathogen. Overall, we see that cells are better able to clear attenuated strains of Mtb and this effect is maintained in cells treated with sphingolipid synthesis inhibitors. This indicates that Mtb secreted effectors are particularly important for the bacterial survival in sphingolipid deficient cells. These data all show an importance for sphingolipids in protecting cells from Mtb and provide a potential target for future antibacterial therapies.

42 Genetic insights into PFAS toxicity: unravelling resistance mechanisms

Dharshana Lakshminaryanan Central Washington University

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds structurally similar to fatty acids, with hydrogen atoms replaced by fluorine atoms, resulting in stable carbon-fluorine (C-F) bonds. This stability makes PFAS resistant to environmental degradation, earning them the nickname "forever chemicals." Used for over 80 years in consumer and industrial products for their water-repellent and heat-resistant properties, PFAS are found throughout the environment. Epidemiological studies suggest human exposure to PFAS may lead to adverse health outcomes, including endocrine disruption and cancer, though the cellular and molecular mechanisms behind their toxicity remain unclear. Identifying the molecular targets of PFAS toxicity is crucial for developing treatments and safer alternatives. In our research, we use Salmonella enterica serovar Typhimurium (Ames strain) due to its ease of mutagenesis. By exposing this bacterium to perfluorooctanoic acid (PFOA), we have isolated twelve mutants capable of surviving in concentrations of PFOA that inhibit wild-type growth. Our study focuses on evaluating the mutants' tolerance to both PFOA and GenX™, a "next generation" PFAS. Since the mutants exhibit varying PFAS tolerance, multiple genetic changes are likely involved, suggesting various molecular targets. We are currently working on constructing genomic libraries from these mutants that will be screened in the wild-type S. Typhimurium using media containing PFOA at levels that are otherwise non-permissive for growth. This approach aims to identify specific genes whose products are targets of PFAS toxicity. Due to the conservation of bacterial gene products across species, our findings may offer insights into PFAS toxicity mechanisms relevant to other organisms.

43 Mechanisms of biofilm formation and dispersal in the anaerobic opportunistic pathogen Finegoldia magna

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Aggregate biofilms play a crucial role in establishing hard-to-treat, chronic infections because they are resistant to antibiotic treatment and host immune responses. Understanding the processes that promote biofilm formation (aggregation) and dispersal is critical for controlling biofilms and resolving chronic infections. Most biofilm research has focused on readily culturable facultative anaerobes. However, most human microbiota is comprised of understudied obligate anaerobes, many of which are opportunistic pathogens that form biofilms during chronic infections. We are examining biofilm regulation in the obligate anaerobe, Finegoldia magna, which colonizes healthy skin but is an opportunistic pathogen frequently isolated from chronic wound infections. F. magna readily forms aggregate biofilms, even in liquid culture. F. magna biofilms are sensitive to protease treatment, suggesting a primary role for surface proteins in mediating cell-cell adhesion. Using experimental evolution, we identified a putative adhesin required for F. magna aggregation. We are also determining how nutrient cues regulate F. magna biofilms, since growth in a high-density biofilm intensifies nutrient competition. We identified nutrients that promote biofilm dispersal and showed that physical disruption of F. magna biofilms enable cultures to achieve higher cell densities. These findings support the model that biofilm growth incurs a fitness cost that is balanced by benefits such as antibiotic survival or immune cell evasion. Promoting in vivo biofilm dispersal is an exciting new approach for combatting chronic infections. By studying these processes in diverse organisms, we aim to identify novel strategies for controlling biofilm formation in anaerobes that contribute to human health and disease.

44 The Effects of Salinity on the Cutaneous Microbiome and Bd Infection of the Pacific Tree Frog (Pseudacris regilla)

Hannah Kim Eastern Washington University

Anthropogenic climate change and infectious diseases are leading causes of amphibian population decline around the world. Changes in precipitation and salinity have altered important watershed systems, reducing habitat availability and altered physiological performances impacting amphibian survival. The salt refugee hypothesis however, proposes that amphibians have stronger abilities to defend against pathogens in higher saline environments. The Moro Cojo Slough State Marine Reserve (MCSSMR) (California, USA) is a key breeding ground for endangered/threatened amphibian species. To increase conservation where sensitive populations reside, the prevalence and intensity of Batrachochytrium dendrobatidis (Bd) and the composition of skin microbial communities were evaluated within the common Pacific Tree Frog (Pseudacris regilla) across a salinity gradient. We hypothesize amphibians found in higher salinity will have lower Bd rates and higher bacterial diversity. Thirteen skin swab samples were collected from P. regilla at 10 sites with saline concentrations from .5 - 30 ppt for Bd prevalence and intensity (using quantitative PCR) and bacterial community composition (by amplifying the V4-V5 region of the 16S rRNA gene using the Illumina Miseq platform). Statistical analyses, including Adonis method, Kruskal-Wallis test, and PERMANOVA test, will evaluate the interaction between salinity, Bd presence/absence, and microbial diversity. Spearman rank correlation and Mantel test will explore correlations between salinity, Bd infection intensity, and bacterial community structure. These results will provide valuable insight for amphibian conservation efforts where environmental conditions continue to change.

45 Experimental, metabolic, and mathematical framework for understanding microbial communities in the gut.

Matthew Kinahan University of Idaho

Microbes do not exist alone, rather they are one of many community members in an ecosystem. One ecosystem of critical importance is that of the gut. The gut microbiome has numerous implications in human health and deviations from healthy community compositions can have detrimental impacts. Community composition is governed by many factors with a driving factor being resource competition. Is it possible to predict how a community will compete for resources, what they will secrete, and what their steady-state composition will be? Using a bottom-up approach with bacteria representing major phyla within the gut we have experimentally determined their resource utilization profiles on common monosaccharides and disaccharides, revealing unique niches. Using metabolic modeling, we can predict how these bacteria will impact their environment through metabolic byproducts and get a sense for particular synergistic effects. Through experimental and metabolic models we can parameterize a consumer-resource model that predicts bacterial communities grown in a continuous culture. This will allow us to find steady states within the community and potentially control these communities. This system provides an experimental and theoretical framework that can be used to test hypotheses and confirm model predictions.

46 Preliminary results of in vitro gut microbiota fermentation of cranberry seeds and presscake and gut and vaginal microbiota modulation from cranberry juice consumption in healthy women

Ahhria Kirkendall Washington State University

Studies have found that understanding the physiological mechanism(s) of cranberry consumption and the effects on the microbiota and metabolites may provide more insight into potentially recommending cranberry juice consumption as an alternative to antibiotics from a regulatory standpoint (Jepsen, Williams, & Craig, 2014; Williams et al., 2023). To substantiate ongoing investigations, the Nutrition and Gut Microbiome Lab conducted an in vitro fermentation with cranberry (Vaccinium macrocarpon) seeds and presscake as well as are conducting a randomized crossover, double-blinded, placebo-controlled, dietary intervention on the effects of oral cranberry juice consumption in women of 18 years and older (n=50). Research goals encompass: 1) analyzing gut and vaginal microbial composition as well as urinary metabolites before and after clinical beverage intake, and 2) identifying potential microbial or metabolic pathways for UTI prevention. The preliminary analysis of the in vitro fermentation revealed that cranberry seeds induced a strong increase of Bacteroides and Clostridium spp. likely driven by the fiber and pectin contents with a modest bifidogenic effect. This effect is expected to derive from the phenolic contents of the cranberry seeds (Rodríguez-Daza et al., 2020). For the in vivo pilot study (n=7) of the ongoing dietary intervention (n=50), there is a large heterogeneity in gut and vaginal microbiota observed, as expected. The most consistent trend observed is a significant reduction of Proteobacteria in the vaginal microbiota, especially with one of the clinical beverages, which would be hypothesized to be beneficial for UTI prevention (Al Othaim, Marasini, & Carbonero, 2021). The clinical beverages remain double-blind.

