Microbial Early-career Researchers Association

Research Symposium

2023

Monday, April 10, 2023

Campus Instructional Facility (CIF)

Welcome to the 3rd Annual Microbial Early-career Researchers Association (MicroERA) Research Symposium

MicroERA proudly welcomes you to our third annual research symposium! This event brings together trainees from across campus. Presenters this year represent 9 units and highlight the broad reach of microbial systems research at Illinois.

The Microbial Early-career Researchers Association serves graduate, undergraduate, and postdoctoral researchers by providing opportunities for professional development and networking. MicroERA is a growing organization and is always actively recruiting members. Any undergraduate, graduate student or postdoc that is interested in microbial sciences research is welcome. Join our email list and follow us on social media to keep up to date with our activities.

Currently, MicroERA is seeking trainees to join the executive board so it can continue its mission. Email MicroERA.UIUC@gmail.com with your interest or talk to one of our board members today!

Sincerely,

MicroERA Board and Symposium Planning Committee

Elizabeth Brandley
Stewart Montgomery
Kristen Farley
Ben Levine
Lucy Chou Zheng
Lydia Okyere
Vince Kelly
Tori Boyle
John McMullen

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Campus Instructional Facility (CIF)
Address: 1405 W Springfield Ave, Urbana, IL 61801

Schedule of Oral Presentations

In lighting style talks, presenters are given 5 minutes to present their work with an additional 2 minutes reserved for questions.

Moderator: Stewart Montgomery

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Keynote

Emergent Metabolic Dynamics in Microbial Communities

Arthur Prindle, Ph.D
Department of Biochemistry and Molecular Genetics, Feinberg School of Medicine,
Northwestern University

Emerging research of the human microbiome has generated new insights into the role of human-associated microbes in health and disease. In particular, microbes that colonize the gastrointestinal tract play a central role in host metabolism, immunity, and homeostasis, and can change in response to external perturbations such as dietary alterations, chemical exposures, and physiological or psychological stressors. However, the microbiome field currently lacks essential knowledge for how microbial cell-to-cell interactions give rise to higher-order community behavior, which is a key roadblock in the path towards next-generation microbiome-based products and therapies. In this talk, I will describe our various efforts to address these challenges, including how our multi-scale microfluidic platform for microbial community analysis is contributing to the new field of bacterial electrophysiology. My lab is currently deciphering the underlying mechanisms of bacterial biofilm electrophysiology with the goal of better understanding and engineering natural microbial community behavior. These next-generation microbial community analysis and engineering tools provide a foundational platform for bridging synthetic biology technologies to the microbiome field.

Arthur Prindle is an Assistant Professor at Northwestern University jointly appointed in the Department of Biochemistry and Molecular Genetics and the Department of Chemical and Biological Engineering. Dr. Prindle received his B.S. degree in Chemical Engineering from Caltech and his Ph.D. in Bioengineering from UCSD. His research group mainly develops enabling technologies to characterize microbial communities and to engineer these microbes with the capacity to monitor and improve human health. Dr. Prindle has been recognized with a number of awards including the Career Award at the Scientific Interface (CASI) from the Burroughs Wellcome Fund, the Pew Biomedical Scholarship, the Packard Fellowship in Science and Engineering, the NSF CAREER award, the NIH Maximizing Investigators' Research Award (MIRA), and the Early Career Award for Scientists and Engineers from the Army Research Office (ECASE-Army). He joined Northwestern University in 2017 as a member of the Center for Synthetic Biology. https://sites.northwestern.edu/prindle/

Poster Withdrawn

Associations of maternal dietary inflammatory potential and gut microbiome

Suzanne Alvernaz[1], Elizabeth Wenzel[2], Unnathi Nagelli[1], Lacey Pezley[3], Jhalak Modi[1], Apoorva Tummala[1], Mohit Jain[4], Jack Gilbert[5,6], Pauline Maki[2,7,8], Lisa Tussing- Humphreys[3], Beatriz Peñalver-Bernabé[1,9]

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[9]Department of Urology, University of Illinois, Chicago

