Leveraging A Global-Scale Atlas of Phage Host Range to Discover Novel Precision Antimicrobials

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Background: A growing global antimicrobial resistance (AMR) crisis fueled by the overuse of traditional antibiotics has created an urgent need not only for new antimicrobials, but entirely new approaches for sustained therapeutic development against existing and emerging pathogens. At the same time, the world faces the looming threat of catastrophic climate change, and methane emissions from agriculture are a key driver in overall greenhouse gas emissions.

Methods: We have adapted a unique genomic technology, proximity ligation sequencing, to harness the vast genetic diversity of microbial viruses (phages) to discover large-numbers of protein-based interventions against microbes. Proximity-guided metagenomics allows us to pinpoint the microbial targets of phages in situ, recovering the genomes of both microbe and phage without culturing or isolation of either party. Inherently, each lytic phage genome encodes one or more proteins (endolysins) which can lyse their host cell. Each of these proteins represents a powerful tool in the targeted destruction of a selected organism, and because we know which species of microbe each phage infects, we can identify candidate lysins from proximity-guided metagenomic data alone.

Results and **Discussion**: As part of our global-scale metagenomics project, we used our ProxiMeta platform to recover the target-resolved genomes of hundreds of thousands of phages across the tree of life. We will share published and unpublished work describing our discovery and evaluation of these lysins, and their applications in selectively removing methanogenic archaea in ruminants, and microbes involved in human disease.

Ultra-high throughput single-microbe sequencing enabled by semi-permeable capsules

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Background
Whole-genome and targeted sequencing open a window to understanding the diversity and function of unculturable microorganisms. On the one hand, metagenomic sequencing is attractive for its straightforward library preparation from bulk samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of cells in parallel. Here, we demonstrate how Semi-Permeable Capsule (SPC) technology enables a virtually unlimited number of processing steps on genetic material from thousands of individual microbes in parallel and allows for ultra-high-throughput single-cell sequencing.

Methods
This study aimed to demonstrate the use of SPCs for barcoding >10,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality. For proof-of-concept evaluation, we encapsulated well-characterized E. coli and B. subtilis bacteria into SPCs, lysed cells at alkaline conditions (pH 13), amplified their genomes, and employed a split-pool approach to add unique cellular barcodes.

Results
Upon sequencing of an aliquot of ~3,000 cells, important technical metrics, such as cross-contamination and genome recovery, were measured to assess the performance of the workflow. The results showed excellent genome retention within SPCs, with <1% of cross-contaminated genomes. Genome recovery analysis yielded a median coverage of 90% at a median sequencing depth of 8X for B. subtilis cells (1690 cells sequenced), and a median coverage of 63% at a median sequencing depth of 3X for E. coli cells (2023 cells sequenced) with clear indication for higher recovery at saturating sequencing.

Discussion
We conclude that the compartmentalization of microbial cells into SPCs allows the generation of high-quality whole-genome data at scale, and further apply the method on environmental samples.

Microbiome species profiling at scale with the Kinnex kit for full-length 16S rRNA sequencing

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Background: Targeted 16S sequencing is a cost-effective approach for assessing the bacterial composition of metagenomic communities and is particularly useful for low bacterial biomass samples. However, due to high similarity in 16S rRNA genes among related bacteria, sequencing the entire 16S gene (~1.5kb) with high accuracy is essential for precise species and strain identification. Comparative studies have shown that PacBio full-length (FL) 16S sequencing outperforms other methods for taxonomic resolution and accuracy. The Kinnex 16S rRNA kit, based on the multiplexed array sequencing (MAS-Seq) method, processes amplified, barcoded 16S amplicons and outputs a sequencing-ready library that results in up to a 12-fold throughput increase compared to standard FL 16S libraries for significantly higher throughput (up to 1,536 amplicons per SMRT Cell).

Methods: We tested the Kinnex 16S rRNA kit on a diverse range of samples including standards, feces, saliva, plant, wastewater, and swabs (skin, oral, vaginal, and veterinary wound). We analyzed the data using the HiFi-16S-workflow, that provides a FASTQ-to-report analysis for FL 16S HiFi reads.

Results: The results show that Kinnex 16S sequencing can yield >30k average reads per sample at 1,536-plex on a single Revio SMRT Cell or at a 768-plex on a Sequel IIe SMRT Cell. Comparing Kinnex 16S to standard FL 16S datasets, we found a high correlation and no bias in community compositions and were able to assign up to ~99% of denoised reads to species. In addition, because of the higher number of reads per sample, Kinnex 16S allows for more recovery of lower abundance species.

Discussion: With the Kinnex 16S rRNA kit, researchers may now multiplex more samples to dramatically reduce cost per sample or to profile each sample deeper with more reads/sample. The additional reads/sample along with better taxonomic resolution is advantageous for numerous environmental sample types which are often highly diverse.

Utilizing microbial genomic DNA libraries to discover modified nucleoside eraser enzymes

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Background: The number of known RNA modifications is ever increasing with over 170 modifications identified to date. The enzymes that install these modifications are called writer enzymes, whereas the enzymes that remove the modifications are called eraser enzymes. Many writer enzymes are known and well characterized. However, few eraser enzymes have been identified and studied.

Methods: To identify new eraser enzymes, we have designed a genetic selection system using auxotrophic strains of Escherichia coli deficient in either purine or pyrimidine biosynthesis. Using these strains, we can screen for the ability of E. coli to grow on minimal media supplemented with a modified nucleoside as the sole source of purines or pyrimidines. If E. coli is unable to grow on the provided modified nucleoside, then genomic DNA plasmid libraries can be used to identify enzymes from other species that can remove these modifications.