47 Nutrient limitation supports stress tolerance in anaerobic opportunistic pathogens

Francia Lopez Palomera University of Oregon

Around 2% of the U.S. population has a chronic, nonhealing wound. The most common wound complication is infection, and importantly, wounds cannot heal until these infections are resolved. Obligate anaerobic bacteria are among the most prevalent and abundant colonizers of chronic wounds, yet we know little about the physiologies of these organisms. The abundance of obligate anaerobes in chronic wounds correlates with poorer outcomes, including limb amputation, yet these anaerobes persist despite oxygen and antibiotic therapies. Two of the most prevalent obligate anaerobes in chronic wound infections are the opportunistic pathogens Finegoldia magna and Anaerococcus vaginalis. Despite their clinical significance, little is known about the strategies that F. magna and A. vaginalis use to withstand stresses in the wound environment. We showed that nutrient limitation, which causes growth arrest, enables these pathogens to tolerate antibiotic and oxygen exposure. Nutrient-limited cultures of F. magna and A. vaginalis adopt antibiotic tolerant states, such that these cultures survive high antibiotic doses. Additionally, we found that F. magna and A. vaginalis withstand high oxygen exposure for 24-48 hours in nutrient-limited conditions where metabolic activity is low. Furthermore, F. magna displays greater sensitivity to oxygen in nutrient-replete compared to nutrient-limited conditions, supporting the model that metabolic activity sensitizes these pathogens to oxygen. Understanding how host environments modulate bacterial physiology in ways that enable these pathogens to survive otherwise lethal stresses could guide the development of more effective therapies for treating chronic wound infections.

48 Citrobacter rodentium effector Tir reduces the efficiency of intestinal epithelial cell extrusion following activation of intestinal epithelial inflammasomes

Marin Miner OHSU

Upon infection by intestinal pathogen Citrobacter rodentium, two cytosolic innate immune sensors, the NAIP-NLRC4 inflammasome and Caspase 11 are triggered. This leads to inflammatory cell death termed pyroptosis and intestinal epithelial cell (IEC) extrusion. This response protects from Salmonella and Shigella infection in mice. Extrusion relies on extensive actin remodeling to force the infected cell out of the epithelium while still maintaining the integrity of the intestinal barrier. It has yet to be determined if bacterial secreted effectors that polymerize actin can impact host cell extrusion. One C. rodentium effector, Translocated Intimin Receptor (Tir), nucleates actin for pedestal formation, making it a candidate for restricting extrusion. Our data demonstrates that the elimination of Tir's actin nucleation site (TirY471) leads to an increased extrusion phenotype. Wildtype mice show decreased epithelial colonization when infected with Tir mutant C. rodentium compared to wildtype bacteria. This difference is absent in Nlrc4-/- mice, indicating that Tir is reducing NLRC4 induced extrusion. Our preliminary data also indicate additional inflammasome pathways in epithelial cells that are activated upon C. rodentium infection. We have shown the presence of ASC specks during infection of primary IECs deficient in NLRC4 and Caspase 11. This could explain the evolutionary benefit for C. rodentium to target a downstream process of NLRC4 rather than NLRC4 itself. Elucidating the inflammasome pathway redundancy in IECs is crucial to understand the impact of bacterial effectors on host defense.

49 Populus endophytes from a semi-arid environment enhance plant heat tolerance

Morgan Raimondo University of Washington

Given the urgent environmental challenges posed by climate change, enhancing the resilience of plants to elevated temperatures is critical for climate adaptation and mitigation. Endophytes, the beneficial microbes residing within plant tissues, have been shown to support plant health, yet their role in conferring heat tolerance remains underexplored. This research investigates endophytes isolated from P. trichocarpa in the semi-arid Yakima River area of Washington State, hypothesizing that these microbes can enhance heat tolerance in their host plants. We inoculated sterile P. trichocarpa with individual endophyte strains (n=14) and assessed growth parameters under simulated arid conditions. Results indicated that inoculated plants exhibited significantly greater total chlorophyll content and improved growth compared to controls (p-value < 0.05). Notably, two novel strains—Microbacterium (YR-2A) and Pseudomonas (YR-21Y)—showed considerable potential for enhancing thermal stress resistance. De novo whole genome sequencing of these strains was performed to elucidate the genetic mechanisms underlying their beneficial effects, revealing the presence of stress tolerance genes like ACC deaminase gene (acdS), which modulate plant ethylene levels. These findings underscore the potential of leveraging endophyte-plant partnerships to enhance climate resilience in forestry and agricultural systems in a rapidly changing environment. By understanding the molecular mechanisms behind these symbiotic relationships, we can utilize microbial partnerships to enhance plant resilience in the face of increasing environmental stressors.

50 Pathogen-driven evolutionary innovations in the mammalian immune protein lactoferrin

Titas Sil University of Oregon

The emergence of new protein functions is a fundamental aspect of evolution, yet the mechanistic details underlying this process remain elusive. Host immune proteins can evolve rapidly in response to pathogens and provide ideal models to study the evolution of new functions. Specifically, lactoferrin is an innate immune protein from the transferrin family, which arose in mammals through gene duplication of transferrin and acquired new functions. Both lactoferrin and transferrin restrict cellular pathogens by limiting iron availability, however, lactoferrin has gained additional iron-independent antimicrobial properties. Its N-terminus, rich in positively charged amino acids, directly interacts with microbial surfaces, leading to bacterial lysis and biofilm disruption. To explore the origins and molecular mechanisms underlying these novel functions, I computationally reconstructed ancestral lactoferrin sequences and structures. Since the duplication event that led to modern lactoferrin, a trend of increasing positive charges in the N-terminus has been observed. In human and bovine lactoferrins, this positively charged N-terminal region is proteolytically cleaved to produce antimicrobial peptides. Using purified peptides from ancestral and modern lactoferrin sequences, I found that ancestral peptides have comparatively weak antimicrobial activity against both planktonic bacteria and biofilms. This suggests a gradual enhancement of lactoferrin's antimicrobial potency during mammalian evolution coinciding with increasing electrostatic potentials. Future work is focused on identifying the molecular mechanisms underlying these differences, and how bacteria evolve resistance to lactoferrin. This research will elucidate the functional evolution of lactoferrin in the context of host-pathogen interactions and advance knowledge about antimicrobial peptides as potential therapeutics for pathogens.

51 Influence of Synthetic Nasal Media on phage-biofilm dynamics in S. aureus

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In vitro studies of microorganisms often fail to replicate key aspects of their natural environments. Factors such as nutrient limitation and interactions with other microbes can shape microbial physiology as well as evolution and ecology in those environments. Hence, environmental conditions are crucial to consider in laboratory experiments, as they can reveal important interactions often missed in traditional in vitro studies. The gram-positive bacterium Staphylococcus aureus, an opportunistic pathogen in humans that often causes skin and soft tissue infections, is also a frequent commensal colonizer of the human nares. Within the host environment, S. aureus adheres to host tissues, forming biofilms and interacts with resident viral predators, such as bacteriophages. Previous studies on S. aureus adaptation are largely restricted to nutrient rich media in planktonic conditions that lack the spatial complexity of the host environment. The major goal of my research is to define how environmental conditions such as nutrients or spatial structure shape bacterial-phage ecology and evolution. Recently I have been applying Synthetic Nasal Media (SNM) to address these questions, a defined media that simulates the nutrient environment of the nares which S. aureus resides. Growth of S. aureus in SNM can facilitate biofilm formation, although population growth was limited due to nutrient availability in this host-like environment. Growth in SNM did not inhibit phage infection and will provide a model to simulate host conditions during experimental evolution. Through this work I hope to define how changes in the host environment impact interactions between phages and their bacterial prey.