Background: Pregnancy involves changes in a wide variety of physiological systems, including the gut microbiota.
Aim: To use a systems approach to characterize associations among dietary inflammatory potential, a measure of diet quality and the gut microbiome during pregnancy
Methods: 46 pregnant women were recruited prior to 16 weeks of gestation. Participants completed a food frequency questionnaire (FFQ) and provided rectal swabs. Dietary inflammatory potential was assessed using the Dietary Inflammatory Index (DII). Rectal samples were analyzed using amplicon sequencing.
Results: Here, we present findings from the first trimester regarding the impact of inflammatory diets on the gut microbiome

Chemotherapy-induced changes in gut microbial composition disrupt entero- hepatic bile acid metabolism

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[2]Department of Animal Sciences, College of ACES, University of Illinois at Urbana-Champaign

Chemotherapy-induced bile acid (BA) malabsorption highly contributes to associated gastrointestinal side effects, but the role of gut microbes that metabolize BA remain understudied. We aimed to understand how chemotherapy-induced changes in microbial composition affect enterohepatic BA metabolism. Mice received I.P. paclitaxel (Chemo) or vehicle control (Veh). Germ-free mice received a gut-microbial transplant from these mice - Chemo-GMT or Veh-GMT. Chemo-GMT reduced expression of most BA-related enzymes, transporters, and receptors in the liver, ileum, and colon (vs Veh- GMT) as observed in Chemo vs Veh. This suggests that the chemotherapy-altered microbiome contributes to dysfunctional BA metabolism and thus associated gastrointestinal side effects.

Raffinose family oligosaccharide utilization by *Bacteroides thetaiotaomicron*

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The human gut microbiome plays a crucial role in health and disease. Members of the genus *Bacteroides* are known for breaking down a wide range of polysaccharides using Polysaccharide Utilization Loci (PUL). This process has been well studied in the model commensal *Bacteroides thetaiotaomicron* (*B. theta*). However, the systems required for utilizing smaller oligosaccharides are poorly understood. Previously, we showed that an alpha-galactosidase (BT1871) encoded within PUL24 is essential for *B. theta* to utilize Raffinose Family Oligosaccharides (RFOs). We have identified at least two independent mechanisms by which *B. theta* upregulates expression of BT1871 for efficient RFO usage. First, a genomic rearrangement resulting in *BT1871* gene duplication and fusion to an adjacent rRNA locus. Second, we isolated strains with spontaneous nonsense mutations in the gene encoding the PUL24 anti-sigma factor. Using RT-qPCR, we show that PUL24 expression is slightly upregulated in the presence of RFOs and may be further activated by a CRP like fashion.

Exploring plasmid stability in a nested legume-rhizobium mutualism undergoing nitrogen deposition

Sierra L. Bedwell[1,2], Isabelle M. Lakis[1,2], Kevin D. Ricks[4], Allison R. Higgins[1,5], Rachel J. Whitaker[1,2], Katy D. Heath[1,2,3]

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[5]Department of Crop Sciences, University of Illinois Urbana-Champaign

Nitrogen fixation involves a nested mutualism between plants, bacteria, and plasmids, wherein rhizobia fix nitrogen with plasmid-encoded genes and receive metabolites from their host legumes in trade. Our lab has shown that long-term nitrogen deposition leads to symbiosis degradation and the evolution of less beneficial rhizobia. We hypothesize that, under nitrogen deposition conditions, plasmid abundances will be lower in rhizobia in and out of association with plants. We will use ddPCR to compare rhizobial plasmid populations through environments with varying levels of long-term nitrogen deposition. We propose that exploring this question will broaden understanding of mutualism response to environmental changes.

Microbial delivery of antibodies to combat porcine respiratory disease complex

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Porcine respiratory disease complex (PRDC) is responsible for billions of dollars in loss to the meat industry every year. PRDC results from interactions between host microbiota, environmental stress, and pathogens. Current treatment for PRDC relies on antibiotics, which leads to antimicrobial resistance and does not address viral disease. To develop a non-antibiotic alternative response to PRDC, we aim to engineer members of the host microbiota to secrete neutralizing antibodies against viral pathogens implicated in disease. To achieve this, we will use a modular synthetic biology platform to engineer species of pig respiratory commensal bacteria, then return these engineered strains to the host respiratory niche to provide antibiotic-free protection against viral pathogens that drive PRDC.

