Genome-wide search for host range mechanisms for generalist plant pathogens

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Background: The bacterial wilt pathogens in the Ralstonia solanacearum species complex are generalist plant pathogens that impact food security in the global tropics. Our central question is: what are the genetic factors that limit or expand phytopathogen host range? Based on global impact and well-documented epidemiological studies, we are developing the Ralstonia phylotype IIB-4 strains into a model clade to decipher host range mechanisms.

Methods: We collected more than 19 Ralstonia IIB-4 strains that were isolated from diverse locations/epidemics: Martinique Island, Colombia, Peru, Brazil, and ornamentals imported into Florida. We quantified virulence of each strain on four plant species that are natural hosts for some-but-not all IIB-4 Ralstonia: tomato cv. Moneymaker, melon cv. Sweet Granite, impatiens cv. Beacon Orange, and banana cv. Dwarf Cavendish. We used Illumina sequencing to assemble draft genomes of 12 new strains and inferred phylogeny of our 19 strains and other publicly available IIB-4 genomes. We used SCOARY to investigate whether presence/absence of any genes was associated with the level of virulence on the different host plants.

Results: Phylogenetic analysis revealed that there are at least 5 subclades of IIB-4 Ralstonia. The patterns of virulence correlated with phylogeny. SCOARY did not reveal obvious candidates.

Discussion: SCOARY works best with a larger dataset. We have sequenced more Ralstonia isolates from our collection and identified additional IIB-4 strains. Virulence assays on these new strains are ongoing. Additionally, because IIB-4 strains are reported to break genetic resistance of tomato cv. H7996, we are phenotyping all strains on this tomato line as well.

Investigating growth mechanisms required by xylem-invading bacteria under varying levels of plant defense responses

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Background: Ralstonia spp. are the world's second most devastating phytobacteria, causing severe crop yield losses. Breeding for resistance to these bacteria is an important means of disease control. However, pathogen virulence evolves constantly, overcoming crop resistance. Here we investigate the genetic factors that benefit or hinder bacterial growth under varying levels of plant defense responses. We hypothesize that bacteria use common and unique genetic mechanisms to grow under varying levels of plant defense responses.

Methods: To explore mechanisms benefitting or hindering bacterial fitness inside the host, we are using a powerful functional genomics approach called random barcoded transposon mutant sequencing (RB-TnSeq). This approach can quantify the fitness of thousands of mutants during competitive growth in complex naturalistic environments. First, we screened tomato cultivars for their levels of quantitative disease response to Ralstonia sp. Second, we inoculated tomato cultivars with the RB-TnSeq library. Using Illumina sequencing, we quantified the change in the relative abundance of bacterial mutants before and after growth in eight different tomato cultivars. This quantification allows us to identify genetic factors that benefit or hinder bacterial growth in the selective environment inside the plant host.

Results: Analysis of the relative abundance of bacterial mutants in selective conditions reveals that Ralstonia sp. use common and unique genetic mechanisms under varying levels of plant defense pressure.

Discussion: Ongoing analysis of additional replicates of RB-TnSeq experiments in the Ralstonia-tomato model system will help us unravel genetic mechanisms that benefit or hinder bacterial growth under varying levels of plant defense responses. Our next step is to validate these mechanisms and understand their functions under the pressure of plant defense responses.

Global map of evolutionary dependencies between antibiotic resistance and virulence genes in E. coli

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Genes conferring antibiotic resistance or virulence phenotypes frequently undergo horizontal gene transfer in bacteria, contributing to the emergence of new multidrug resistant pathogenic variants. Mounting evidence indicates that pre-existing genome content variations influence the successful acquisition of such genes. However, the underlying evolutionary dependencies among specific genes, i.e. when one gene facilitates or hinders the acquisition of a second gene, remain poorly understood. Here we chart a high-resolution map of evolutionary dependencies between resistance and virulence genes by phylogenetic analysis of more than 20,000 Escherichia coli genomes. Our map reveals that (1) resistance genes generally facilitate each other's gain; (2) key virulence genes lack such a general pattern; and (3) contrary to some previous results, there is no overall negative dependency between the acquisitions of key virulence and resistance genes, indicating largely independent evolution between these two traits in E. coli. Strikingly, we found that the presence of efflux pump resistance genes in a genome strongly increases the chance of acquiring various other classes of resistance genes, making these efflux pumps an indicator of potentially emerging new multidrug resistant strains.

Pesticide stress induces rapid ecological changes in bacterioplankton species and gene composition without consistent evolutionary changes

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Background: Evolutionary changes depend on shifts of heritable traits and are traditionally considered slow compared to ecological changes, related to shifts in species abundance. Nevertheless these two processes may overlap, particularly in bacteria, that exhibit short generation time and high mutation rates. Here, we studied the ecological and evolutionary responses of freshwater bacterial communities to pesticide contamination. We hypothesized that ecological responses, such as increase in abundance of resistant organisms, would be accompanied by similar evolutionary changes within populations, as ecologically successful organisms might have experienced adaptive evolution involving genome-wide selective sweeps.

Methods: We designed a 8-week experiment to study the intersection of ecology, genetic and evolutionary responses of bacteria exposed to herbicide contamination. 1,000L freshwater mesocosms were treated with different concentrations of a glyphosate-based herbicide (GBH) and water samples were filtered at 11 timepoints for DNA extraction. To test ecological hypotheses, we assessed taxonomic diversity through 16S rRNA amplicon sequencing and functional diversity by measuring the use of organic carbon sources with EcoPlates. To test evolutionary hypotheses, we performed shotgun metagenomic sequencing, reconstructed metagenome-assembled genomes, annotated them for the presence of known functional genes and inferred single nucleotide variants within genomes.