52 Giant virus NUDIX hydrolase facilitates host gene shutoff during infection of amoeba

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All life must protect itself from viruses. To detect and resolve viral infection, organisms have evolved a wide diversity of antiviral solutions. Viruses have likewise evolved countless strategies to successfully navigate and subvert their hosts and propagate their genetic material. These virus-host arms races have been explored in bacteria and multicellular eukaryotes, however to date there are no known antiviral systems in microbial eukaryotes. The unicellular eukaryotic amoeba and their giant viruses provide an opportunity to identify novel antiviral systems through identification of common viral immune evasion strategies and their subsequent disruption. Infection of Acanthamoeba castellanii by the giant Marseillevirus dramatically reduces host transcript levels by two hours post-infection, suggesting a virally induced host shutoff program. Our screen of Marseillevirus nucleases and virion-associated proteins revealed one ORF, ORF242, which reduced expression of a luciferase reporter by ~80%. ORF242 is a member of the NUDIX hydrolase family —which includes viral mRNA de-capping enzymes. We predict that ORF242 leads to transcription shutoff by removing the 5’ caps of host mRNA and leading to degradation by the cellular ribonuclease Xrn1. Through mutational analysis, we show that the NUDIX domain of ORF242 is required for reduction of the luciferase reporter – as is the case for other viral NUDIX hydrolases involved in host shutoff. This function is conserved in other giant viral ORF242 homologs. Ultimately, we will generate virus mutants to further investigate the mechanism of ORF242 in vivo. The consequent mutation of this mechanism will allow identification of anti-viral factors and novel immunity pathways.

53 Stressed for success: How pathogen persistence in ticks is supported by ATF6 regulation of stomatin

Kaylee Vosbigian Washington State University

Ixodes scapularis, the North American deer tick, can transmit seven pathogens including the causative agent of Anaplasmosis, Anaplasma phagocytophilum. How tick-borne pathogens interact with their hosts has been primarily studied in mammals. Comparatively less is known about tick-pathogen interactions. We found that the transcription factor, ATF6, is activated during Anaplasma infection and supports Anaplasma colonization of ticks. What ATF6 regulates to support Anaplasma is not known. We identified an ATF6 binding site in the promoter region of stomatin and found that stomatin expression is increased in ticks infected with Anaplasma. We have demonstrated that ATF6 binds to the stomatin promoter and initiates transcription of a luciferase reporter gene. When ATF6 is activated via a pharmacological activator or transcriptionally decreased by RNAi we found corresponding changes in expression of stomatin in tick cells. Furthermore, we found that stomatin expression supports Anaplasma growth in larvae. We have linked stomatin expression to cholesterol levels in Anaplasma and in tick cells suggesting stomatin aids Anaplasma growth through promoting cholesterol incorporation into Anaplasma. Overall, our findings uncovered a previously unknown host mechanism that supports Anaplasma pathogenesis in ticks.

54 Spatial distribution of bacilli and virulence factors in the Mtb-infected lung

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The lung granuloma is a hallmark feature of tuberculosis (TB) that many believe is the focal point of interactions between host and bacterial factors, ultimately determining disease outcomes. Elucidating these interactions is critical to understanding TB pathogenesis with major implications for diagnostics and therapy. From a histological perspective, host components within granulomas have been well-characterized; however, the virulence factors secreted by Mycobacterium tuberculosis (Mtb) within these contexts are less understood regarding their localization and abundance. Here, we generate nanobodies against lipoarabinomannan, ESAT-6, CFP-10, ESAT-6/CFP-10 heterodimer, and Ag85. We apply these nanobodies to immunohistochemical staining of mature granulomas from Mtb-infected rabbits and Rhesus macaques (RMs). The Mtb-specific nanobodies strongly detect antigen with (sub-)cytoplasmic morphology in the lymphocytic cuff and within macrophage populations at the periphery of the granuloma, demonstrating widespread dissemination of virulence factors beyond the myeloid core. While ESAT-6 staining suggests cytoplasmic diffusion, targeting ESAT-6/CFP-10 dimers results in distinct compartmental staining in CD68+CD163+ cells. Bacterial puncta are also stained within the core; however, there is heterogeneity regarding which bacteria stain positively when targeting a given antigen. This heterogeneity is highlighted with multiplexed fluorescence incorporating the nanobodies, RNAScope, and a commercial antibody against TB lysate, suggesting complex regulation of antigen expression by Mtb that is dependent on local granuloma immune dynamics. In addition to serving as proof-of-concept for nanobody immunostaining, these data overall highlight complex virulence factor dynamics that have been underappreciated in the Mtb-infected lung.

55 Temporal Dynamics of Rhinovirus-Mediated Protection Against Lethal Influenza A Virus Infection in Mice

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Coinfection by different respiratory viruses is common and can alter disease severity compared to single virus infections. Clinical studies suggest that rhinovirus (RV) coinfection with influenza A virus may reduce disease severity, but the mechanisms and temporal dynamics remain poorly understood. Previous studies in mice have shown that RV provides protection against subsequent lethal influenza A virus (PR8) infection when given 2 days prior to challenge, but the duration of this effect remains undefined. Here, we systematically investigated the temporal window of RV-mediated protection against PR8 in a mouse model.
Female BALB/c mice were inoculated with RV at various time points (2, 4, 6, 8, 10, 14, and 30 days) before challenge with PR8. RV prevented PR8-induced mortality when administered up to 14 days before PR8 challenge. Protection against weight loss was most robust at the 2-day interval, where mice gained and maintained weight above baseline, while longer intervals (4–14 days) showed progressively increasing weight loss, which remained significantly less severe than mock/PR8 controls. When administered 30 days before PR8, RV provided no benefit; both groups showed similar weight loss and mortality patterns.
The reduced severity of PR8 observed up to 14 days after RV infection represents a significantly longer window of heterologous protection than previously recognized. These findings provide a foundation for understanding viral interference mechanisms in respiratory infections and have important implications for both our basic understanding of heterologous immunity and potential therapeutic approaches for preventing severe respiratory viral infections.

56 Investigating the Function of Desulfovibrio piger Sulfur-Reducing Genes in Hydrogen Sulfide Synthesis

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The human gut microbiota contributes to host homeostasis through various metabolic processes and interactions. Gut microbiota dysbiosis can occur through pressures enacted by dietary and disease related factors. Sulfur-reducing bacteria (SRB) are ubiquitously found in the human gut microbiota yet overabundance of these organisms contributes to inflammatory disease exacerbation due dangerously high production of their primary metabolite, hydrogen sulfide (H2S). H2S synthesis occurs through a pathway called dissimilatory sulfate reduction (DSR) which has been well characterized in many SRB. Desulfovibrio piger, an SRB that has been implicated in obesity and several inflammatory diseases, has not been well studied from the perspective of its DSR pathway. While genomic studies reveal high similarity between D. piger and other well characterized SRB, the exact genetic control of its DSR pathway is not yet known. This study will employ clonal transformation of Escherichia coli to determine which putative D. piger DSR genes are required for H2S synthesis. Six putative DSR genes/gene sets will be transformed into E. coli and the resulting mutant strains will be tested for gain of H2S synthesis function. The results of this study will provide clarity on the genetic control of this disease exacerbating pathway in a bacterium which has not been previously characterized.