Comparative analysis of the *Streptococcus pneumoniae* competence regulon induction *in vitro* versus *in vivo* during pneumonia-derived sepsis

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Streptococcus pneumoniae (pneumococcus) is a major human pathogen. The pneumococcal competence system is required for genetic transformation and virulence. The competent state develops naturally by quorum sensing through accumulation of the competence stimulating peptide. *In-vitro*, a short burst of competent state augments the expression of three distinct phases of "early, late and delayed" genes. However, our *in-vivo* studies indicate pneumococcus undergoes a prolonged competent state during lung infection. Therefore, we aim to unravel the differences in competence state *in-vitro* verses during pneumonia. Increased understanding of the *in vivo* competence induction may lead to novel therapy against pneumococcal pneumonia.

Distinct small RNAs regulate premature Rho-dependent transcription termination of the bacterial cyclopropane fatty acid (*cfa*) synthase mRNA

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In *Escherichia coli*, certain small RNAs (sRNAs) require RNA chaperone Hfq to properly regulate target mRNA molecules. Hfq facilitates base pairing of these sRNAs with their mRNA targets to alter gene expression and cellular physiology in response to various stimuli. While Hfq-dependent sRNAs are well known post-transcriptional regulators, we now know that sRNAs can further regulate target expression co- transcriptionally by influencing Rho-dependent transcription termination. Rho is a global regulator of transcription termination in *E. coli* that is highly conserved among most sequenced bacteria. Rho primarily binds to sequences within nascent mRNAs called Rho utilization (*rut*) sites to subsequently terminate transcription. In this study, we characterize sRNA-dependent positive and negative co-transcriptional regulation of the *E. coli* cyclopropane fatty acid (*cfa*) synthase gene. A long isoform of *cfa* mRNA possesses a 212-nt long 5'UTR that is subject to regulation by multiple Hfq-dependent sRNAs. We found that a *cfa* translational fusion has significantly higher activity in a *rho* mutant, suggesting *cfa* mRNA is subject to Rho-dependent transcription termination within its 5'UTR or early coding sequence (CDS). A moderately conserved 16-nt tract within the *cfa* long 5'UTR was found to be critical for Rho-dependent regulation and is

likely part of a *rut* site, further supporting this hypothesis. The sRNAs RydC and CpxQ were previously shown to activate or repress *cfa* expression, respectively, by modulating the RNaseE- dependent decay of *cfa* mRNA. The regulation of *cfa* by these sRNAs is impaired in the *rho* mutant, suggesting they may also modulate the premature Rho-dependent transcription termination of *cfa* mRNA. The goal of our future experiments is to further elucidate the molecular mechanisms of co-transcriptional regulation utilized by these Hfq-dependent sRNAs.

Reassortment in human rotavirus A

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Human rotavirus A is a segmented RNA virus that causes severe diarrhea in infants and children. When a host cell is infected with more than one viral strain, the segments can get exchanged making new variants. This process, known as reassortment, plays a major role in generating genetic diversity. However, little is known about the frequency and the impact of reassortment on rotavirus diversity. We used nucleotide sequence data from the NCBI virus variation database to investigate changes in alleles within rotavirus genotypes. Our analyses suggest that there are sub-genotypes within segment genotypes that could involve in intra-genogroup reassortment events.

Crimson and Clover: *Methylobacterium* interactions with *Rhizobia* and *Trifolium Hybridum*

Allison Higgins[1,3,4], Katy Heath[2,3,4], Sierra Bedwell[1,3,5], Izzy Lakis[1,3,5]
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[4]GEMS Biology Integration Institute

With a rapidly changing climate, plant fitness has become a new focus in the field of agriculture. Clovers are a critical cover crop. Clover has an observed symbiotic relationship with *Rhizobia* and *Methylobacterium*. *Mehtylobacterium* has been observed in a variety of crops to increase yield and general fitness. In a past experiment, we treated plants with several strains of *Methylobacterium* and a beneficial *Rhizobium* strain, and did not find a significant effect. We did, however, observe a red veining in plants treated with both symbionts. In this experiment, we hope to observe this veining more closely to understand the mutualisms.