Results and Discussion: Community composition shifted primarily in response to GBH treatments without affecting carbon substrate utilization, suggesting functional redundancy for this trait. GBH also selected (directly or indirectly) for an increase in antimicrobial resistance genes encoding efflux pumps, and species abundance after a high GBH pulse were better predicted based on efflux pumps than the presence of known glyphosate resistance mutations in the target enzyme. Finally, population genomic analyses showed that intra-specific diversity varied idiosyncratically across populations that reacted similarly to GBH, showing that ecological changes occurring over short timescales are not always accompanied with directional evolutionary changes.

Impact of antibiotic regimens for the treatment and prevention of travelers' diarrhea

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Background: International travel, travelers' diarrhea (TD), and antibiotic use disrupt the healthy gut microbiome and increase risk for colonization by antibiotic resistant organisms. Two recent clinical trials evaluated the efficacy of three single-dose antibiotics to treat (TrEAT TD), or once or twice daily prophylaxis to prevent (PREVENT TD) TD on deployed military personnel. While these trials showed the regimens are comparable at treating or preventing TD, we hypothesized they may have different adverse impacts on the gut microbiome and resistome.

Methods: To evaluate this, we performed shotgun metagenomic and whole genome sequencing on 424 fecal samples and 54 diarrheagenic E. coli isolates collected from 167 military personnel traveling from the US/UK to Kenya/Honduras.

Results: We observed no significant difference in microbiome richness or ARG abundance between TrEAT groups, but note that TrEAT subjects had low gut microbiome richnesses which remained low for up to 3 weeks post-diarrhea. For the PREVENT cohort, we found the twice-daily arm was associated with decreased microbiome richness and increased ARG abundance post-travel. However, E. coli relative abundance increased 3.8-fold in the placebo group. Lastly, we observed antibiotic-resistant E. coli persisting within subjects and spreading to other as far as 10 months apart, and identify non-pathotyped E. coli as potent reservoirs ARGs.

Discussion: In this study we demonstrate the risks and benefits of antibiotics for the treatment and prevention of TD. We show that different antibiotic treatments are comparable in their impact on the microbiome, but all result in a lower microbiome richness that does not recover. We also show that twice-daily antibiotic prophylaxis to prevent TD significantly disrupts the microbiome, but placebo had increased risk for acquisition of diarrheagenic E. coli. These findings can be used to inform treatment guidelines for future travelers.

Assessing Microbial DNA in Blood: Does Fraction Matter?

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Introduction: Distinct bacterial populations have been discovered in the bloodstream of prostate cancer (PC) patients, which may be indicative of disease severity. The majority of studies have relied on plasma as a proxy for blood, even though other components of blood may also have microbial DNA. We sought to determine the microbial load and composition in each fraction of blood (plasma, buffy coat, red blood cells) in non-cancer compared to PC patients.

Methods: Blood was drawn from men with high-grade PC, low-grade PC, and without cancer (n=5 per group). Samples were processed in triplicate and aliquoted into plasma, buffy coat, red blood cell pellet (RBC) and whole blood prior to freezing. Negative controls and a mock microbial dilution series were processed alongside blood samples. Microbial DNA was extracted using the QiAMP DNA mini kit. Droplet-digital PCR was used to measure 16S rRNA copies and microbial DNA composition was determined using synthetic long read 16S rRNA gene sequencing (Loop Genomics).

Results: Plasma had the lowest copies of 16S rRNA genes with a median of 21.3 copies/μL (interquartile range [IQR] of 112.5 copies/μL), with RBCs having slightly more (median 38.7, IQR 135.9 copies/μL). Buffy coat followed RBCs (median 104.72, IQR 178.9 copies/μL), and as expected, whole blood had the highest (median 134.9, IQR 153.4 copies/μL). Compositionally, Actinobacteria was the most abundant phylum followed by Firmicutes and Proteobacteria. Initial sequencing demonstrated that whole blood had the most observed species (median 10, range 9-10) and plasma had the least (median 5, range 5-6).

Discussion: The results from our study indicate plasma contains the least amount of microbial DNA and fewest number of species, indicating a potential limitation of prior work. Future directions include evaluating the compositional differences in the fractions and identifying associations with disease status.

Genome wide reconstruction of gene family evolution in microbes using xenoGI and the DTLOR model

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Background: The evolution of gene families is a key part of how microbes evolve and adapt to new niches. Duplication, transfer, loss (DTL) based reconciliation algorithms are an important method for understanding gene family evolution. Such methods take a species tree and a gene tree as input and reconcile them to reconstruct the history of events such as duplications and losses. One deficiency of existing DTL methods is that they do not account for the fact that members of a gene family may enter the species tree multiple times.

Methods: We have developed a new DTL based model called DTLOR. In order to allow multiple entries into the species tree we introduce two new events, O (origin) and R (rearrangement). We also keep track of the syntentic location of gene families. A python implementation of this model is included in the xenoGI software package and can be applied to all the gene families in a given clade of microbes.

Results: We used simulations to assess the effectiveness of xenoGI at reconstructing the evolution of gene families. Precision and recall values suggest that effectiveness varies across events. For example, the highest precision values (in the 90s) are for origin and duplication events and the lowest are for transfer and rearrangement events (in the 30s). We will also present some example cases identified in enteric bacteria.

Discussion: Analysis of simulation output and application to real data suggests that DTLOR as implemented in xenoGI is effective at reconstructing gene family evolution in clades of closely related microbes.

Genomic Analyses of Longitudinal Mycobacterium abscessus Isolates in a Multi-Center Cohort Reveal Signatures of In-Host Parallel Adaptation

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Background: Nontuberculous mycobacteria (NTM) are ubiquitous in the environment and are increasingly causing opportunistic infections. Mycobacterium abscessus (MAB) is one of the major NTM lung pathogens which disproportionately affect patients with cystic fibrosis (CF). MAB often causes chronic lung infections spanning years with low rates of treatment success. Understanding in-host adaptive behavior of MAB will inform strategic treatment development.

Methods: We leveraged a cohort of 175 longitudinal isolates from 30 patients with MAB lung infection in two hospital centers to identify genomic correlates of in-host adaptation. Utilizing whole genome sequencing, we characterized the relatedness of isolates both within our cohort and in the broader global context of MAB genomes. We then further investigated genes undergoing parallel adaptation in the host lung environment. Finally, we test the phenotypic consequences of parallel mutations by conducting antibiotic resistance and mercury resistance assays.