57 Within-lung Interspecies Plasmid Transfer Causes Rapid Evolution of Extreme Antibiotic Resistance in Cystic Fibrosis

Sardar Karash University of Washington

Antibiotic resistance is linked to poor outcomes in people with cystic fibrosis (PwCF) chronically infected with Pseudomonas aeruginosa (Pa). We analyzed longitudinal Pa isolates from PwCF (N=15) and found the emergence of extremely tobramycin (Tob) resistant Pa within lungs initially infected with sensitive Pa. The extreme resistance suddenly emerges, durable for years within the lungs, and has MICs 100-1000 folds above resistance breakpoint. Here, we sought to identify the evolution and mechanisms of the extremely resistant Pa that emerges within the lungs. Complete genome comparisons of early sensitive and extremely resistant Pa pairs revealed that both are clonally related; however, the extremely resistant Pa carry conjugative resistance plasmids absent in their early sensitive clones. This clonality suggests that plasmids are acquired within the lungs. While the plasmids differ, nearly all contain an identical novel transposon we named Tn-CF1, which encodes an aac(3) resistance gene responsible for extreme resistance. Long-duration passaging experiments showed that plasmid carriage was stable and incurred no apparent fitness cost. In three PwCF, we identified co-infecting bacteria from which sensitive Pa likely acquired the plasmids. Co-infecting species, such as Achromobacter, Stenotrophomonas maltophilia, and Pseudomonas putida, carried identical plasmids several months before they were acquired by sensitive Pa. These co-infecting bacteria can conjugate the plasmids into sensitive Pa ex vivo, resulting in extreme Tob resistance. In summary, we show that co-infecting pathogens can transfer antibiotic resistance plasmids to highly sensitive Pa within the lungs of PwCF, leading to the sudden onset of extreme, durable, and low fitness-cost resistance.

58 A link between chemotaxis and carcinogenesis: Helicobacter pylori motility and chemotaxis restrict delivery of the oncogenic bacterial effector CagA within the gastric glands

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Helicobacter pylori is a major cause of gastric cancer and uses chemotaxis, i.e. directed swimming motility to infiltrate the gastric glands. H. pylori promotes cancer development by injecting cytotoxin-associated gene-A (CagA) effector into gastric glands, dysregulating stem cells and leading to metaplasia and dysplasia. Since these long-lived cells reside deep within the glands, we wondered whether swimming motility and chemotaxis are required for H. pylori to access this cell population, and thus, be involved in gastric cancer development.

To test this, we developed swine gastric explants as a metabolically inert reductionist model system, enabling us to easily visualize all gastric mucosa cells that become CagA+. Using fluorescence microscopy, we compared H. pylori colonization patterns and CagA delivery of H. pylori strains G27 and SS1. We found both strains effectively colonize the gastric surface and pits, and a minority population deeply infiltrates into the gastric gland base, like human infections. Isogenic mutants deficient in chemotaxis generally infiltrated more deeply into the glands.

In swine infections in vivo, a system that models key anatomical and physiological features of the human stomach, chemotaxis-deficient strains caused more severe gastritis and ulceration than WT. Together, our data suggest that: (1) chemotaxis restricts gland infiltration and limits H. pylori pathology, which may assist the pathogen in its goal to be a lifelong colonizer of its human host, and (2) although it may take decades for the oncogenic effects of CagA to manifest, cellular dysregulation by this bacterial effector may begin immediately upon infection.

59 Evaluation of Peptide Binding for Oligopeptide Binding Proteins (OppA) in Borrelia Burgdorferi

Arti Kataria National Institutes of Health

Lyme disease (LD) is one of the most frequently diagnosed vector-borne infections globally caused by Borrelia burgdorferi (Bb). Bb contains no pathways for de novo synthesis of amino acids (AA) making it heavily reliant upon AA transporters (BbOpp). Indeed, the BbOpp system has been shown to be essential for spirochete viability, making Bb the only bacterial system to date that has been shown to be peptide dependent. The Opp system orchestrates peptide acquisition through oligopeptide-binding proteins (OppA), permeases (OppBC, OppBC), and NBD containing proteins (OppDF). Bacterial OppAs are known for promiscuous binding via the peptide backbone, allowing for a diverse repertoire of peptide binding partners. Herein, we began a large-scale study to evaluate the peptide binding capabilities of the individual Bb OppAs. Structural and modeling data show some variations within binding determinants that suggest each OppA may display specific affinities. For high-throughput binding analyses, we have employed thermal shift assays, which suggest that OppA2 is a promiscuous binder showing binding to >80 peptides. Whereas OppA1, OppA4 and OppA5 showed positive binding with 22, 14 and 4 peptides respectively demonstrating the selective nature of OppA proteins. Alternatively, OppA3 appears extremely selective. We have solved the crystal structures of OppA2 and OppA3 at a 2.4 Å and 1.9 Å resolution, respectively. Consistent with the results of our TSA screens OppA2 was loaded with an endogenous tetrapeptide while OppA3 structure was open. We will continue characterizing the binding strategies for these proteins as well as identifying the unique ligands for OppA3.

60 Inflammasomes prevent intestinal inflammation in Vibrio parahaemolyticus-infected mice

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Since mice are profoundly resistant to many human enteropathogens, including Salmonella, Shigella and Vibrio spp., we lack adequate small animal models that recapitulate the human gastrointestinal diseases caused by these bacteria. Identifying factors that protect mice from disease is not only pivotal for improving these mouse models but may also shed light on the mechanisms involved in human pathogenesis.
Recent work demonstrated that mouse resistance to Shigella and Salmonella is mediated by inflammasomes, cytosolic innate immune complexes that assemble in response to pathogen infection or noxious stimuli and initiate inflammation and a lytic cell death termed pyroptosis. Inflammasomes expressed in mouse, but not in human, intestinal epithelial cells (IECs) sense Salmonella or Shigella invasion and trigger the inflammasome-mediated extrusion of infected cells to prevent disease in mice. Importantly, inflammasome-deficient mice develop human-like diarrheal disease following oral Shigella or Salmonella infection.
Vibrio parahaemolyticus (Vp) is a facultative intracellular pathogen that similarly invades IECs and causes gasteroenteritis in humans but no disease in mice. Whether inflammasomes sense and restrict Vp infection in mice is unknown. Here, we use in vitro and in vivo systems to show that Vp activates murine inflammasomes and that inflammasomes indeed protect mice against intestinal inflammation following oral Vp infection. Future work is aimed at identifying additional incompatibilities that limit Vp disease in mice.

61 Crosstalk between three CRISPR-Cas types enables primed type VI-A adaptation in Listeria seeligeri

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CRISPR-Cas systems confer adaptive immunity to their prokaryotic hosts through the process of adaptation, where sequences are captured from foreign nucleic acids and integrated as spacers in the CRISPR array, and thereby enable crRNA-guided interference against new threats. While the Cas1-2 integrase is critical for adaptation, it is absent from many CRISPR-Cas loci, rendering the mechanism of spacer acquisition unclear for these systems. Here we show that the RNA-targeting type VI-A CRISPR system of Listeria seeligeri acquires spacers from DNA substrates through the action of a promiscuous Cas1-2 integrase encoded by a co-occurring type II-C system, in a transcription-independent manner. We further demonstrate that the type II-C integration complex is strongly stimulated by preexisting spacers in a third CRISPR system (type I-B) which imperfectly match phage targets and prime type VI-A adaptation. Altogether, our results reveal an unprecedented degree of communication among CRISPR-Cas loci encoded by a single organism.