Results: We assessed the ability of the E. coli purine and pyrimidine auxotroph strains to grow on 19 different modified nucleosides. The auxotrophic E. coli strains were unable to grow on 16 of the compounds. We performed a genetic selection on these 16 compounds using 7 diffident genomic DNA libraries. From these selections, we identified 8 genes that allowed E. coli to grow on 2-thiouridine or 4-thiouridine. One of the enzymes from Thermococcus kodakarensis, named TudS_T for 4-thiouridine desulfurase, is the first example of a 4-thiouridine eraser enzyme from archaea shown to have activity in vivo.

Discussion: Combining genomic DNA libraries with auxotrophy-based genetic selection enables the discovery of novel enzymes. TudS_T does not show significant sequence or structural homology to known 4-thioruridine desulfurases and could not be identified using standard bioinformatic searches. This approach has allowed us to identify evolutionarily divergent enzymes and uncover new aspects of archaeal biology.

Adagio: A user-friendly graphical interface for building fully reproducible, infinitely scalable, plugin-based data pipelines

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Background: Biological insights in microbial genomics research are often gained by analyzing large, multi-omic, datasets. Workflows for handling such data are complex, involving many different tools. QIIME 2 improves workflows by providing 1) data-provenance tracking, allowing users to retrospectively determine exactly how any result was generated, 2) semantic typing that indicates acceptable input types, validating which data tasks apply to, thereby reducing errors; and, 3) plugin architecture that allows third parties to contribute new functionality. To broaden the use of provenance-tracked, semantically typed, plugin-based data pipelining beyond scientists with command line skills, developers at Novonesis and Cymis collaborated to create Adagio.

Methods: Adagio is a graphical user interface-based data processing pipeline builder. Data can be processed using any cloud-based or on-premise computational resources that support containerization; Adagio provisions machines, verifies authentication, and runs pipelines in the background. Pipeline statuses can be fetched at any time. Account infrastructure allows separate groups to control projects, files, and plugins (modular software tasks).

Results: Novonesis biologists and software engineers have collaboratively built and run amplicon, metagenomic, and genomic pipelines using Adagio. Novonesis' microbiome pipeline ingests sequence data through a REST interface and generates reports of taxonomy and metabolic potential (including custom statistical analyses and figures) in less than 10 hours.

Discussion: Adagio allows biologists to reliably analyze data using verified methods. Adagio's flexible infrastructure provides scientists with coding skills the opportunity to develop custom plugins while others can use and build pipelines from verified tasks, drawing on open-source plugins written by QIIME 2's developer community. Once developed, plugins can be re-used, enabling rapid turnaround on large analyses and adherence to FAIR data principles. Further, Adagio is data-agnostic, enabling users to process non-sequence/non-biological data. Finally, Adagio's validated methodology and coupled graphical and command line interfaces makes it a powerful tool for teaching bioinformatics best practices.

Patterns of plasmid inheritance and diversity within core and variable elements of Rhizobium leguminosarum genomes

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Background: Understanding the ecological and evolutionary rules governing host-microbe interactions depends on our fundamental understanding of bacterial inheritance patterns in natural populations. In addition to the mobile genetic elements that carry the canonical genes for symbiosis, many symbiotic bacteria have multipartite genomes, i.e., genomes more than one replicon that can vary in size, copy number, gene content, propensity for horizontal gene transmission, diversity, and even structure within replicons of the same type. The dynamics of the myriad of players in multipartite genomes can affect interactions with hosts in ways we are only beginning to understand.

Methods: Here we generate 62 closed genomes of Rhizobium leguminosarum bv. trifolii from natural populations and use a combination of genomics, phylogenetics, and population genomics to assess patterns of diversity and inheritance between and within plasmids in a local, recombining population.

Results: We find that, of the four plasmids in the core mobilome, two (type II & III) have more stable size and gene presence and track the chromosome phylogeny (display more vertical transmission), while others (I & IV) show ample size and gene presence-absence variation, propensity for horizontal transmission, and even structural changes that result in novel plasmid types. Further, we associate horizontal transmission of the pSym (type IV plasmid) with the evolutionary changes in the symbiotic benefits of Rhizobium for plant hosts.

Discussion: Our study demonstrates how plasmid transmission, diversity, and structure can vary even among replicons in the same bacterial cells in natural populations of an important soil bacterium. Moreover, plasmid transfer can be linked to the evolution of important phenotypes such as symbiotic partner quality in response to environmental change.

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

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

Methods
Antimicrobial susceptibility testing and whole-genome sequencing of 415 carbapenem-resistant Klebsiella pneumoniae sequence type 258 isolates collected from 2014-2015 across 20 US long-term acute care hospitals. To identify features associated with resistance emergence and spread, we evaluated the fraction of non-susceptibility explained by carbapenem resistance-associated genotypes, executed an unbiased genome-wide association study (GWAS), and performed regression modeling of patient characteristics.