Results: We found highly related isolate pairs (<10 single nucleotide polymorphisms) across hospital centers with low likelihood of transmission. We further annotate non-random parallel mutations in 23 genes, and demonstrate altered macrolide susceptibility co-occurring with a nonsynonymous whiB1

mutation. Finally, we highlight a 23kb mercury resistance plasmid whose loss in the host confers phenotypic susceptibility to organic and non-organic mercury compounds.

Discussion: Here we highlight genomic processes through which MAB is adapting to promote its own survival within the host. Many of these events occur in parallel across patients and hospital sites. In the absence of evidence of recent transmission, we suggest highly infectious strains of MAB exhibit low rates of mutation. Further, the within-lineage polymorphisms we observed have phenotypic effects, potentially benefiting fitness in the host, at the detriment of environmental survival. Our study provides novel insight into within-host behavior of MAB infections, and highlights evidence of parallel adaptation to a pathogen lifestyle.

Use of recombinant bacteria with unique tags as spike-in controls for the quantification of microbiome content

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Advanced sequencing and bioinformatics technologies have revolutionized microbiome research in remarkable ways, opening up applications in diagnostics, therapeutics, and environmental sciences. Despite the promise of these technologies, the analysis of metagenomic data remains challenging due to the technical biases introduced throughout the metagenomics workflow—from sample preparation to bioinformatic analysis. Further, the natural complexity of microbial communities themselves has challenged microbiome researchers in their ability to make meaningful, quantifiable, reproducible, and comparable measurements across different laboratories. To help promote assay standardization and validation, ATCC has developed innovative spike-in standards for microbiome research. These controls are prepared as whole cell or nucleic acid mixtures comprising three genetically engineered bacterial strains (derived from Escherichia coli, Staphylococcus aureus, and Clostridium perfringens), each containing a unique synthetic DNA tag that can be detected and quantified in routine 16S rRNA gene amplicon and shotgun sequencing assays. To demonstrate the utility of these spike-in controls in microbiome research, we conducted studies where we mixed them with whole-cell or gDNA mock communities containing different bacterial strains at various ratios. The resulting data showed that the unique tags of all three bacteria were identifiable and quantifiable by shotgun and 16S rRNA amplicon sequencing. These proof-of-concept experiments support the utility of using spike-in controls with a unique 16S rRNA tag to monitor the full process from DNA extraction to data analysis of a microbiome workflow for both 16S rRNA and shotgun metagenomics assays.

Mutation rates and adaptive variation among the clinically dominant clusters of Mycobacterium abscessus

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Mycobacterium abscessus (Mab) is a multi-drug resistant pathogen increasingly responsible for severe pulmonary infections. Analysis of whole genome sequences (WGS) of Mab demonstrates dense genetic clustering of clinical isolates collected from¬ disparate geographic locations. This has been interpreted as supporting patient-to-patient transmission, but epidemiological studies have contradicted this interpretation. Here we present evidence for a slowing of the Mab molecular clock rate coincident with the emergence of phylogenetic clusters. We find that clustered isolates are enriched in mutations affecting DNA repair machinery and have lower spontaneous mutation rates in vitro. We propose that Mab adaptation to the host environment through variation in DNA repair genes affects the organism’s mutation rate and that this manifests as phylogenetic clustering. These results inform our understanding of niche switching for facultative pathogens, and challenge the model of transmission as the major mode of dissemination of clinically dominant Mab clusters.

Identifying genes associated with aerobic and anaerobic growth in S. enterica and non-model E. coli strains

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The response to changing oxygen availability is well studied in Escherichia coli K-12, but the extent that this understanding can be applied to other facultative anaerobes is unclear. Here we investigate the genomic variation across 182 isolates of Escherichia and Salmonella and use a pan-genome association study to link genetic variations to growth rates in aerobic and anaerobic conditions. This study will give us a set of genes predicted to be responsible for the oxygen response in our strains. E. coli and Salmonella strains were grown in technical triplicate in MOPS minimal media with 20% glucose for at least ten hours. Optical density measurements were done every 16 minutes with continuous shaking. Genomes were sequenced with the NovaSeq6000 system with read lengths of 2 x 150bp. Ordered contigs were then annotated using custom scripts that combined results from various gene prediction tools. Orthologous genes were identified using OrthoMCL and BLASTP and the pangenome was constructed using the Roary software. A gene presence/absence table and the growth rates were input into the Scoary2 software to associate orthogenes to the growth rates in aerobic and anaerobic conditions. Clusters of orthologous genes were used to categorize the associated genes. About 23% of the genes predicted to be associated with growth rates in both conditions were metabolism-related; additionally, ~20% were genes of unknown function. Interestingly, we identified a pattern in some subclades with notable differences in their associated gene presence and absence compared to the rest of our strains. These results give us insight into genes predicted to be responsible for the oxygen response and will also help us select a subset of strains for further analysis, including using RNA-seq. The genes of unknown function with predicted association may now also be targets for further study.

Breaking the fourth wall on meta-analysis in microbiome data

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Background: Establishing a common understanding of specific terms is key to successful team science and an important process for transdisciplinary fields. Microbiome analysis draws on established disciplines including microbiology, epidemiology, computer science, ecology, statistics, genetics, oceanography, geology, psychology, and chemistry among others. As a result, the interpretation of specific terms may reflect a research group's primary disciplinary background. One example of this problem is the definition of the term, "meta-analysis".

Methods and Results: Within medical and epidemiologic research, a "meta-analysis" typically accompanies a "systematic review" and refers to a synthesis exercise that relies on a systematic, structured search of the literature, team-based extraction of study summary statistics, and risk-of-bias ratings to draw a conclusion based on all available evidence. However, within microbiome research, "meta-analysis" describes combination of individual-level data from multiple sources, where "different" may be defined as multiple geographic locations by a single research group; the use of a secondary convenience dataset to provide contextualization for primary results; or a systematic search process and data extraction.