62 Phage infection progression pathway and a phage-resistance landscape revealed with single-cell transcriptomics in Bacteroides fragilis

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Phenotypic heterogeneity in an isogenic microbial population may produce differences in vulnerability to bacteriophage infection across individual cells. Using bacterial single-cell RNA sequencing, we profiled the transcriptomes of ~50,000 cells from cultures of a human gut commensal and opportunistic pathogen, Bacteroides fragilis, infected with a lytic bacteriophage. We quantified the asynchronous progression of phage infection in single bacterial cells and reconstructed the infection timeline, characterizing both host and phage transcriptomic changes as infection unfolded. We also discovered a subpopulation of bacteria that remained uninfected and determined the heterogeneously expressed host factors that protected these bacteria from phage infection. Specifically, we found that phase-variable expression of capsular polysaccharides (CPS) provided the most potent protection. As a secondary line of defense, we discovered configurations of the phase-variable restriction-modification system I (RM-I) and other phage-defense systems, which protected against bacteriophage in conjunction with CPS variants. By acting together, phase-variable CPS and defense systems create an adaptive phenotypic landscape where rare protective combinations enable the survival and re-growth of bacteria expressing these phenotypes. The emerging model of complementary action of several antiphage defense mechanisms explains the development of phenotypic resistance and showcases the potent role of phase variation in bacterial anti-phage defenses.

63 Characterization of a putative D-Alanine-D-Alanine carboxypeptidase (BB0605) in Borrelia burgdorferi peptidoglycan remodeling

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Borrelia burgdorferi (Bb) is a spirochete known for causing Lyme disease, a tick-borne illness prevalent in North America and Europe. Notably, Bb exhibits auxotrophy for many essential amino acids (AAs) which it satisfies using a robust peptide transport system. Given this unusual feature, we are particularly interested in how the AAs it requires contribute to cellular functions, particularly in the synthesis of peptidoglycan (PG). PG serves a vital role by supporting structure and osmosensitivity. Spirochetes possess a uniquely thin PG layer, which affords flexibility and planar waveform motion. Bb's PG includes low cross-linking between peptides and the non-canonical AA and sugars. PG remodeling is a dynamic process involving synthesis, degradation, and modification of these components. A key player in this process is bb0605, which encodes a putative penicillin-binding protein (PBP) crucial for PG remodeling. BB0605 is a homolog of E. coli's DacD, a D-Alanine-D-Alanine carboxypeptidase (DD-CP) essential for the cross-linking of PG. DD-CPs are enzymes that play a critical role in the final steps of PG synthesis by cleaving the terminal D-Alanine residue from D-Alanine-D-Alanine residues. Using a bb0605 transposon mutant (bb0605tn), we are evaluating the role bb0605 plays during in vitro growth and the enzootic cycle. We aim to confirm bb0605's role in PG remodeling using various approaches, including enzymatic approaches (recombinant protein purification and functional assays), PG analysis (electron microscopy and mass spectrometry), and contributions to the enzootic cycle (in vivo studies). Through these investigations, we hope to elucidate the importance of this PBP to Borrelia physiology.

64 Cracking the Caseum Code: Investigating Mycobacterium tuberculosis persistence in a host model

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Killing roughly 1.5 million people every year, tuberculosis remains the world's deadliest infectious disease. The alveolar granuloma, a hallmark of TB infection, serves as the body's attempt to wall off Mycobacterium tuberculosis (Mtb) cells and prevent spread. At the heart of these complex immune structures exists a cheese-like substance called caseum, an acellular material containing dead foamy macrophages. Despite this hypoxic and necrotizing environment, some populations of Mtb are able to survive, exhibiting a non replicative state and tolerance to anti-TB drugs. The upregulation of Rv3290c (lat), a Lysine ε-aminotransferase, in nutrient starved conditions suggests its potential role in this phenomena. In order to explore lat expression in TB infection, a caseum surrogate was developed using THP1 macrophages to mimic a tuberculous lesion environment. Using a CRISPR interference platform, gene expression of lat was knocked down and survival within the caseum surrogate model was assessed by enumeration of colony-forming units (CFU). Over the course of 42 days, Mtb exhibited a survival defect upon transcriptional silencing of lat. These results highlight the potential necessity of lat in nutrient starved conditions and further implicate it as a gene of interest. In addition, there has been speculation of lrp (leucine-responsive regulatory protein - Rv3291c), to work in tandem with lat in managing intracellular amino acid levels and mediating stringent response. More studies are required to determine the genes essential for persistence. Future work aims to characterize lat's mechanism of protection, as well as identify other interacting genes.

65 Characterization of microbial activity using automation and AI-powered image analysis

Lorenzo D'Amico, PhD Triton Bio, Inc

Visual analysis of microbial growth and activity in culture is a necessary but laborious task in research, environmental, industrial microbiology laboratories worldwide. Current automated plate imaging solutions are purpose-built for diagnostic applications and cannot be readily accessed by academic, environmental and industrial researchers. Therefore, microbiologists use personal smartphones or "DIY" imaging systems to acquire end-point measurements. This creates critical operational and scientific challenges with documentation, reproducibility, and missed scientific discoveries – especially in resolving spatial and temporal details of microbial activity in culture.

To address these challenges, Triton Bio developed a bench-top incubator with an automated computer vision system for microbiological samples. This system captures images of Petri dishes over time within a temperature controlled enclosure (up to 45C). A motorized stage is used to position six specimens beneath a mounted camera. The system is connected to a cloud environment so that specimens can be monitored remotely and large datasets can be easily stored and analyzed using automated computer vision software. Importantly, the imaging-incubator is compact (only 14" wide x 14" deep x 7" high) and scalable for both smaller and large labs.

Preliminary data indicates that autonomous image segmentation software systems can be applied to 1) detect and quantify growth kinetics of individual colonies for various fungi and bacteria, 2) quantify morphological features such as biofilm rugosity, and 3) extract colorimetric information from colonies with little human intervention. This automated imaging-incubator and the companion AI vision software have the potential to accelerate research timelines and unlock new avenues of microbiology.

66 Membrane remodeling during alphavirus infection activates a novel viral sensing pathway

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Successful infection by viruses requires they evade detection by the host's immune system long enough to effectively proliferate. Viruses have evolved multiple mechanisms to either escape from or antagonize host immune proteins. To avoid detection, alphaviruses hide their viral RNA by remodeling the host's cell membrane, sequestering RNA synthesis within invaginations called spherules. Our lab observed that this membrane remodeling activity is sufficient to activate a host inflammasome, which is a cytosolic protein complex important for detecting pathogen infection. However, the specific components of this inflammasome and its mechanism of sensing membrane remodeling is unknown. Using CRISPR Cas9, we knocked out genes encoding potential inflammasome components in HEK293T cells and tested whether edited cells responded to alphavirus membrane remodeling. We identified two cell death pathway kinases, RIPK1 and RIPK2, that have essential roles in detecting membrane remodeling. These host genes have not previously been characterized as elements of inflammasomes. We suggest that this may reveal a novel viral sensing pathway that arose in host cells to overcome viral escape and could be used to detect other pathogens that remodel host membranes.