The broad-spectrum metallophore staphylopine sensitizes *Staphylococcus aureus* to copper poisoning during infection

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Transition metals play dual roles as essential nutrients and toxic elements in biological systems. *Staphylococcus aureus* relies on small molecules, or metallophores, to acquire zinc. Such metallophores often have broad-spectrum metal chelating abilities. However, the contribution of the non-cognate metal uptake on metal homeostasis and bacterial pathogenesis is poorly understood. The current investigations revealed that metallophore usage enables copper entry into *S. aureus*, sensitizing it to copper toxicity in culture and during infection. This study demonstrates that the limited specificity of metallophores is not advantageous to microbes in all aspects and can drastically increase the threat posed by metal intoxication.

Tandem mobilization of anti-phage defenses alongside staphylococcal SCC*mec* cassettes

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Bacteria have evolved diverse immune systems that protect against their viral predators (phages). Little is known about how these systems are horizontally transferred among different species. This study investigates how two anti-phage defense systems in *Staphylococcus epidermidis*, Nuclease-helicase immunity (Nhi) and CRISPR-Cas10, are mobilized and transferred to other staphylococcal species. Data generated thus far support the hypothesis that these defenses are excised and circularized as independent cassettes along with staphylococcal cassette chromosome *mec* (SCC*mec*), a known mobile genetic element of *Staphylococcus* species that carries different antibiotic-resistant genes.

Understanding InvR-mediated post-transcriptional regulation of SPI-1 transcriptional activator HilA and discovering potential sRNA regulators of SPI-1 in *S. Typhimurium*

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Bacterial small RNAs (sRNAs) are short, typically non-coding RNAs that regulate gene expression for quick adaptation to stress and virulence conditions. *Salmonella* Pathogenicity Island 1 (SPI-1) encodes essential virulence genes for *Salmonella* invasion of intestinal epithelial cells. Many environmental and host-associated signals control SPI-1 gene expression, but in most cases, the molecular mechanisms remain unclear. A growing body of evidence suggests that some signals control regulation of SPI-1 at a post-transcriptional level. *Salmonella* has ~350 sRNAs, many of which are known to play important roles in post-transcriptional regulation of many pathways and systems, including SPI-1. Here, we show that the SPI-1-encoded sRNA InvR acts as a negative feedback on SPI1 expression. HilD activates both *invR* and *hilA*, encoding the major SPI-1 transcription factor. InvR negatively regulates translation of the *hilA* mRNA. While most sRNAs that block translation do so through direct interference with ribosome binding to the mRNA, our study shows that InvR regulates *hilA* translation by a different mechanism. The *hilA* mRNA 5' untranslated region (UTR) is ~300-nuclotides (nt) in length. Genetic and structural studies demonstrate that InvR base pairs at a site that is 120- nt upstream of the *hilA* ribosome binding site (RBS). We carried out Global sRNA Target Identification by Ligation and Sequencing (GRIL- Seq) using the long *hilA* 5' UTR as bait to identify new potential sRNA binding partners for *hilA*. These analyses identified known sRNA binding partners, InvR and PinT as well as additional candidate sRNAs. Interestingly, the GRIL-seq data suggest that there may be a second InvR-*hilA* 5' UTR binding interaction near the *hilA* RBS. We are testing the model that InvR binds to two distinct sites on *hilA* mRNA to uncover the molecular mechanism of regulation by InvR. Our results highlight the complexity of sRNA regulatory inputs controlling SPI-1 and *Salmonella* virulence.

Knowledge attitude and practices of para-vets about ticks and tick-borne diseases in Pakistan

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Recent global changes have accelerated the spread of ticks and tick-borne pathogens, affecting animals and humans. According to a livestock survey in Pakistan, there are 41.2 million buffaloes, 49.6 million cattle, 78.2 million goats, and 30.9 million sheep. Among this massive population of ruminants, tick infestation of 34.83% (buffalo), 57.11% (cattle), 51.97% (sheep), and 46.94% (goats) is reported. Most livestock farmers rely on para-vets for assistance with any animal health problem. We designed a survey to assess para-vets' Knowledge Attitude and Practices (KAP) toward tick-borne diseases, which will ultimately help us to give insight into their practices for controlling and preventing tick-borne diseases.