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

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

Integrative genomic reconstruction of carbohydrate utilization networks in bifidobacteria: global trends, local variability, and dietary adaptation

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Bifidobacteria are among the earliest colonizers of the human gut, conferring numerous health benefits. While multiple Bifidobacterium strains are used as probiotics, accumulating evidence suggests that the individual responses to probiotic supplementation may vary, likely due to a variety of factors, including strain type(s), gut community composition, dietary habits of the consumer, and other health/lifestyle conditions. Given the saccharolytic nature of bifidobacteria, the carbohydrate composition of the diet is one of the primary factors dictating the colonization efficiency of Bifidobacterium strains. Therefore, a comprehensive understanding of bifidobacterial glycan metabolism at the strain level is necessary to rationally design probiotic or synbiotic formulations that combine bacterial strains with glycans that match their nutrient preferences. In this study, we systematically reconstructed 66 pathways involved in the utilization of mono-, di-, oligo-, and polysaccharides by analyzing the representation of 565 curated metabolic functional roles (catabolic enzymes, transporters, transcriptional regulators) in 2973 non-redundant cultured Bifidobacterium isolates and metagenome-assembled genomes (MAGs). Our analysis uncovered substantial heterogeneity in the predicted glycan utilization capabilities at the species and strain level and revealed the presence of a yet undescribed phenotypically distinct subspecies-level clade within the Bifidobacterium longum species. We also identified Bangladeshi isolates harboring unique gene clusters tentatively implicated in the breakdown of xyloglucan and human milk oligosaccharides. Predicted carbohydrate utilization phenotypes were experimentally characterized and validated. Our large-scale genomic analysis considerably expands the knowledge of carbohydrate metabolism in bifidobacteria and provides a foundation for rationally designing single- or multi-strain probiotic formulations of a given bifidobacterial species as well as synbiotic combinations of bifidobacterial strains matched with their preferred carbohydrate substrates.

Persistence of Pseudomonas aeruginosa in ICU sink drains

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Antimicrobial-resistant organisms (AROs) are a significant concern for human health, especially in intensive care units (ICUs), which care for medically vulnerable patients. Concerningly, AROs, including the opportunistic pathogen Pseudomonas aeruginosa, have been shown to persistently colonize ICU sink drains. Previous work revealed the presence of P. aeruginosa sequence type (ST) 1894 from ICU sink drains through a year of sampling, though this work was limited to isolate analysis, which can be insufficiently sensitive to identify all Pseudomonas present. To address this limitation and to assess P. aeruginosa persistence without requiring isolates, we performed shotgun metagenomic sequencing of 32 Cetrimide agar plates from 14 sink drains, each grown from E-swabs of ICU sink drains collected over three weeks from two wards of an acute care hospital in St. Louis, Missouri. We expected to detect strain-level evidence of P. aeruginosa persistence from these sink drain plate sweeps, particularly of ST1894. If successful, this plate sweep method will increase sensitivity and throughput of ICU environmental surveillance by avoiding the need for time-consuming and insensitive isolate analysis. Consistent with previous work, P. aeruginosa was identified repeatedly from 5 sink drains at multiple timepoints. Specifically, this included the stem cell transplant ICU soiled utility room (weeks 1 - 2) and a patient room (weeks 2 and 3) and the surgical ICU housekeeping closet (weeks 1-3), a patient room (weeks 1 – 3), and soiled utility room (weeks 1 and 3). Incidentally, we observed significantly higher Shannon diversity and species richness in samples from soiled utility rooms and housekeeping closets compared to those from patient rooms. Our results suggest that persistent P. aeruginosa colonization can be detected using plate sweep methods rather than isolate cultures, and work is ongoing to generate hybrid long- and short-read assemblies to identify persistent strains of P. aeruginosa.

Development of Plasmids and Genetic Systems in Human Gut Commensals

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Background: The gastrointestinal tract is home to a highly diverse commensal microbial community that drives numerous host phenotypes, including digestion, nutrient absorption and immune function. Despite the introduction of anaerobic culturing techniques, multi-omics, and germfree animal models, a key bottleneck to mechanistic studies on the gut microbiota is the lack of suitable genetic tools. One common member of the human gut microbiota is *Blautia obeum*, an anaerobic, gram-positive bacterium that has been shown to use multiple mechanisms to drive strong infection resistance against gut pathogens such as Vibrio cholerae, the causative agent of the severe diarrheal disease cholera. There are currently no genetic tools established for *B. obeum*, which limits the mechanistic dissection of these anti-pathogen activities.

Methods: To solve this problem, we have used bioinformatic techniques to identify putative episomes, replication systems, and antibiotic markers to generate genetic tools for *B. obeum*. We have synthesized shuttle vectors able to replicate both in lab Escherichia coli cloning strains and able to be transferred to *B. obeum* via conjugation.

Results: We were able to successfully generate recombinant vectors that can be transferred into *B. obeum*.

Discussion: Replicating plasmids allow us to optimize methods to transfer recombinant DNA into previously intractable gut microbes and can form the basis of genetic modification including targeted and untargeted mutagenesis systems. Our pipeline to identify genetic elements that can be combined into conjugatable episomes allows us to begin to examine specific genetic determinants of *B. obeum* function and can be extended to other bacteria in the gut microbiota that also lack genetic systems.

A future for single cell genomics: technical improvements and applications to natural systems

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Characterization of microbiomes using culture independent approaches, including amplicon sequencing, metagenomics, and single cell genomics, have significantly expanded our understanding of microbial diversity and function. Yet, each method has limitations: amplicon sequencing lacks functional insights, metagenomics and the subsequent reconstruction of metagenome assembled genomes (MAGs) often miss key genomic elements like 16S rRNA genes and mobile genetic elements (MGEs) and collapse population heterogeneity, and single cell sequencing is hindered by biased amplification resulting in partial genome recovery. Here, we introduce a novel whole genome amplification (WGA) approach, primary template-directed amplification (PTA), which utilizes exonuclease-resistant terminators to facilitate quasilinear template amplification, which significantly increases single cell genome quality, as compared to currently used multiple displacement amplification (MDA) protocols. This advancement is pivotal for environmental genomics and human microbiome studies, allowing more accurate analyses of uncultivated genomes, including microbial population studies and a deeper understanding of microbe-MGE interactions. With this poster, we will demonstrate the value of PTA and discuss the potential of combining PTA with semi-permeable capsules in microfluidic instruments for high-throughput single-microbe sequencing, thereby enhancing overall throughput and data quality.