67 Disruption of Sphingolipid homeostasis: the effect of ORMDL knockout on Zika replication

Audrey Hinchliff OHSU

Zika virus's (ZIKV) ability to significantly alter the lipid regulation in host cells is not well known – especially its effect of sphingolipid composition. Sphingolipid synthesis begins with a rate limiting step in which L-serine and palmitoyl CoA is condensed by serine palmitoyltransferase (SPT). From there it is converted to dihydroceramide then ceramide (Cer). SPT is negatively regulated by three ORMDL isoforms (ORMDL1-3). From previous research we have seen that ZIKV will alter sphingolipid composition and will increase ceramide levels through multiple pathways. To investigate this relationship further, we used ORMDL1/2/3 triple knockouts (TKO) in a549 cells to see how increased levels of ceramide from the de novo pathway specifically would affect ZIKV replication. Using plaque assays, we found that there is more viral replication occurring in the ORMDL TKO than the control cells.

68 Brewing up a solution to spoiled beer with killer yeasts

James Mackenzie University of Idaho

Undesired secondary fermentation by diastatic strains of Saccharomyces cerevisiae can result in spoilage by hyperattenuation, off-flavors, and explosive packaging. Diastatic yeasts cause spoilage by secreting an extracellular glucoamylase enzyme encoded by the STA1 gene. A survey of US brewers found that diastatic contamination has been experienced by 53% of brewers. Critically, there are no methods to prevent or remediate a diastatic yeast contamination event. An antifungal “killer toxin” named K1, produced by S. cerevisiae, is capable of inhibiting 91% of diastatic strains. To test whether killer toxins could prevent spoilage, a diastatic yeast contamination event was simulated in 1,000 liters of finished beer in collaboration with Rhinegeist Breweries. Adding a killer yeast strain to the contaminated beer was successful in stabilizing pH and temperature and preventing hyperattenuation. Our research focuses on producing hybrid strains of brewer’s yeast that can produce killer toxins. Rare mating of killer yeasts and brewing strains has enabled the isolation of K1 toxin-producing “killer” brewing strains. Initial tests of killer brewing strains showed they were as effective against diastatic yeast as the parental K1 killer yeast strain. Our next steps are to test the fermentative properties of killer brewing strains and the ability to produce high-quality beer with an acceptable shelf life. These innovative killer brewing strains give brewers a powerful tool to keep their brand consistent and shielded from diastatic contamination. This protection not only saves costs tied to product loss and lawsuits but also safeguards their reputation—essential in an increasingly competitive industry.

69 Genomic Insights into Veillonella Parvula PK1910 and Comparative Analysis with Veillonella Atypica

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Bacteria within Veillonella are common members of the oral microbiome. Members of Veillonella are associated with periodontal diseases, including peri-implantitis. Here, we report the complete genome sequence of Veillonella parvula strain PK1910, isolated from subgingival plaque. PK1910 is the first Veillonella strain to have a genetic system developed and the completion of this genome provides a reference for microbiome studies and allows for more examination of this strain’s impact on periodontal diseases. V. parvula PK1910 single colonies were inoculated in 5mL of BHI broth supplemented with sodium DL-lactate, porcine hemin, and vitamin K1, and was incubated under anaerobic conditions. Genomic DNA was extracted for hybrid ONT and Illumina WGS sequencing. Library preparation was performed with the Illumina Nextera DNA Flex Kit and paired-end 2x150bp indexes. ONT sequencing was ran on PromethION P24 with R10.4.1 flow cell. Illumina sequencing was run on NextSeq 2000. The de novo assembly using Filtlong, Miniasm, Flye, and Polypolish yielded the final singular, closed contig. Annotations identified of 2,049 genes, with 1,979 coding sequences, 48 tRNAs, four 5S rRNAs, four large subunit (LSU) 23s rRNAs, and 4 small subunit (SSU) 16S rRNAs. Veillonella parvula is predominately found in the subgingival plaque whereas Veillonella atypica prefers the supragingival plaque. Pangenome analyses between members of these two species reveal that Vp contains more genes encoding for coping with reactive oxygen stress including oxyR and katA despite Vp dominating in a more anaerobic environment which may support the observation that Vp can synergistically support growth of anaerobic periodontal pathogens.

70 Diverse mechanisms of type I-B anti-CRISPR proteins in Listeria seeligeri

Luke Oriolt University of Washington

Bacteria and the viruses that infect them (phages) are engaged in an evolutionary arms race governing infection and immunity. Bacteria have evolved diverse immune systems to protect themselves from infection, and in turn, phages have evolved factors that inhibit specific immune systems. Central among these are anti-CRISPRs (Acrs) which are small proteins or RNAs that inhibit the activities of prokaryotic CRISPR-Cas adaptive immune systems. CRISPR-Cas systems are classified into six types based on their nuclease content and mechanism of action. The spectrum of Acr activity is typically limited to a single CRISPR type. Here, we have conducted a screen of Acr activity in natural isolates of Listeria seeligeri, which resulted in the discovery of 15 Acr families inhibiting type I-B CRISPR, the most common CRISPR type in Listeria spp. Through a series of genetic and biochemical assays, we have characterized the mechanism of action of these Acrs and identified Acrs that inhibit a variety of different steps of type I-B CRISPR immunity by observing their effects on cas gene expression, Cas protein complex assembly, guide RNA association, target DNA binding, and nuclease recruitment. Taken together, our results highlight the diverse strategies taken by phages and mobile genetic elements (MGEs) to subvert prokaryotic immune systems and illustrate similar strategies that can be engineered to control CRISPR-mediated genome editing.

71 Marseillevirus T19 utilizes two distinct mechanisms which inhibit gene expression in Acanthamoeba castellanii

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To date, the vast majority of virus-host interactions in eukaryotes has focused primarily upon viral infection of multicellular eukaryotes, leaving much to be discovered about the interactions between microbial eukaryotes and their viruses. As a result, the field of microbial eukaryotic antiviral system remains rich with the potential to uncover both conserved and novel antiviral mechanisms across the vast kingdom Eukarya. In my own investigations of the relationship between viruses and their microbial eukaryote host, I have turned to the model of Marseillevirus infection of Acanthamoeba species. More specifically, I focused on how Marseillevirus strain T19 impairs host transcription of Acanthamoeba castellaniiNeff. Guided by established models of virus-induced host transcriptional shutoff in related viruses as well as previous literature demonstrating host-transcriptional shift by Marseillevirus T19, I hypothesized that Marseillevirus T19 actively antagonizes host transcription levels and sought to determine what mechanisms may be responsible. Here I present one such investigation which utilizes translational inhibition via drug treatment with nourseothricin to demonstrate that there are at least two distinct mechanisms that Marseillevirus T19 uses to inhibit transcription in its host Acanthamoeba castellaniiNeff: one mechanism which is dependent upon host translation machinery, and one which is not dependent.