Excess cation stress and tolerance mechanisms in bacteria

Yumi Iwadate[1], James M. Slauch[1]
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Elucidating bacterial physiology at stationary phase will help us find novel strategies to prevent bacterial infections, as bacteria are often in a non-replicating state both inside and outside of host environments. We have demonstrated that excess cations, namely polyamines and magnesium, stress cells as they enter stationary phase, but the mechanisms of this stress are not understood. In this study, we report two uncharacterized *Salmonella* genes that, when mutated, cause lethality in stationary phase. Further characterization will inform us of the nature of cation stress and how cells normally prevent damage in these conditions.

Exploring anti-phage defenses in integrative conjugative elements encoded by staphylococci

Vanessa Jones[1], Asma Hatoum-Aslan[1]
[1]Department of Microbiology, University of Illinois at Urbana-Champaign

Due to its prevalence as a pathogen and growing antimicrobial resistance, *S. epidermidis* is a prime candidate for the therapeutic use of bacterial viruses (phages) to eradicate antibiotic-resistant infections. However, *S. epidermidis* encodes anti- phage defenses that could undermine the effectiveness of phage treatment. We seek to expand our search for new anti-phage defenses in *S. epidermidis* by identifying new mobile genetic elements and searching for defenses within them. Here, we discovered a putative integrative conjugative element (ICE) in *S. epidermidis* ATCC12228, and will attempt to identify novel and diverse anti-phage defenses encoded within it.

Cell size regulation in bacteria-connection between nutrients and chromosome replication

Ezza Khan[1], Paola E. Mera[1]
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A fundamental question in biology is, how cells sense their environment and regulate cell size. Unlike other species, *Caulobacter crescentus* does not exhibit nutrient dependent increases in size. Our preliminary data revealed that when *Caulobacter* express *dnaA* constitutively, cell size is reduced 25- 30% in minimal media. Thus, the levels of DnaA might impact cell size when grown in minimal media. To determine what region might contribute to the observed phenotype, we constructed DnaA variants. Analysis of these strains indicate the involvement of domain IV and N-terminus in size regulation. These findings suggest cell cycle-dependent regulation of DnaA contribute to the coordination between nutrient availability and cell size regulation.

Pseudomonas aeruginosa volatile organic compounds cause airway mucus hypersecretion by activating the AhR signaling and by polarizing toward a M1 macrophage, neutrophils, and type 17 immune responses

Shanny H. Kuo[1], Jaishree Sharma[1], Som G. Nanjappa[1], Gee W. Lau[1]
[1]Department of Pathobiology, University of Illinois at Urbana-Champaign

Pseudomonas aeruginosa (PA) volatile organic compounds (VOCs) are found abundantly in the breath of diseased lungs, serving as biomarkers for acute exacerbations. The importance of VOCs in the lung pathogenesis is unknown. By using both an air-liquid interface cell culture and a mouse model, we tested the hypothesis that PA VOCs activate AhR signaling and/or dysregulate immune response to drive mucus hypersecretion in diseased lungs. VOCs increase AhR and mucus expression while downregulate FOXA2, which regulates mucus homeostasis. VOCs induce a combination of M1 macrophage/neutrophilic/Th17 response, as confirmed by depletion of macrophages or neutrophils, and by the IL-17$^{-/-}$ mouse approach.

Uncovering the role of TSP8 in attachment of *Cryptosporidium* sporozoites

Joshua Lain[1], Sumiti Vinayak[1]
[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana Champaign

The protozoan parasite *Cryptosporidium* is a leading cause of diarrhea in children, immunocompromised individuals, and agricultural animals. There is no effective vaccine or drug available to prevent or treat infection. We lack knowledge of the molecules used by the parasite for attaching to the epithelium. We have identified a *Cryptosporidium* thrombospondin adhesive domain containing protein that may play a role in host cell attachment. Super-resolution and expansion microscopy of sporozoites suggests that this protein is localized on the surface of the parasite. Functional studies are underway to determine the role of this protein.