Benchmarking pipelines for extracting strain-level diversity to improve transmission inference

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The rise of multi-drug resistant organisms like carbapenem-resistant Enterobacterales (CRE) poses a significant threat to hospitalized patients, causing 13,100 infections and 1,100 deaths in the U.S. in 2017. Whole-genome sequencing has become crucial for tracking infections and guiding prevention practices, but standard methods that sequence isolates from a single colony miss multi-strain colonization and intra-strain diversity, hindering transmission network reconstruction. Population deep sequencing (PDS), selective agar plates, and strain deconvolution methods offer an increased capture of CRE's genetic diversity, but are not thoroughly evaluated for CRE surveillance. We evaluated the mGEMs pipeline for strain deconvolution due to its ability to distinguish closely related lineages based on probabilistic taxonomic classification of metagenomic reads. Our study examined how database structure, the number of reference sequences clustered via multi-locus sequence typing (MLST), impacts mGEMs performance. Single strain samples included 1,592 isolates from 717 patients' rectal swabs, while multiple strain samples contained synthetic mixtures of pairs of isolates at different abundance ratios. In addition, variant callers were evaluated for their ability to detect 5,000 spiked minor variants from single strain samples created with Varben. Lineages were assessed for the preservation of variants after mGEMs deconvolved single strain samples. Using all sequences in the reference database gave the highest F-1 score, 0.52 and 0.66, for single and mixed samples, respectively. GATK had the highest average F-1 score, among variant callers, followed by LoFreq, Freebayes, and mpileup (0.94, 0.84, 0.76, and 0.71 respectively). mGEMs incorrectly assigned reads to different lineages which caused false positives and negatives variants, while true positive variants remained consistent across ratios. Our findings indicate that database structure and the abundance of lineages affects mGEMS performance. Future work will focus on incorporating additional strain deconvolution tools and exploring the impact of coverage on their performance.

Using (Randomly Barcoded) Transposon Sequencing to Unravel the Molecular Biology That Supports the Water Survival of Bacterial Wilt Pathogens

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The Ralstonia solanacearum species complex are the causal agents of bacterial wilt, a disease that affects around 400 plant species. These pathogens have a remarkable ability to survive in surface water. When left in sterile, de-ionized water, Ralstonia isolates have survived for decades. To investigate how these pathogens survive in de-ionized water, we set up a long-term genetic screen where we inoculated water microcosms with random barcoded transposon mutant libraries. We sampled and sequenced (RB-TnSeq) these microcosms out to 180 days. Sequencing of the barcodes allowed us to determine what genes support water survival and what genes hinder water survival. Water microcosms were set up with mutant libraries of three different species of Ralstonia: R. solanacearum IBSBF1503, R. pseudosolanacearum GMI1000, and R. syzygii PSI07. Of these strains, our preliminary analysis has identified 213 (IBSBF1503), 160 (GMI1000), or 92 (PSI07) genes that support water survival. Several of these genes are known or putatively known to be involved in gene regulation pathways. Targeted knockout mutants of several genes were made and used to inoculate water microcosms to confirm that the genes support water survival. These knockout mutants were GMI1000 ΔphaR (polyhydroxybutyrate metabolism regulation), R. solanacearum K60 ΔrpoS (stress response regulation), and GMI1000 ΔphcA (quorum sensing master regulator). Each targeted knockout mutant had decreased culturability after water survival compared to the wild type. The difference in culturability depended on time and the growth stage of the cells used to inoculate the water microcosms. Experiments are also ongoing to validate the fitness defects of ΔphoU, ΔprrAB, and ΔRS_RS10425 (putative sigma factor) mutants. Our work can help strengthen the foundation for the development of managing bacterial wilt pathogens as little is known about the biology that supports the water survival of Ralstonia.

Characterization of diverse chlorhexidine efflux pumps using multiplexed functional metagenomic Barcoded Tagmentation (BarTa) libraries

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Background: The COVID-19 pandemic saw an increased use of disinfectants and their extensive use alongside other biocides may select for antibiotic cross resistance, particularly through the expression of efflux pumps with broad substrate repertoires, which poses a public health concern. It is resource-intensive to identify which compounds act as substrates for diverse efflux pumps in various environments by sequence homology alone. This can be overcome using sequence naïve functional metagenomic selections in which metagenomic DNA is shotgun cloned into an expression host, optimally resulting in the host organism gaining phenotypes corresponding to expression of each potential gene from a given microbiome.

Methods: We used functional metagenomic selections to identify efflux pumps that confer decreased sensitivity to the disinfectant chlorhexidine and investigated their ability to confer antibiotic resistance as well. To do so for multiple source samples simultaneously, we modified our METa assembly method for making functional metagenomic libraries to include barcoding of input metagenomic DNA during the tagmentation process to prepare Barcode Tagmentation (BarTa) assembly libraries.

Results and discussion: This method allowed us to prepare three functional metagenomic libraries containing metagenomic DNA from across 44 sources and to successfully demultiplex >95% of reads from each source following selection for chlorhexidine resistance. These selections captured genes for both known antimicrobial efflux pumps as well as transporter genes not previously associated with antimicrobial resistance, some of which also resulted in significant decreases in tetracycline, ciprofloxacin, and colistin susceptibility. Multiplexed functional metagenomic libraries have the potential to greatly decrease the per library time and resource costs of functional metagenomics. Application of this approach will facilitate the exploration and comparison of antibiotic resistance genes from different environments.