72 How Sublethal Acaricide Exposure Elevates Pathogen Burden in Ticks

Sarah Wright Washington State University

Tick-borne diseases including Lyme disease (Borrelia burgdorferi) and Anaplasmosis (Anaplasma phagocytophilum) cause significant morbidity to people and animals. There are at least 20 different pathogens that are transmitted by ticks in the United States, demonstrating the importance of developing new strategies to limit the impact of ticks on human health. Historically, pesticides that target ticks and mites, termed “acaricides”, have been used to deter tick infestations. Sublethal doses of acaricides cause higher expression and activity of antioxidant enzymes in ticks. Previously, we found that Nrf2, a master regulator of antioxidant gene networks, promotes pathogen persistence in ticks. Given this link, we asked if acaricide treatment impacts the ability of ticks to harbor and transmit pathogens. We found that pretreating tick cells with acaricides resulted in an antioxidizing environment within the cell. The Nrf2 pathway is triggered by oxidative stress and induces the expression of antioxidant genes, which ultimately promotes pathogen survival. When pretreating tick cells with sublethal doses of acaricides we found it caused a 20-fold increase in A. phagocytophilum burden. We found that Nrf2 is key in regulating the relationship between acaricides and A. phagocytophilum burden. When treating uninfected nymphal Ixodes scapularis ticks with sublethal doses of acaricides and then feeding them on a mouse infected with B. burgdorferi we see a significant increase in pathogen burden. Given that acaricide resistance is on the rise, with up to 90% resistance in some tick species, these findings have important implications for re-examining current control measures for ticks and tick-borne diseases.

73 Navigating contradictions: Salmonella Typhimurium chemotactic responses to conflicting effector stimuli

Arden Baylink Washington State University

Chemotaxis controls swimming motility and colonization of many intestinal bacteria, yet how enteric pathogens navigate the complex chemical landscape of the gut, which contains contradictory chemoattractant and chemorepellent stimuli, remains poorly understood. We find that Salmonella Typhimurium requires chemotactic sensing of two opposing signals in the intestinal lumen—the microbial metabolite and bacteriostatic chemorepellent indole, and the nutrient chemoattractant L-Ser—for efficient invasion of colonic tissue. Despite feces being the major biological source of the chemorepellent indole, accumulating to millimolar levels, non-typhoidal Salmonella are actually strongly attracted to fecal material because chemoattraction to L-Ser and other attractants override indole chemorepulsion. This behavior is orchestrated through the chemoreceptor Tsr, which coordinates a spectrum of distinct rearrangements in the bacterial population structure based on the ratio of L-Ser to indole. Through seeking niches with the highest L-Ser to indole ratio, S. Typhimurium presumably optimizes nutrient access and avoids regions of high-competitor density.

74 Evolution of enhanced anaerobic biodegradation in an in-situ derived microbial community from oil polluted sediment

Klas I. Udekwu University of Idaho

Ecosystem functions mediated by microbes-such as polycyclic aromatic hydrocarbon (PAH) degradation can be enhanced by continuous evolution of in-situ derived microbes. Such enrichment strategies can ostensibly be exploited for facilitating bioremediation of anoxic oil-contaminated sediments and soils. We evolved a community of microbes derived from sediment cores obtained from an aqueous oil-dump with naphthalene as sole carbon input under aerobic and anaerobic conditions. We found that anaerobic degradation of naphthalene was enhanced in the presence of activated carbon primarily and describe the microbiome changes effected by -oxic and anoxic selection.
Our results show proof of principle for use of evolutionary principles for selecting community function at least under anaerobic conditions, the rate-limiting degradative processes critical for optimal bioremediation.

75 Multi-omics comparison of Lactobacillus iners and crispatus and their response to simulated vaginal fluid

Christopher Whidbey Seattle University

Stable presence of Lactobacillus species and low community diversity are characteristics of a healthy vaginal microbiome, while diverse vaginal microbiota and low levels of Lactobacillus are associated with preterm birth and bacterial vaginosis (BV). Lactobacillus are thought to act in a protective manner through acidifying the vaginal tract by lactic acid production and competition for adhesion space and resources, however, the molecular mechanisms by which they do so are poorly defined. The two most common species of Lactobacillus found in the vaginal microbiome are Lactobacillus iners and Lactobacillus crispatus, where L. iners is less associated with positive health outcomes and is thought to be less protective. Despite its importance, much less is known about L. iners biology. L. iners is more common in individuals of African descent, while L. crispatus is more common in individuals of European ancestry. Thus, understanding exactly how L. iners interacts with the host and other vaginal microbiota could be a route to developing microbiome-focused therapeutics capable of addressing long-standing health disparities. As a first step toward this goal, we have characterized and and compared the genome, transcriptome, and proteome of L. crispatus JV-V01 and L. iners UPII-143D grown in standard medium or in simulated vaginal fluid. These studies provide an -omics level view of two key vaginal microbes and will help lay the foundation for future studies focused on the vaginal microbiome.

76 K9 Citizen Science Approach for Tick and Tick-Borne Pathogen Surveillance in Eastern Washington

Kaden O'Keefe Gonzaga

Ticks, as vectors, play a pivotal role in transmitting pathogens to humans and animals, making their surveillance essential, yet this is lacking in Eastern Washington. We designed a three-pronged citizen science approach to collecting ticks in order to cast a wide net for sample collection and to raise awareness of tick-borne pathogens in this region. Ticks were gathered from one of the following: local dog owners who frequent hiking trails, local dog groomers, and field research students. The goal is to enhance our understanding of the prevalence and diversity of tick species and tick-borne pathogens in this region. The study utilized a DNA-based PCR approach to identify bacterial pathogens and distinguish Dermacentor andersoni and Dermacentor variabilis, the two most common tick species found in Spokane, WA. The following bacterial species were targeted: Ehrlichia chaffeensis (ehrlichiosis), Francisella spp. (tularemia), and Rickettsia rickettsii (Rocky Mountain spotted fever). From our canine hiking data, 76 hikes were recorded, with 57 tick samples collected from 9 of 15 dogs. Dog groomers and field researchers collected 143 and 61 ticks, respectively. In total, 261 tick samples were collected with over 90% being D. variabilis. Francisella spp. were the most common (although predominantly endosymbionts), and lower frequencies of the other bacterial pathogens were found. These findings will help inform veterinarians and dog owners about the relative risk to humans and canines of contracting tick-borne pathogens when using urban trails in Eastern Washington.

77 Investigating a Fish Die-Off Mystery: Impacts of Aeromonas contamination in rainbow trout raised in elementary classrooms

Reina Geforos Gonzaga

During the 2023-2024 academic year, elementary schools in Spokane, WA participated in a national program where students learn about salmonids and their role in the ecosystem. Teachers and students raise trout fry with careful attention paid to temperature and oxygen content in the water. Surprisingly, four schools in our school district documented a die-off in trout fry during April 2024. Our lab studies the bacterial fish pathogen, Aeromonas, and we were contacted to help investigate potential causes of the fish die-off. We hypothesized that Aeromonas was responsible for the die-off based on the observed clinical signs such as red gills of affected fish. To test for the presence of Aeromonas, samples were collected from three different schools (two affected, one unaffected). Water samples (tap and tank) were taken from all of the schools and dead fish were collected from an affected school. These samples were cultured on an Aeromonas selective medium and putative Aeromonas colonies were maintained for pure culture. In total, 27 of 27 fish samples contained Aeromonas, and all tank water sampled were positive for Aeromonas, including the unaffected school. All Aeromonas isolates were characterized to determine species, screened for virulence factors, and tested for antibiotic sensitivity. These results will help clarify which species of Aeromonas were associated with the diseased trout fry and the mystery of the unexpected die-off.