Discussion: Given the ambiguity of this term, we invite the microbiome research community to comment on how they define meta-analysis. We hope this discussion becomes a catalyst to create a consistent definition across the research field.

Fully-automated library preparation for long read WGS technologies on a novel digital microfluidics system

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Long-read sequencing has come to play a vital role in generating contiguous, quality genomes to be used for a multitude of applications such as detection of large structural variants, deep coverage of long repeat regions, microbial community studies, and de novo assemblies. Because they do not include PCR amplification, long read libraries are able to avoid a common source of base composition bias in sequencing data. Protocols from Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) have been growing in popularity in academic and clinical settings, though the manual preparation of these libraries requires hours of benchtop work and can be complicated to complete. Miro Canvas is a digital microfluidics platform that offers a low-throughput, small-footprint automation solution for long and complex library preparation protocols. The Canvas is a true walkaway technology, minimizing the hands-on time required for library prep to reagent preparation and instrument setup. PacBio SMRTbell Express Template Prep 3.0 and ONT Genomic DNA by Ligation are fully automated on Miro Canvas, and can construct libraries from 1-5 μg of high molecular weight DNA input. Key metrics such as read length distribution, read quality, number of reads, read length N50, and variant detection are comparable between the Miro Canvas protocols and their manual counterparts. Additionally, the Canvas protocols include bead-based size selection, reduction in reagent volumes by up to 75%, and the small footprint of the system pairs well with portable ONT sequencers. Long read technologies on Miro Canvas consistently demonstrate high-quality results with the added benefit of simplified protocol setup in a fraction of the hands-on time required for the completion of benchtop protocols.

Gut Microbial Correlates of Serious Bacterial Infections in Febrile Term Infants

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Background: Infants in the first 60 days of life (DOL) are highly susceptible to serious bacterial infections (SBI), consisting of urinary tract infections (UTIs), bacteremia, and meningitis, yet they remain challenging to diagnose and prevent. The gut microbiome and its association with SBI in term infants remains understudied. We sought to determine if there are specific gut microbial correlates of SBI.

Methods: We studied a group of febrile term infants <60 DOL who presented to 14 US emergency departments and were enrolled in the Pediatric Emergency Care Applied Research Network (PECARN) Biosignatures II study. 40 infants with SBI and 80 infants without documented SBI were chosen, and shotgun sequencing was performed of the gut metagenome and of cultured isolates from the gut and extraintestinal site (blood, urine, cerebrospinal fluid). We used Metaphlan3 to profile taxonomic composition and MaAsLin2 to perform generalized linear mixed modeling. InStrain was used for detection of the extraintestinal pathogen within the gut metagenome.

Results: No significant differences were found in gut microbiome alpha or beta diversity and enterotype classification between groups. Escherichia coli abundance in the gut was greater for infants with UTI caused by E. coli as determined by MaAsLin2 (p-value<0.001). We recovered an isolate from the gut that matched the SBI isolate in 57.5% of cases (23/40), based on species identification and multi-locus sequence typing and serotyping. A strain isogenic to the pathogen was detected in the gut of two additional cases (population average nucleotide identity >99.99%, breadth>0.5) by InStrain.

Discussion: While community signatures of SBI were not apparent, analyzing both the gut metagenome and cultured isolates allowed detection of the pathogen in the gut in more than half of infants with SBI. These findings prompt consideration of new opportunities for surveillance to prevent SBIs among colonized, pre-symptomatic infants.

Intensive care unit water sources are persistently colonized with multi-drug resistant bacteria and are the site of extensive horizontal gene transfer of antibiotic resistance genes

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Background: Contamination of hospital sinks with microbial pathogens presents a serious threat to patients, but our understanding of colonization dynamics is largely based on outbreaks. In a previous survey of intensive care unit (ICU) surfaces, we observed extensive contamination by both common nosocomial pathogens and opportunistic, water-associated pathogens. This led us to hypothesize that water sources were acting as a reservoir for these environmental multidrug-resistant organisms (MDROs). Here, we investigate the colonization patterns of MDROs in ICU sinks and water from two hospitals in the United States and Pakistan collected over 27 months. We additionally collected samples from geographically-matched rooms in homes and shared office spaces.

Methods: We used selective culture and isolation to recover 822 bacterial isolates, which were subjected to Illumina sequencing and antimicrobial susceptibility testing. A subset of 60 were additionally sequenced using Oxford Nanopore long-read technology.

Results: ICU water sources in both countries had a high burden of Pseudomonas, Acinetobacter, and Stenotrophomonas spp. Genomic analyses revealed long-term colonization by Pseudomonas spp. and Serratia marcescens strains across multiple rooms for more than two years. Isolates recovered from ICU rooms were enriched in antibiotic resistance gene (ARG) abundance and diversity compared to home and work rooms. Nanopore sequencing uncovered

extensive cross-species horizontal gene transfer (HGT), predominately within Enterobacterales. Plasmids that harbor diverse and clinically-important ARGs are shared among multiple Enterobacterales and Acinetobacter species over a period of 19 months, and these plasmids confer phenotypic resistance to multiple beta-lactams, including carbapenems.

Discussion: In this environment, antibiotic resistance in Pseudomonas spp. is maintained by strain colonization, while HGT maintains AR elements within A. junii, A. johnsonii, and Enterobacterales independent of colonization. These results imply that even transient MDRO appearance in these reservoirs poses a risk to patients, and emphasizes the importance of proactive surveillance to prevent infections.