Eukaryotic metagenome-assembled genomes recovered from seagrass leaves include a novel chytrid in the order Lobulomycetales

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Background: Fungi play pivotal roles in terrestrial ecosystems as decomposers, pathogens, and endophytes, yet their significance in marine environments is often understudied. Seagrasses, as globally distributed marine flowering plants, have critical ecological functions, but knowledge about their associated fungal communities remains relatively limited. Previous amplicon surveys of the fungal community associated with the seagrass, Zostera marina (ZM) have revealed an abundance of potentially novel chytrids.

Methods: In this study, we employed deep metagenomic sequencing to extract metagenome-assembled genomes (MAGs) from these chytrids and other microbial eukaryotes associated with ZM leaves.

Results: Our efforts resulted in the recovery of five eukaryotic MAGs, including a single fungal MAG in the Chytridiomycota (75% BUSCO completeness), three MAGs representing diatoms in the Bacillariophyta (95%, 88% and 44% BUSCO completeness) and one MAG representing a haptophyte algae in the Prymnesiophyceae (61% BUSCO completeness). Whole-genome phylogenomic assessment of these MAGs suggests they all largely represent under sequenced, and possibly novel eukaryotic lineages. Of particular interest, the chytrid MAG was placed within the order Lobulomycetales, consistent with the identity of the dominant chytrid from previous ZM amplicon survey results. Annotation of this MAG yielded 5,650 gene models of which 77% shared homology to current databases. With-in these gene models, we predicted 121 carbohydrate-active enzymes and 393 secreted proteins (103 cytoplasmic effectors, 30 apoplastic effectors) paving the way for in-depth ecological exploration of the role of this chytrid within the ZM ecosystem. Exploration of orthologs between this MAG and existing Chytridiomycota genomes is currently ongoing and promises further insights into its evolutionary and ecological adaptations.

Discussion: Overall these five eukaryotic MAGs represent substantial genomic novelty and valuable community resources. Ongoing and future work will continue to unravel their evolution and ecology, contributing to a deeper understanding of the roles of fungi and other microbial eukaryotes in the larger seagrass ecosystem.

Alzheimer's and the gut: exploring microbial and metabolic effects of a TREM2 mutation in mice

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Background: Through genome-wide association studies, the microglial gene TREM2 has been associated with Alzheimer's disease (AD) pathology. The MODEL-AD consortium has produced transgenic mice carrying the hypofunctional TREM2R47N mutation, a suitable model to study how the mutation interacts with AD pathogenesis. We thus sought to investigate the impact of TREM2R47N on metabolism and the microbiome in the context of AD.

Methods: Plasma, brain, liver, and cecal samples were collected from B6J (WT), 5xFAD, TREM2, and 5xFAD*TREM2 mice at 4- and 12-months of age. Lipid and polar metabolite abundances were measured in plasma, brain, and liver via targeted LC-MS. Cecal microbiomes were sequenced via shotgun metagenomics. We used multivariate permutational analysis of variance to test the metabolomes and microbiomes for significant differences based on various factors (i.e. genotype, sex, and age) and used fold-change and linear model regression analyses to identify differentially abundant analytes across these factors. We conducted pathway analyses in MetaboAnalyst to identify enriched biochemical pathways.

Results: We found that TREM2R47N induced alterations in amino acid and glycerolipid metabolic pathways, along with central carbon and nucleotide metabolism, in the plasma and liver of 4-month-old mice. TREM2 mutants were also associated with significantly increased 5-methyl-tetrahydrofolate abundances, and decreased triacylgylceride abundances, in the brain. The microbiome analysis is ongoing, and results are expected by the time of this meeting.

Discussion: That the TREM2 mutation can affect the metabolism of tissues outside of the brain suggests that it may have wider physiological effects than previously thought. The observed increase in 5-MTHF in the brains of TREM2 mutant mice indicates that TREM2 may affect folate metabolism and the production of homocysteine and glutathione. We expect that our microbiome analysis and subsequent correlation analyses between differentially abundant metabolites and microbes will further enhance our understanding of the underlying pathological cascade of AD.

Exploring plasmid diversity in soil ecosystems

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Background: Plasmids are extrachromosomal DNA molecules capable of autonomous replication, often carrying accessory genes with advantageous traits to microbial hosts. They can be transferred and impart these traits to new hosts, frequently traversing long phylogenetic distances. Interest in plasmid biology has increased in recent years, with studies uncovering their ecology in microbial populations. However, research has focused on plasmids from clinical or other human-associated environments, consequently, we lack a comprehensive understanding of plasmid diversity in natural environments, such as soil ecosystems, particularly regarding the adaptations and functions driving their persistence. Therefore investigating plasmids in soils can provide insights into the evolutionary and ecological dynamics of soil organisms.

Methods: We retrieved 9,431 previously identified putative plasmids from genomes of soil bacteria and archaea, sourced from PLSDB, Refsoil+ and IMG/PR and 7,549 soil metagenomic and metatranscriptomic datasets from IMG/MER and MGnify. We then used geNomad to identify plasmid sequences and determined broad and specialized functions through comparison against several databases. Additionally, we investigated the conjugative capacity and putative hosts of these plasmids, and potential conflicts through CRISPR arrays.

Results: In total we identified 8,810 putative plasmids from genomes and 98,728 from metagenomes, which were dereplicated into 98,281 plasmids and clustered into 57,265 groups. Even though we describe a great amount of novel sequences, determined through phylogenetic gain, we do not saturate diversity. We identified enriched functions distinguishing isolate and metagenomic plasmids, as well as specific Pfams and KOs differentiating plasmids from different soil ecosystems. Plasmids encode several specialized functions related to BGCs and AMRs, with metagenomic plasmids exhibiting more stress-related resistances.