Investigating the roles of RNA-binding proteins in human gut symbiont, *Bacteroides thetaiotaomicron*

Hellan Lee[1], Amanda N. D. Adams[1], Patrick H. Degnan[2], Carin K. Vanderpool[1]
[1]Department of Microbiology, University of Illinois Urbana-Champaign
[2] Department of Microbiology, University of California, Riverside

The human colon harbors an extremely dynamic bacterial population in the body, with *Bacteroides* species being some of the most dominant gut colonizers. While many highly developed sensing and regulatory systems have been discovered in *Bacteroides* and how they contribute to *Bacteroides* fitness in dynamic intestinal environments, little is known about RNA-based regulation in *Bacteroides*. We hypothesize that our recently discovered novel family of RNA binding proteins (RBPs) in *Bacteroides* may play a role in post-transcriptional regulatory processes. Using RNA-immunoprecipitation sequencing, we identified 16S rRNA as one of the putative target RNA, suggesting RBPs' roles in ribosome biogenesis.

Determining TipN's role in the cell cycle

Morgan Letzkus[1], Paola E. Mera[1]
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Caulobacter crescentus is the model organism to study polarity and cell cycle regulation. TipN is a polar protein that has been implicated in multiple cell cycle events. I have shown that the C-terminus, but not the N-terminus, of TipN is involved in promoting unidirectional translocation of *oriC*. Further, the C- terminus appears to be involved in maintaining the integrity of the cell membrane and involved in antibiotic resistance via TEM and confocal analysis, growth curves, and viability assays. My project aims to elucidate the mechanism(s) and domain(s) of TipN that are important in chromosome segregation, cytokinesis, and morphology.

Airway protein citrullination by the *Pseudomonas aeruginosa* pyocyanin

Shi Qian Lew[1], Woosuk Choi[2], Gee W. Lau[1]
[1]Department of Pathobiology, University of Illinois at Urbana-Champaign
[2]Department of Pediatrics, David Geffen School of Medicine, University of California at Los Angeles

Pyocyanin is a redox-active toxin of *Pseudomonas aeruginosa* copiously secreted in chronically-diseased lungs, resulting in mucus hypersecretion. Here, we demonstrate that pyocyanin induces protein citrullination in the human bronchial epithelial cells (HBECs). Pyocyanin increases the intracellular calcium levels, leading to elevated expression of peptidylarginine deiminases 2 (PAD2) that catalyze protein citrullination. We are characterizing selective citrullinated proteins induced by pyocyanin, including DOK2. We are currently performing molecular and cellular characterizations to decipher the importance of DOK2 citrullination to goblet cell metaplasia and mucus hypersecretion as well as lung injury resolution during *P. aeruginosa* infection.

Fructan chain-length influences enteric microbiota-host GABAergic signaling

Benjamin A. Levine[1], Brett R. Loman[2]
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Functional gastrointestinal disorders (FGIDs) are associated with lower colonic mucosal GABA, a neurotransmitter that influences intestinal motility. Eliminating dietary fiber is a common strategy to reduce FGID symptoms. However, dietary fiber conveys many benefits like reducing luminal pH, which stimulates enteric microbial GABA synthesis. Therefore, we examined whether fiber types influence enteric microbiota-host GABAergic signaling. Mice received either a fiber-free, cellulose (inert fiber control), scFOS (short-chain fructan), or inulin (long-chain fructan) diet for two weeks. Gene expression, pH, and metabolite results from ileum and colon indicate that fructan chain-length influences enteric microbiota-host GABAergic signaling in an intestinal segment-dependent manner.

Influenza viral aggregation forming polyploid potentially increases virus mutational robustness

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RNA viruses bear high-level mutation rates among all biological systems. It is unclear how viruses tolerate such mutation rates while maintaining their infectivity and evolutionary potential. We previously demonstrated that lowering neuraminidase activity could increase the overall mutational robustness of hemagglutinin in influenza virus.
Given that neuraminidase activity is required to prevent viral aggregation, we hypothesize that a higher proportion of aggregation forms infectious units with more copies of genes, leading to a better tolerance of mutations. We explored the feasibility of using flow cytometry to study viral aggregation. This result could add more complexity to virus population diversity.