Longitudinal analysis of the microbiome and metabolome in the 5xfAD mouse model of Alzheimer's disease

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Microbial exposures impact the onset and progression of Alzheimer’s disease (AD) through the mediation of inflammation, the exchange of small-molecules across the blood-brain barrier, and stimulation of the vagus nerve. A baseline understanding of AD model animal microbiomes is therefore vital for improving the efficacy of our animal models and improving AD research quality. Here we describe our evaluation of the microbiome and metabolome in longitudinal fecal, cecal, and plasma samples from the 5xfAD transgenic mouse model. We performed DNA extraction and shotgun Illumina sequencing on cecal and fecal samples from 5xfAD and wild-type B6J (WT) animals from 4–18 months of age. We also performed metabolomics on plasma and feces from a subset of the same animals. We observed significant sex, age, and cage-specific differences in the microbiome. Bacteria with significantly higher abundances in 5xfAD mice include multiple Alistipes spp., two Ligilactobacillus spp., and Lactobacillus sp. P38. Those with lower abundances included multiple Turicibacter spp., Lactobacillus johnsonii, and Romboutsia ilealis. In contrast to previous findings of depleted serotonin in persons with AD, plasma measurements revealed elevated serotonin in older 5xfAD animals relative to their WT littermates, although the differences were small in comparison to the large decrease in serotonin associated with aging across models. 5xfAD animals also exhibited significantly lower plasma concentrations of carnosine and the lysophospholipid lysoPC a C18:1. Correlations between the fecal microbiome, fecal metabolome, and plasma metabolome were also explored. Taken together, these findings strengthen the link between Turicibacter abundance and AD and provide a basis for further microbiome studies of murine models for AD.

To infinity and beyond: exploring viral diversity in extreme environments

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Background: Despite their rampant abundance on the planet, relatively little is known about viruses in extreme environments, and whether viruses facilitate adaptation of their hosts to harsh conditions. In an effort to catalog viruses across extremes (e.g., temperature, salinity, aridity), we explored viral diversity in three datasets: (i) 50 metagenomes from terrestrial Cyanobacteria co-cultures isolated from hot desert environments, (ii) 191 metagenomes from Antarctic rocks from ice-free areas until recently thought incapable of supporting life, and (iii) 42 metagenomes and metatranscriptomes associated with seagrasses, the only existing marine plants.

Methods: We identified viral sequences in assembled metagenomes/metatranscriptomes using VirSorter1/VIBRANT or VirSorter2, assessed their quality using CheckV, grouped them into viral OTUs (vOTUs) at >95% similarity and then clustered vOTUs with RefSeq genomes using VContact2. We mapped reads to vOTUs to obtain relative abundance and predicted bacterial hosts using NCBI BLAST. Exploratory analysis was performed by undergraduate interns using KBase and R.

Results: We identified 814 viral sequences associated with the terrestrial Cyanobacterial co-cultures, over 100,000 sequences associated with endolithic Antarctic rock communities, and 8047 sequences associated with seagrass leaves, roots and associated sediment. However, of the viral sequences identified, only 72, 73 and 54 sequences, respectively, clustered with viral RefSeq genomes. Across all three datasets, the majority of viral sequences clustering with RefSeq genomes belonged to the order Caudovirales. Further within datasets, few vOTUs were shared across samples, and communities were largely dominated by taxonomically unclassified viral clusters, likely representing novel viral genera.

Discussion: Overall, these results are consistent with viruses in extreme environments being underrepresented in reference datasets. This foundational work expands knowledge of viral diversity in these habitats and provides catalogs of viral sequences for future work, such as investigating the role of viral auxiliary metabolic genes in adaptation of hosts to extreme environments.

Gain some, lose some: bacterial gene content shifts associated with growth temperature adaptation

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Life thrives across a wide span of temperatures, yet every organism is restricted to growing within a narrow optimum range. While some genomic features that contribute to adaptation to optimum growth temperatures (OGT) are known, specific sets of genes contributing to temperature preferences are unknown and the evolutionary processes leading to changes in OGT are poorly understood. The bacterial phylum Thermotogota is an excellent system for studying the evolution of OGT. It comprises mesophilic, thermophilic, and hyperthermophilic members that collectively grow between 20°C-90°C. Using genome-wide association studies, we identified a set of gene families associated with OGT in members of the Thermotogota phylum. Mapping family gains and losses on the species phylogeny revealed that families present in thermophiles with OGT ≥65°C were also present in the phylum's last common ancestor, whereas families found in species with OGT <65°C were gained later. Additionally, the gene families found in hyperthermophiles were lost on branches leading to Thermotogota with OGT <65°C. Additionally, the latter taxa frequently acquired genes horizontally, based on evidence from phylogenetic analyses. We hypothesize that adaptation to lower temperature necessitates both the acquisition of genes absent in thermophiles and loss of unnecessary genes. Our findings are in concordance with several analyses that suggest a non-hyperthermophilic nature of early microbes and a later evolution of both hyperthermophily and mesophily. Some of these gene families are physically and functionally associated with each other, with most of these gene networks contributing to metabolic functions. However, many of these genes encode proteins of unknown function, and their experimental characterization will advance our understanding how OGT diversity evolved in Thermotogota and throughout the tree of life.

The evolution of the binary toxin locus in Clostridioides difficile

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Background: Clostridioides difficile (Cd) is a pervasive nosocomial pathogen and significant cause of healthcare-related diarrhea. Cd infection (CDI) is often provoked by microbiome perturbations (ie. antibiotics or chemotherapy), but Cd strain identity contributes to differences in colonization outcome. Cd genomes are highly mosaic, and the ramifications of this genetic diversity in context of disease severity remains poorly understood. Our previous data indicate that the presence of the binary toxin locus, an accessory locus purported to increase virulence, may be associated with CDI.

Methods: Diagnostic data from patients colonized with toxigenic Cd with a history of least one diarrheal stool was analyzed from three prospective/retrospective Cd studies at Barnes Jewish Hospital in St. Louis. To capture distinct presentation of disease, I examined 178 patient-isolate pairs from patients who had at least one diarrheal episode and whose stools were either enzyme immunoassay positive (EIA+) for toxin (n=71) or EIA negative (EIA-, n=107). Whole-genome sequencing and isolate genomics were used to examine correlates of disease severity.