Conclusion: This work provides a comprehensive overview of plasmids in soil environments, revealing key functions exclusive or enriched in certain soil ecosystems.

Crimson and Clover, Over and Over: The Phylogenetics of Methylobacteria in Trifolium hybridum's Rhizosphere

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With a rapidly changing climate, the effects of symbiotic bacteria and fungi on plant fitness and plant yield have become a new focus in both natural and agricultural populations. Yet many questions remain about even common and important members of the plant microbiome and the taxonomic scale at which they vary across plant compartments. Legumes are critical crops, cover crops, and members of natural communities, famously associating with nitrogen-fixing Rhizobium but also forming symbiotic relationships with other common taxa including bacteria in the genus Methylobacterium. Methylobacterium, known for their iconic red pigment and highly variable effects on plant growth and fitness, is associated with the phyllosphere and rhizosphere alike. We have developed a culture collection of more than 300 Methylobacterium strains collected from a long-term nitrogen experiment at Kellogg Biological Station, which has tested the effect of fertilization on plant communities for more than 30 years in order to investigate the implications of modern agricultural practices on Methylobacterium phylogenetics. We observed four distinct morphotypes evenly distributed across both nitrogen addition and control plots, as well as compartment. To follow up on this observation, we sequenced the 16S rRNA of a subset of these strains (n=90). Using these data sets, we hope to more deeply understand the eco-evolutionary implications of long-term nitrogen addition on communities of Methylobacterium associated with clover.

Bacteriophage isolation and characterization from IBD fecal samples

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Introduction

The gut microbiome plays an integral role in our health. Disruptions to this system can lead to diseases such as inflammatory bowel disease (IBD) which encompasses two conditions, Crohn's disease (CD) and ulcerative colitis (UC). IBD is characterized by inflammation of the gastrointestinal tract. These diseases are correlated with a decrease in the microbial diversity and an increase in tailed bacteriophages (phages) in the gut. It’s likely that phages modulate the gut bacterial population, but little is known about how the viromes impacts IBD.

Methods

We have a collection of 1186 fecal samples, with many longitudinal series, from 352 patients, taken throughout IBD progression. Each sample has a full virome and a corresponding 16S metagenome. We developed a method to enrich for and isolate phages in aerobic and anaerobic microbes from the fecal samples. We are also able to track phage contigs bioinformatically through the longitudinal sample series.

Results

We hypothesize that phages play a vital role in remodeling the gut microbiome, and thus have an incompletely understood role in IBD. We seek to understand these dynamics by coupling wet lab and genomics methods. So far, we have seen Enterococcus and Enterocloster phage enrichments lyse recipient bacteria. From these enrichments, DNA was isolated and sequenced, allowing us to track phage contigs in multiple patient sample viromes. Most of these phages are temperate and may encode lysogenic conversion factors that mediate interactions with host’s tissues and with other members of the gut microbiome.

Discussion

We hope to better define the role of viruses in disease progression by tracking viral contigs in fecal samples. Our goal is to identify which phages are associated with disease, and the mechanism(s) by which they promote disease or protect from it.

Eco- and Evolutionary-Genomics of Freshwater Cyanobacteria Populations

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Climate change, agricultural runoff, and species invasions have caused multiple disruptive ecological shocks in Lake Mendota, a freshwater eutrophic lake. The lake suffers from noxious cyanobacterial blooms that produce toxins. The ability to comprehend and predict blooms depends on a better understanding of cyanobacteria ecology and evolution. Cyanobacteria are an ancient and diverse phylum of bacteria that are ubiquitous globally with community composition varying by environment. Recent work suggests that genomes within defined bacterial species have a high amount of genomic diversity in the wild, where ecological and evolutionary processes intersect. Using cyanobacterial genomes recovered from a metagenomic time series spanning 20 years of sampling in Lake Mendota, we have the unique opportunity to investigate the mechanisms maintaining this diversity. A total of 125 curated cyanobacterial genomes were recovered from the metagenomes and used to map reads in the 464 metagenome samples. We found that an Aphanizomenon genome had the highest coverage across the dataset by far, and the second highest mean coverage. Microcystis populations had markedly higher microdiversity across the genome. We examined the population-level diversity of the cyanobacteria through time to ask whether new strains have emerged over the 20 years. Preliminary results indicate that low frequency genes with high variability in the populations, indicative of the flexible or cloud genome, are involved with prokaryotic host defense such as phage defense mechanisms and surface structure variants. These findings are important for understanding how variation in genomic content reveals evolutionary dynamics, selective pressures that arise from ecological interactions in populations, and local adaptation in wild bacteria.

Understanding and Engineering P-trap colonization by Enterobacter ludwigii

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Background

People spend over half their time in built environments. In recent years, the healthiness of these environments has attracted increasing attention, with hospitals being a significant focus. This project focuses on hospital sinks, which contain a high abundance of antibiotic-resistant pathogens, and Enterobacter ludwigii (Elu), a common bacterium in sink p-traps. By understanding its Elu's biology and developing engineering tools, we aim to design probiotics for p-traps that reduce the carriage of antibiotic-resistant organisms.

Methods

To understand the metabolic requirements for survival in the sink P-trap, we first tested the growth of three different hospital P-trap derived Elu isolates on media mimicking the P-trap and evaluated their resistance to sodium dodecyl sulfate (SDS). Additionally, we quantified biofilm formation by these strains, sequenced their genomes, and annotated their resistance genes. Finally, we tested a variety of sequences from environmental gram-negative microbes to curate a promoter library for Elu.