Characterizing the role of a transporter protein in *Cryptosporidium parvum*

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Cryptosporidium parvum is a leading diarrheal parasite in young children, immunocompromised individuals, and neonatal calves. The parasite forms a membranous feeder organelle at the host-parasite interface that is speculated to play a role in scavenging metabolites from the host intestinal epithelial cells. However, the molecular mechanisms of metabolite transport are not well understood. We have identified a *C. parvum* major facilitator superfamily (MFS)-domain containing transporter protein that is expressed throughout the parasite lifecycle. Expression was found in asexual stages (trophozoites and meronts) and microgamonts. Our goal is to understand the precise localization and function of this transporter in parasite biology.

Time-resolved RNA-seq analysis to unravel the *Streptococcus pneumoniae* competence induction during pneumonia-derived sepsis

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Pneumococcus is a major human pathogen. The regulation of pneumococcal competence regulon has been extensively interrogated *in vitro*, but poorly understood during lung infection. We utilized a combination of IVIS guided imaging and RNAseq to monitor the development of competence state during pneumonia-derived sepsis. Upregulation of competence-specific genes was observed as early as 12 hpi, supporting the association of pneumococcal competence regulon to host adaptation and stress response. Among others, we found that the pneumococcal histidine triad (Pht) family of genes participate in the regulation of pneumococcal competence induction.

Type IV pili and shear force coordinate surface departure of *Pseudomonas aeruginosa*

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Type IV pili facilitate surface colonization, however, their role in surface departure remains underexplored. Here, we use microfluidics and individual cell tracking to characterize *Pseudomonas aeruginosa* type IV pili in fluid flow. We show type IV pili promote surface departure by generating unstable surface contact. We demonstrate that increasing shear force decreases surface departure by restricting surface motion and cell tilting. Our results support a model where type IV pili and shear force coordinate *P. aeruginosa* surface departure. By studying bacteria in conditions resembling hosts, our work sheds light on the importance of understanding how shear force impacts host-pathogen interactions.

The role of the partitioning protein ParA in the initial stages of chromosome separation

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Bacteria must coordinate the cell cycle concurrently to preserve life. In this study, we explored the partitioning protein ParA in the initial separation of *ori-parS* loci, following chromosome replication initiation. We identified a ParA variant (R195E) with a modification in its DNA-binding domain that results in cells retaining their *ori-parS* loci at the stalked pole inhibiting chromosome segregation. Our data suggest that anchoring of the chromosome to the pole is transiently aided by ParA and disrupting DNA binding blocks chromosome release. Overall, our data contribute to the multiple roles of cell cycle proteins to preserve the cell cycle.

Discovery and characterization of anti-phage defense system in *Staphylococcus epidermidis*

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Bacteria have evolved an arsenal of diverse defense systems to counter bacteriophages. Generally, these defense systems remain clustered in the bacterial genome as 'defense islands'. Anti-phage defense hunting has gained attention lately owing to the wealth of molecular tools that can be developed. *S. epidermidis* is a skin-dwelling opportunistic pathogen infamous for nosocomial infections. Preliminary functional screening of *S. epidermidis* 6593473 defense island revealed that a particular genomic region encodes for anti-phage defense. We aim to elucidate the minimal genetic construct, the phage determinants, and the mechanism underlying immunity to gain deeper insights into the newly discovered anti-phage defense system.

Variation in resolution of genetic conflict leads to variation in spontaneous induction of a lysogen

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The evolution of *Pseudomonas aeruginosa* in cystic fibrosis chronic infections has been well-studied, but the effect of this evolution on its phages is unclear. In this study, we explored the impact of the absence of susceptible cells on coevolution of a virus- bacterium pair. We passaged a lysogen without susceptible cells and measured viral transmission parameters. We hypothesized that viral vertical transmission would increase, decreasing lysogen spontaneous induction. We found that spontaneous induction was decreased in evolved viruses due to avoidance of self-targeting by CRISPR, and that spontaneous induction variation is due to variation in the genetic mechanism of avoidance.