79 Engineering probiotic bacteria as antibiotic and anti-biofilm therapeutic delivery vehicles

Delaney Shea OHSU

Pseudomonas aeruginosa chronically infects the lungs of up to 80% of cystic fibrosis (CF) patients, accelerating morbidity and mortality. Biofilm-forming pathogens such as P. aeruginosa are up to 1000x more antibiotic-resistant than free-floating bacteria due to the physical protection of the biofilm and altered cellular metabolic states within the biofilm. To overcome the dual treatment challenges of protective biofilms and antibiotic-resistant P. aeruginosa, we engineered probiotic Lactobacillus plantarum to secrete both biofilm-degrading enzymes (BDEs) specific to P. aeruginosa biofilm components, as well as P. aeruginosa-specific pyocins. We demonstrate the efficacy of these BDEs and pyocins for breaking down biofilms and killing P. aeruginosa in model biofilms. BDE and pyocin efficacy was demonstrated both in a purified form and when secreted directly from L. plantarum. L. plantarum may be delivered safely into the lungs via inhaler or oral supplement. We have also engineered a non-replicative bacterial membrane vesicle (BMV)-based delivery system to carry BDEs and pyocins, and shown its efficacy in degrading biofilms. BMVs are ~100nm lipid bilayer-enclosed nanoparticles secreted by bacteria for interbacterial communication which can be engineered to deliver cargo to pathogens, potentially increasing drug uptake. Ongoing work includes collecting anti-biofilm BMVs from engineered probiotic Escherichia coli Nissle. We will evaluate both engineered bacteria and BMVs for their ability to degrade biofilms and kill biofilm-forming P. aeruginosa in infected sputum samples from CF patients. Optimizing the ability of probiotic bacteria to eradicate P. aeruginosa biofilms presents a more effective option for treating chronic pulmonary infections than conventional antibiotics.

80 Investigating the role of the K1 preprocessed toxin on toxin immunity

Katherine Hill University of Idaho

Only four main antifungal drug classes are currently prescribed and an increase in antifungal resistant infections makes developing new therapeutics essential. Killer yeasts, such as certain Saccharomyces cerevisiae strains, produce antifungal proteins known as killer toxins, representing a novel approach to treating fungal infections. K1 is a killer toxin that can inhibit the growth of the opportunistic pathogen Candida glabrata. However, many species of pathogenic yeast are resistant to K1. The exact mechanism of immunity toward K1 is unknown, but it is speculated that it involves interactions between the immature K1 preprotoxin (the preprocessed version of the toxin) and the cell surface protein Kre1p. The immature K1 contains a N-terminal pre and pro-region (named δ), and then the α followed by the γ and ß domains respectively. Immunity has been found to only require the α domain and 39 amino acids of the γ domain, but it is unclear what degree of immunity is provided, and if altering the length of the shortened γ domain impacts it. To quantify immunity, I have created truncated preprotoxins with differing lengths of γ. During this process, I made a new discovery that the truncated preprotoxin has a potential underlying toxicity. These findings change the way we understand how the K1 killer toxin functions, and raised questions about how killer yeasts protect themselves from their own toxins. Understanding the mechanism of immunity will aid in the specific targeting of killer toxins to pathogenic fungi and the potential application to fight human diseases.

81 Amendment of marine oil-polluted sediments with activated carbon alters microbial community structure and enhances biodegradation

Björn Brindefalk University of Idaho

82 Temperate phage tolerance by the type VI-A CRISPR-Cas system in Listeria seeligeri

Marshall Godsil University of Washington

83 Activation of c-di-GMP and cAMP signaling during Pseudomonas aeruginosa biofilm formation

Xuhui Zheng University of Washington

The formation of bacterial biofilms has been implicated in several different types of chronic infections. After initial attachment, a critical, first step in biofilm formation is a cell inducing a surface sensing response. In the Gram-negative opportunistic pathogen Pseudomonas aeruginosa, two second messengers, cyclic diguanylate monophosphate (c-di-GMP) and cyclic adenosine monophosphate (cAMP), are produced by different surface sensing mechanisms. While c-di-GMP supports a sessile lifestyle by promoting the synthesis of biofilm matrix materials, cAMP triggers virulence and surface motility by upregulating T3SS and type IV pili. Given the disparate cellular behaviors regulated by these second messengers, how newly attached cells coordinate these pathways remains unclear. Some of the uncertainty relates to studies using different strains, experimental systems, and usually focusing on a single second messenger. Here, we developed a tri-color reporter system to simultaneously monitor intracellular c-di-GMP and cAMP levels at a single-cell level. We found that c-di-GMP or cAMP is selectively activated in the two most commonly used experimental systems to study surface sensing. By further examining the conditions that differentiate a c-di-GMP or cAMP response, we demonstrate that the presence of an aqueous phase overlaying the surface controls whether a c-di-GMP or cAMP response is initiated. Specifically, exposure to air on an agarose surface activates cAMP signaling through type IV pili and the Pil-Chp chemosensory system, while c-di-GMP response requires flagellar-driven fast-swimming bacteria attaching to the surface from an aqueous phase. Collectively, this work indicates that c-di-GMP and cAMP signaling responses are dependent on the surface context.

84 Decoding the evolutionary tug-of-war between MxA Loop L4 and Influenza A nucleoprotein

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Myxovirus resistance protein 1 (MxA) restricts influenza A viruses (IAV) by binding the IAV nucleoprotein (NP). Evolution-guided functional studies have suggested that the unstructured Loop L4 of MxA interacts with IAV NP. However, because Loop L4 is intrinsically disordered, the interaction between NP and MxA is poorly understood. Using protein modeling and molecular dynamics, we identified a hydrophobic pocket in NP that might represent a critical site for interaction with Loop L4. Based on this prediction, we hypothesize that mutating interaction residues at or proximal to this hydrophobic pocket in NP will weaken or disrupt its interaction with MxA and affect MxA restriction of IAV. To test this hypothesis, we are mutating NP residues. We will test these NP variants using a minireplicon system to investigate how these changes affect NP function in the absence of MxA, and how they affect escape from MxA restriction. Reduced MxA restriction of influenza's replication machinery would indicate that the mutated NP residues are critical for MxA's ability to bind and restrict NP, supporting the hypothesis that this hydrophobic pocket and Loop L4 are at the interface of this interaction. These findings will enhance our understanding of the MxA-NP interaction and may inform future strategies for targeting viral resistance mechanisms in IAVs.

85 A non-canonical role for Caspase-1 in controlling antimicrobial resistance of intracellular Salmonella

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Caspase-1 is a key effector molecule involved in inflammasome activation and has a well-established role in restricting the growth of pathogens by triggering a form of cell death called pyroptosis. Here we reveal a non-canonical, cell death-independent role for caspase-1 in controlling the transcriptional state and drug resistance of an intracellular pathogen. Using Pathogen-sequencing, a method for sensitive transcriptional profiling of minuscule numbers of intracellular bacteria from infected macrophages, we show that host caspase-1 decreases the resistance of intracellular Salmonella to endogenous cationic antimicrobial peptides, and to a cationic polypeptide antibiotic used as a last-line drug in Gram-negative bacterial infections. These effects of caspase-1 were independent of its enzymatic activity but dependent on its ability to repress the activation of a two-component signal transduction system in intracellular bacteria. These effects were also independent of caspase-11. Our data suggest an activity- and inflammasome-independent role for caspase-1 later in infection in restricting intracellular Salmonella which evade initial inflammasome-dependent restriction by caspase-1. Our findings thus take host caspase-1 beyond the well-studied inflammasomes and tie it to signal transduction and drug resistance of an intracellular pathogen with possible implications for host-directed therapy to combat antimicrobial resistance.

86 Investigating the interactions of HNS and phage proteins during lytic phage infection

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