Results

Contrary to our expectations, we found that biofilm formation was anti-correlated with SDS resistance, and that the fastest-growing, most SDS-tolerant Elu strains were less prevalent in real p-traps, suggesting that to survive periodic insults of detergent, biofilm formation is highly advantageous.

Discussion

Moving forward, we have built a genomic DNA library to reveal genes and pathways that are critical for Elu's attachment and adhesion to plumbing surfaces. To construct a model Elu system for future studies and applications, we are knocking out its antibiotic resistance genes and integrating genomic barcodes. By integrating biological and engineering research on Elu, we aim to provide a comprehensive example of how to understand bacterial behavior in an unexplored environment and systematically develop a ""probiotic"" for the built environment microbiome.

Identification and Functional Analysis of Predicted Toxins from two strains of Variovorax paradoxus

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Background. Toxin-Antitoxin systems are predicted by genome analysis to be widespread in bacterial genomes. These systems function in a number of roles including virulence, phage defense, and competition. It is possible that some of the predicted toxins are maintained as functional proteins, while others may have drifted into different functions, or have become inactive.

Methods. 44 putative toxins from Variovorax paradoxus strains EPS and VAIC were identified using TASer (https://shiny.bioinformatics.unibe.ch/apps/taser/) which identifies Type I-IV TA system components using curated Hidden Markov Models (HMMs). Predicted toxins fwere cloned into pBBR8k-GFPuv, replacing the fluorescent protein under the control of a pBAD promoter with the toxin of interest. The pemK toxin gene from the pemIK TA pair was used as a positive control, and pBBR8k-GFPuv served as the negative control. Overexpression with arabinose induction was tested using LB medium as well as AB induction medium, and toxin function was examined on solid media and in liquid in comparison to glucose suppressed cultures.

Results. Many of the toxins identified by the prediction software did not show any activity in any of our experiments, although we did see robust toxicity in a substantial fraction. We are currently examining the overall pattern of toxin function to see if TA systems on secondary replicons are more likely to be active than genomic ones.

Discussion. The overexpression analysis allows us to examine toxins in isolation from confounding factors, but is limited to toxins that can impair growth in E. coli. We hope that future studies will be able to be done in Variovorax and that patterns of toxin function will help us understand the extent that they play a role in bacterial growth and function in the environment. These data will also be compared to transcriptomic data collected from these strains.

Does selection for complete genome packaging drive the molecular evolution of packaging interactions in Cystoviruses?

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Background: Reassortment, the swapping of entire genome segments between segmented RNA viruses during coinfection of a host cell, is associated with host switches and increases in pathogenicity and transmissibility. Reassortment occurs during viral genome packaging, so packaging components of coinfecting strains must be compatible for reassortment to occur. Cystoviridae is a family of tri-segmented, dsRNA bacteriophages that infect pseudomonads. Genome packaging in cystoviruses is mediated by binding between the major capsid protein and a noncoding packaging ('pac') region on each segment.

Methods: Here, we use experimental evolution to investigate whether selection for packaging of segments with sub-optimal pac regions can lead to compensatory genetic mutations that alter packaging interactions and genetic exchange. Virions require all segments to replicate, therefore selection to package segments is strong. We generated mutations in pac region of the M segment of the type strain phi6 which were predicted to hinder packaging and used a plasmid reverse genetics system to transfect the host with the mutants. We will experimentally evolve the transfection progeny by passaging, before surveying evolved strain genomes for mutations and determining whether mutations restore packaging and fitness. Lastly, we will investigate whether mutations alter reassortment potential by conducting crosses between the evolved strains or phi6 and other cystoviruses and comparing reassortment rate using Linked-Multilocus Genotype by Sequencing (LMGSeq), a high-throughput method for quantifying reassortment that we developed.

Results: We found that following transfection, one mutant showed a three-order reduction in fitness compared to phi6. Preliminary sequence analysis shows frequent compensatory mutations in pac in the progeny following transfection of this mutant.

Discussion: Sources of variation in reassortment potential of segmented RNA viruses are not fully understood. Our results will provide insight into factors that can influence segment exchange and reassortment rates in RNA viruses more broadly.

Improving identification of strain-sharing for diverse bacterial species in Kenyan children stool microbiomes

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Background: The WASH B trial explored the impact of water, sanitation, and hygiene (WASH) on the welfare of Kenyan children, including on incidence of infections by enteric pathogens, which can be transmitted in community and household settings. We sought to explore the impact of WASH on bacterial strain-sharing more widely using stool metagenomes and StrainGE, a tool suite we developed to identify low abundance strains from complex community samples. While StrainGE had been used to track E. coli strains across human ecologies, it had not been optimized for a broader range of species.

Methods: We used a combination of synthetic spiked metagenomes, longitudinal metagenomic datasets of human stool samples, and machine learning models to establish the metrics and thresholds required for StrainGE to identify instances of strain-sharing between human gut metagenomes for a wide set of species.

Results: We found that the metrics used to establish strain-sharing thresholds for E. coli were not universally effective across various bacterial species. Testing on spiked metagenomes revealed that identifying strain-sharing in samples with conspecific strains was particularly challenging. We demonstrated that training a machine learning model on a broad set of StrainGE metrics improved performance and enabled identification of strain-sharing across multiple bacterial genera. In ongoing analysis, we have observed that WASH resulted in a significantly lower strain-sharing rate between households than a control treatment for Escherichia and Enterococcus strains.

Discussion: We illustrate the challenges of identifying strain-sharing for a broad range of species in metagenomic samples, particularly when these samples contain conspecific strains. We propose an improved solution utilizing a machine learning approach that is revealing new perspectives on the impact of WASH on the spread of microbes in a Kenyan rural setting, and holds promise for investigating strain sharing in other complex community settings.