Results: EIA+ patients were significantly more likely to be colonized with Cd strains containing cdtAB relative to EIA- patients. Using previously collected isolates and representative isolates from NCBI, phylogenetic analysis revealed that cdtAB is generally confined to ST11 and ST1 lineages of Cd, with diverse lineages containing cdtAB pseudogenes. Comprehensive examination of cdtA synteny indicates that cdtAB may be mobilized between isolates with a number of transcriptional regulators.

Discussion: The association of EIA status with the presence of the binary toxin locus confirms the previous literature that CdtAB may exacerbate Cd disease. Surprisingly, the genetic context of the cdtAB locus is not conserved across all isolates, indicating a mobilizable capacity of the locus. Given our previous understanding of toxin regulation in Cd, future work will emphasize the regulatory mechanisms underlying CdtAB protein expression.

Investigating wound microbiome composition in Type 2 Diabetic mice

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Chronic, nonhealing wounds affect many patients with Type 2 diabetes. Microorganisms are believed to play a significant role in the process of wound healing, as more diverse microbiomes induce faster healing. Various commensal and pathogenic bacteria including Staphylococcus, Corynebacterium, and Pseudomonas tend to colonize wounds once they are established on the host. However, how the microbiome influences chronic wounding is not fully understood. To better understand the role of microbes in diabetic wound healing, we performed 16S rRNA sequencing to compare the wound microbiomes of diabetic and nondiabetic mice. Each mouse was anesthetized and wounded with a double 2 mm biopsy punch, and those wounds were swabbed pre-wounding and days 2 and 7 post-wounding. The swabs then underwent DNA extraction, V3-V4 16S rRNA library preparation, and Illumina-based sequencing. ANOVA analyses on the change in wound diameter between the diabetic and nondiabetic mice groups revealed that the diabetic mice wounds healed significantly slower ($p=0.021$) than the control group. NMDS analyses on microbial community compositions revealed distinct community profile shifts between sampling days in nondiabetic mice wound ($p=0.01$) that were not observed in diabetic mice ($p=0.22$). Additionally, the taxonomic charts revealed unique profiles between the control and diabetic mice pre- and post-wounding. Collectively, those data suggest a correlation between wound healing and microbial composition.

Mapper: Fast and accurate sequence alignment via x-mers

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Read mapping quality determines the efficiency and accuracy of interpreting metagenomic data. Most sequence aligners base their search algorithms on splitting sequences into fixed-size pieces (k-mers). However, a known challenge of k-mer algorithms, the choice of kmer size, strongly affects the quality of sequence alignment. Here, we introduce Mapper, a new sequence aligner that addresses this challenge by dynamically incorporating k-mers of various sizes, which we refer to as x-mers. Mapper is 10-15 times faster than previous aligners, while producing fewer false positives and fewer false negatives. Mapper was tested on a diverse collection of microbial genomes and these advantages are consistent across species. In principle, any k-mer-based algorithm, such as similarity search, genome assembly, metagenomic annotation, and multiple sequence alignment, might benefit from incorporating x-mers.

Characterizing the impact of restoration on the Rana sierrae skin microbiome across restoration histories and sites

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Background: The Sierra Nevada yellow-legged frog, Rana sierrae (Rs), has been driven close to extinction in part by the fungal pathogen Batrachochytrium dendrobatidis (Bd). Skin-associated microbes can inhibit Bd and microbial community structure can impact disease outcomes in hosts, indicating that the microbiome should be considered during restoration efforts.

Methods: Here, we leveraged on-going restoration efforts in the Desolation Wilderness to investigate how the Rs microbiome is impacted by history of restoration and restoration site. We sequenced bacterial 16S rRNA amplicons and performed qPCR for Bd from Rs skin swabs across one source site (naturally persisting frogs), one captive site (Zoo population derived from source), and two restoration sites (including frogs translocated from the source, reintroduced from captivity, and new recruits born at restoration sites).

Results: We tested hypotheses about the impact of restoration on the microbiome by comparing Shannon diversity, community structure (Weighted Unifrac; WU), taxonomic composition, and Bd prevalence and intensity at field sites. We found that reintroduced, translocated, and new recruit frog microbiomes did not differ significantly in any comparisons. While the two restoration sites did not differ from each other in most comparisons, WU was significantly different. In addition, captive frogs were different from all other restoration histories and sites in terms of Shannon diversity and WU. Source frogs also differed from one or more restored populations or restoration sites in all comparisons. Finally, all frog microbiomes were dominated by a single amplicon sequence variant, SV1 (Family Burkholderiaceae).

Discussion: Restoration site appears to be more important than history in determining the microbiome of reintroduced and translocated frogs, and both captive and source frogs were distinct from other groups. Moving forward, we will explore what factors at different sites are leading to microbiome differences. These findings may guide future conservation strategies that take Bd-frog-microbiome interactions into account.

Effect of population size on context-dependent trade-offs of antibiotic resistance

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Evolutionary adaptations are generally associated with fitness costs, yet many studies on the costs of antibiotic resistance have failed to identify growth rate deficits in resistant bacteria. Recent work has shown that costs of resistance can manifest as a reduced tolerance of novel environmental conditions. Here, we consider the effects of population size on these context-dependent fitness costs. The relationship between population size and the environmental costs caused by resistance is unclear; it may be that a larger population size means that mutations with lower fitness costs are more likely to arise, or it may be that a larger population size allows for a higher degree of adaptation to specific environments. In this study, we characterize the effect of population size on the growth deficits incurred by chloramphenicol-resistant Escherichia coli in novel thermal conditions. We show that when resistance develops in a larger population, bacteria show greater growth deficits when compared to bacteria evolved in smaller populations. This result is consistent with the hypothesis that the observed difference is due to larger populations becoming more tightly adapted to the experimental conditions, causing a greater decrease in their thermal niche breadth; nonetheless, further studies are needed to confirm this hypothesis and integrate this observation into an evolutionary framework. The results of this study give insight into the interplay between two key ecological factors that could influence antibiotic resistance development. More broadly, further characterizing the fitness costs associated with resistance development can help predict scenarios where resistance will arise.