In-vivo production and delivery of biotherapeutics against respiratory pathogens

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Recent advances in manipulation of commensal bacteria have unlocked the potential to engineer these microbes for therapeutic purposes. While significant progress has been made in the study and manipulation of gut microbiota, other commensal microbes remain relatively underexplored. We aim to engineer commensal human respiratory microbes to produce and deliver viral neutralizing antibody fragments from within the respiratory niche. Previous studies support the use of intranasally delivered antibody fragments as effective prophylactic therapies. We anticipate that introduction of our engineered strains into the respiratory niche will lead to colonization and stable, persistent generation of antibody fragments at the infection site.

Cellular heterogeneity in pre-infection gene expression patterns influences influenza A virus infection susceptibility and IFN induction

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Interferon (IFN) production is among the first lines of defense against influenza A virus (IAV) infection and replication in lung epithelial cells. Our previous work revealed that individual cells within a population exhibit heterogeneity in both susceptibility to IAV infection and IFN expression. We hypothesize this variation arises from the pre-infection cellular states. To test this, we created a collection of A549 subclones to investigate variation in IFN induction and susceptibility to IAV infection. These studies will identify novel host determinants of IAV susceptibility and innate immune responsiveness and will help explain how cellular heterogeneity can influence IAV infection outcomes.

Regulation of phosphate homeostasis in *Staphylococcus aureus* differs from established models

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Inorganic phosphate is essential for life and regulation of phosphate homeostasis is necessary for *Staphylococcus aureus* pathogenesis. In *S. aureus*, the PhoPR two-component system controls phosphate homeostasis. Differing from the paradigm, *S. aureus* possesses an expanded repertoire of regulatory proteins. This translates to the hypothesis that proper regulation of phosphate acquisition and homeostasis is important for *S. aureus* pathogenesis and the increased complexity within these mechanisms expands the environments in which *S. aureus* can live and safely acquire phosphate. Growth and expression assays, and infection studies demonstrate that the regulation of phosphate homeostasis in *S. aureus* differs from established models.

Inverted repeat sequences of an IS element expand the host range of pGK12, a shuttle vector for lactic acid bacteria

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We report an unusual insertion of an insertion sequences (IS), ISLrh, from *Lactocaseibacillus rhamnosus* into pGK12. The resulting plasmid pTRK829 gained the ability to stably replicate in *L. rhamnosus*, unlike pGK12. Examination of transformants of pTRK829 identified a derivative, designated pTRK830, which retained ISLrh but had a deletion elsewhere. Both exhibited high transformation efficiencies in many LAB strains. Sequence and functional analyses of ISLrh revealed that terminal inverted repeats (IRs) of ISLrh and the insertion position are essential for the replication of pTRK830. Moreover, pTRK830 and its derivates were successfully employed to express heterologous gene in three different LAB strains.

CRISPR-mediated dynamics in natural and simulated *Sulfolobus* populations

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CRISPR-Cas system provides archaea and bacteria with adaptive immunity against viruses. As a heritable system, CRISPR is a powerful force shaping microbial diversity. While the structure and diversity of CRISPR arrays have been determined at a single time point, the associated temporal dynamics have not. Here we propose to explore the role of the CRISPR in the eco-evolutionary dynamics within *Sulfolobus* metapopulations and their viruses in time. We take bioinformatics and theoretical approaches to bridge the gap between empirical CRISPR datasets and simulated models. Understanding these dynamics will have great applied importance in phage therapy treatment and infectious disease emergence.

Engineering protein secretion of *Bacteroides* species

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Bacteroides species, one of the most abundant and prevalent bacterial populations in human gut, are capable of long-term, stable colonization of the gastrointestinal tract, making them promising chassis for developing long-term treatment and prevention for chronic diseases. However, the lack of efficient heterologous protein secretion tools prevents their use as engineered, on-site delivery vehicles for protein-based biologic drugs or disease-responsive reporters. Here, we revealed and characterized a group of secretion carriers derived from native secretory proteins of *Bacteroides thetaiotaomicron* that enable the high-titer secretion of functional antibody fragments and reporter proteins in multiple *Bacteroides* species for improving their biomedical applications.