Unveiling Extremophiles: Exploring Protist Diversity in Canadian Alkaline Soda Lakes with Metabarcoding

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Background: In Canada, the Cariboo Plateau region is home to a multitude of evaporative alkaline soda lakes. These lakes are an “extreme” environment, with high concentrations of salts and an average pH of over 10. Despite the extreme abiotic conditions, alkaline soda lakes have exceptionally high levels of primary productivity and organismal abundance and diversity. These soda lake communities are most heavily represented by filamentous cyanobacterial species. Many other species exist in these lakes, across all three domains of life – Bacteria, Archaea, and Eukarya. Microbial eukaryote species, or protists, have been historically overlooked due in part to the assumption that protists lack cellular adaptations to survive in extreme conditions.

Methods: We have conducted a metabarcoding study to identify the diversity of protist species present in three Canadian soda lakes: Good Enough Lake, Probe Lake, and Deer Lake. Metabarcoding was conducted using the 18S rRNA gene with the Illumina Miseq platform. Sequencing data was processed through the DADA2 pipeline with resulting amplicon sequencing variants (ASVs) compared against the Protist Ribosomal Reference (PR2) database.

Results: Taxonomic classification identified a diverse range of protists present in these three Canadian alkaline soda lakes. This study identified over 1,500 ASVs across over 100 different families within the Eukaryota domain. Protistan taxonomic groups were heavily represented in this metabarcoding study, however, animal groups including crustaceans and hexapods were also identified. Approximately 300 ASVs were categorized within the domain Eukaryota with no further taxonomic rank.

Discussion: The findings from this study suggest that Canadian alkaline soda lakes host a high diversity of protistan life. Further, this suggests that protist species have the potential to adapt and thrive in extreme abiotic conditions. This study also suggests there may be a high abundance of novel protistan species waiting to be investigated further.

Decoding Bacterial Transcriptomic Profiles and Functional Responses to Enhancing Efficacy of Phage Activity

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Background: Stenotrophomonas maltophilia (STM) is a recalcitrant Cystic Fibrosis (CF) pathogen that is incredibly difficult to eradicate from CF lungs due to an arsenal of antimicrobial resistance (AMR) mechanisms. Phage therapy—using viruses to eradicate bacteria—offers an alternative approach to treating AMR infections. However, phage resistance has been well documented, demonstrating the bacteria's ability to modulate cell wall structures and employ anti-phage defense systems. Thus, investigating phage-bacterial interactions is vital to mitigating potential risks and identifying key synergistic targets. We hypothesize that gene expression profiles and mutational changes of time-dependent phage-infected bacteria unveil host defense mechanisms and coevolution responses that can be used at synergistic targets.

Methods: RNAseq was performed on time points based on the replication cycle of phage SBP2ϕ2, previously identified using the one-step growth curve. STM was grown to log phase (OD 0.3) and incubated at 37°C, shaking (200 rpm) with either phage SBP2ϕ2 (MOI 10) or SM Buffer (control). Samples were taken at 0, 10, 40, 70, and 110 minutes post phage exposure, with additional 5 and 18 hours of sampling to assess functional changes in bacteria.

Results: For phage SBP2ϕ2, early genes were expressed at 10 minutes, and late genes after 40 minutes. Principal coordinate analysis shows high consistency among biological replicates and significant variation across time points, highlighting the precision and accuracy of the wet lab. Functional assays revealed decreased swim motility and increased biofilm formation. After 110 minutes of phage exposure, we observed modulation of antibiotic sensitivity to two antibiotics that target cell wall synthesis alongside the emergence of phage resistance.

Discussion: This project aims to identify novel synergistic targets associated with phage infection, which will be used to exploit bacterial host vulnerabilities and enhance lytic phage activity. Additionally, we seek to answer fundamental questions about the intricate interaction and co-evolutionary relationship between phage SBP2ϕ2 and STM.

Molecular epidemiology of REPEXH01: a persistent strain of Escherichia coli O157:H7 associated with multiple sources, including leafy greens

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BACKGROUND
Shiga toxin-producing Escherichia coli (STEC) outbreaks cause more than 265,000 infections and cost $280 million annually. This study investigated REPEXH01: a persistent strain of STEC O157:H7 associated with multiple sources, including romaine lettuce and recreational water, that has caused multiple outbreaks since its emergence in late 2015.

METHODS
We applied a variety of computational methods including machine learning, time-calibrated phylogenetics, comparative genomics, and molecular epidemiology to thousands of STEC O157:H7 genomes. We also investigated the antimicrobial resistance determinants, phage content, and the distribution of plasmids in STEC O157:H7.

RESULTS
By comparing the genomes of 729 REPEXH01 isolates to those of 2,027 other STEC O157:H7 isolates, a highly-conserved, single base-pair deletion in espW that was strongly linked ($p < 10-323$) to REPEXH01 membership was identified. Antimicrobial resistance determinants were present in nearly 100 % of REPEXH01 genomes. All REPEXH01 isolates belonged to Manning Clade 8 and nearly all genomes possessed stx2a. Additional molecular markers were identified using machine learning approaches such as random forest and logistic regression models.

DISCUSSION
REPEHX01 belongs to E. coli O157:H7 Manning Clade 8 and most isolates possess stx2a which are associated with more severe outcomes. These factors in addition to harboring multiple antimicrobial resistance determinants underscore the continued need to monitor this strain and understand factors contributing to its emergence and persistence. For example, the single base pair deletion in the espW virulence gene can be useful both as a genomic signature of this strain and as a target for future research to understand the evolution of this strain. Additional research targeting the role of the significance of this mutation on the colonization and survival on leafy vegetables may yield important insights.