In vivo ligand-responsive gene expression in probiotic S. boulardii

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Background: Saccharomyces boulardii (Sb) is a leading probiotic candidate for delivery of biotherapeutics to the mammalian gut. While Sb is a promising probiotic, precise control of gene expression in the species has not been achieved; such control can play a crucial role in maintaining the fitness and survival of the strain during colonization. Developing inducible gene expression systems that can be tuned via the addition of ligands could play an essential role for production and delivery of biotherapeutics.

Methods: Using CRISPR-Cas genome editing, we created an Sb strain that can utilize galactose and raffinose. Using various cloning techniques, we developed 5 ligand-responsive gene expression systems for Sb and characterized their titration curves with aerobic (yeGFP and mKate) and anaerobic (CaFbFP) reporters and measured the expression levels under aerobic and anaerobic conditions using different concentrations of the inducers.

Results: Galactose utilizing Sb allowed tunable gene expression (227-fold induction) when galactose is the sole carbon source with galactose promoter (pGAL1). Other four promoters that are activated in the presence of aTc (pTET), IPTG (pLAC), xylose (pXYL) or copper (pCUP1), achieved 16-, 88-, 10- and 3-fold induction, respectively in the presence of glucose. Under anaerobic conditions, pXYL, pLAC and p pGAL1 exhibited activation profiles similar to the aerobic conditions. Orthogonality assays confirmed that each promoter exhibited ligand-specific activation when tested with ligands other than its own.

Discussion: A challenge in the development of engineered probiotics is control over dosage. Ligand-responsive gene expression systems only activate transcription in the presence of a particular inducer and this regulation by the concentration of inducer enables custom dosage of a therapeutic. This work shows development of ligand responsive expression units for Sb, expanding the applicability of Sb in the gut microbiome.

Fast Average nucleotide identity (ANI) estimates are as good for delimiting Klebsiella species as ANI

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Klebsiella strains are among the most important multi-drug resistant human pathogens. The importance and ubiquity of these organisms call for quick and accurate methods for their classification. Genomic average Nucleotide Identity (ANI) is becoming a standard for bacterial species delimitation. Since complete ANI calculations can be too slow, faster estimates, based on sequence sampling, have been appearing in the literature. In this study we compared 1,189 Klebsiella genomes using measures calculated with Mash, Dashing, and DNA compositional signatures, all of which run in a fraction of the time required to obtain ANI. Receiver Operating Characteristic (ROC) curve analyses showed equal quality in species discrimination for ANI, Mash and Dashing, with Area Under the Curve (AUC) values above 0.99, followed by DNA signatures (AUC: 0.96). Accordingly, groups obtained at optimized cutoffs largely agree with species designation, with ANI, Mash and Dashing producing 15 species-like groups. Testing Mash to map species after adding draft Klebsiella genomes to the dataset, also showed excellent results (AUC above 0.99), suggesting that the whole 13,574 genome dataset could be divided into 26 species-like groups.

ENGINEERING AN INCUBATION ENVIRONMENT THAT MIMICS IN SITU CONDITIONS FOR IN VITRO COASTAL MICROBIOME STUDIES.

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Background: Coastal environments are dynamic and can vary widely on short- or long-term scales depending on location and weather. Incubation equipment that reflects these changes through programmable gradient light and temperature cycles would permit more precise in vitro coastal microbiome studies. Here, we present an open-source incubation environment that mimics in situ conditions for in vitro coastal microbiome studies using a modified shaking water bath that has fully customizable temperature and light gradients that can also mimic real-time field conditions.

Methods: To test how this build emulates real environment experimental conditions, we performed a 48-hour experiment comparing changes to coastal microbiomes at the Ellen Browning Scripps Memorial Pier in La Jolla, CA versus incubation in our build in the laboratory while mimicking live conditions at Scripps Pier. This was followed by metagenomic sequencing across conditions and time points to observe changes to microbial community profiles.

Results: NMDS plots from shotgun sequencing of microbiome samples taken at 0h, 24h, and 48h demonstrated the samples form distinct clusters based on time yet did not form separate clusters between conditions. Analyses of congruence revealed that clustering at each time point between conditions (in situ vs. in vitro) were not statistically different (p-value: 0.2 and 1 between conditions at 24h and 48h respectively). However, there was a significant difference in communities between time points sampled (p-value: 0.01).

Discussion: We developed an open-access build that can be pre-programmed to permit precise in-lab microbiome experiments in a controlled and accessible environment. Further experiments over extended time frames and comparing conventional laboratory testing methods are needed to further explore the efficacy of the programmed conditions, but these data serve as an initial proof of principle for a set of modifications that can be added to common laboratory equipment to bolster complex long-term microbial ecology studies.

Exploring microbial physiology in chronic human infection through metatranscriptomics

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Chronic bacterial infections including cystic fibrosis, chronic wounds, and osteomyelitis affect a significant portion of the population in developed countries and place a large burden on healthcare systems. These infections can last many weeks to years, despite numerous clinical interventions and are frequently recalcitrant to antibiotics. Chronic infections almost always harbor complex polymicrobial communities of commensal and environmental organisms and polymicrobial infections often result in higher bacterial burdens, increased tolerance to antibiotics, and more severe disease compared to single-species infections, a process termed "synergy". Although synergy between microbes has long been recognized, the underlying molecular mechanisms and their impacts on chronic wounds have remained difficult to elucidate. To begin to address this knowledge gap, we assessed 115 metatranscriptomes from human cystic fibrosis sputum and chronic wound infections, isolated from 3 continents and 5 clinics. We first determined the community composition using MetaPhlAn3 and found sputum is more diverse than chronic wounds with a mean of 11.7 and 6.6 species identified, respectively. Further, we found Gram-positive organisms dominated both communities, comprising 67.4% (+/-3.1 SEM) of the species identified in these communities. Further, we found these environments are likely hypoxic as strict anaerobes were found to comprise a mean of 21.7% (+/-2.6 SEM) of the communities in these chronic infection sites. Ongoing analyses are identifying functions critical community functions in each infection site using HUMAnN3, MetaPro, and SAMSA2. Collectively, this data will improve our understanding of microbe-microbe interactions and community functions in chronic infection.

Do you see what I see? Improving color accessibility and organization of microbiome data with the microshades R package

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Background: Color Vision Deficiency (CVD), commonly known as colorblindness, affects 1 in 12 men and 1 in 200 women - approximately 300 million people worldwide. Individuals with CVD do not experience complete loss of color vision, but have reduced ability to distinguish different colors. When creating scientific figures, it is important to consider that individuals with CVD may not perceive all colors as intended. While there are several CVD friendly color palettes available, they are often insufficient for visualizing microbiome data.

Methods: We developed an R package, microshades, to overcome CVD accessibility for microbiome datasets. Microshades includes CVD accessible color palettes and data organization functions. To construct the palettes, hue (type of color), chroma (colorfulness), and luminance (brightness) were adjusted for optimal visual distinction and CVD accessibility. All shades were tested with a CVD simulator (cvdemulator) for accessibility. Data organization functions include grouping data by taxonomic ranking, sorting the data vertically and horizontally, and restructuring the plot legends.

Results: Each microshades color palette contains six hues with five sequential variations of chroma and luminance per hue, for a total of 30 available colors per palette. The microshades_cvd_palettes colors are universally CVD accessible to individuals with the three most common types of CVD (Deuteranope, Protanope, and Tritanope). The individual hues of the microshades_palettes colors are CVD friendly, but when used in conjunction with multiple hues, may not be universally accessible to all forms of CVD.

Discussion: The microshades R package is a visualization tool for microbiome researchers. The package contains two CVD accessible palettes, along with several organization features. The microshades package can be used in conjunction with common microbiome R packages, such as phyloseq, to enhance microbiome data visualization.

Ultrahigh-throughput single-cell genetic profiling of microbiomes

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Background: Single cell genetic heterogeneity in microbiomes underlies many important phenomena, such as evolution, antimicrobial tolerance and resistance, and the dynamics of mobile genetic elements. However, despite the importance of understanding the single cell heterogeneity in microbiomes, tools for single cell genetic profiling of microbes are not yet widely available.

Methods: We have developed a robust, generalizable, and widely accessible method for ultrahigh-throughput single-cell genetic analysis of microbes. Using microfluidic devices, microbial cells are encapsulated into pico-liter droplets, lysed, then targeted regions of the genomes are amplified through a multiplex PCR reaction that simultaneously amplifies regions of interest and connects them with cell-specific barcodes. Libraries are then run on an Illumina sequencer producing uniquely barcoded reads corresponding to amplicons of each cell. This method is designed to be simple, robust, and accessible to most academic laboratories with minimal microfluidics expertise.

Results: To show the broad applicability of this method, we demonstrate three vastly different applications of single cell sequencing on human gut microbes. 1. We capture the single-cell genomic variation within the capsular polysaccharide operons of a single colony of Bacteroides fragilis; 2. we profile the distribution of antibiotic resistance genes among a 25-member gut microbial community; and 3. we monitor the transmission dynamics of 3 naturally occurring self-transmissible plasmids within a community of Enterobacteriaceae.

Discussion: Single cell genetic profiling is a powerful way to study microbiomes, however the field currently lacks robust and widely applicable tools for doing so. This generalizable and accessible tool for single-cell genetic profiling of microbiomes opens up unprecedented opportunities for research in this field.

Impact of polymicrobial interactions on single-cell transcriptomic heterogeneity

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Most microbes reside within complex communities, where microbe-microbe interactions influence their physiology and fitness. In these spatially structured communities, it is hypothesized that subpopulations of cells with altered transcriptional profiles have outsized roles. However, measurements of microbial interactions are almost exclusively performed at the population level. Here, we measure the impact of spatial structure and microbial interactions on transcriptomic heterogeneity using single-cell RNA-sequencing (scRNA-seq). Specifically, we identified the transcriptome of single cells and small aggregates of the oral pathogen, Aggregatibacter actinomycetemcomitans (Aa) grown in biofilms in mono-culture and in co-culture with the cross-feeding and cross-respiring oral commensal Streptococcus gordonii (Sg). Our dataset contains thousands of cells, with an average of ~100 unique protein-coding reads per cell or aggregate. Analysis of these samples is revealing how co-culture in biofilms impacts the extent of transcriptional heterogeneity and the identity of variably expressed genes. These data will be fundamental to our understanding of subpopulations in microbial communities.

Parallel changes in fitness effects and gene essentiality over 50,000 generations of evolution

Anurag Limdi, Sian V. Owen, Cristina Herren, Richard E. Lenski, Michael Baym

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Background: Over evolutionary time, bacteria face changing environments, which may require different sets of genes for survival. As they adapt to a specific constant environment, some genes are modified and lost, which can increase fitness while also modulating the effects of further gene losses. However, whether evolutionary specialization leads to systematic changes in robustness to gene loss is largely unexplored.

Methods: Here, we compare the effects of insertion mutations in Escherichia coli between ancestral and 12 independently derived strains after 50,000 generations of growth in a simple, uniform environment using transposon insertion mutagenesis and sequencing (TnSeq)

Results: We find that epistasis between insertion mutations and genetic background is common, but the overall distribution of fitness effects is largely unchanged. In particular, we see systematic changes in gene essentiality, with more genes becoming essential over evolution than vice versa. The resulting changes often occurred in parallel across the independently evolving populations. A few of the changes in gene essentiality are associated with large structural variations, but most are not.

Discussion: Taken together, our results demonstrate that gene essentiality is a dynamic, evolvable property, and they suggest that changes in gene essentiality are a result of natural selection in this long-term evolution experiment, rather than a mere byproduct of structural changes.

Evaluation of addiction module function of chromosomal Toxin-Antitoxin gene pairs from Variovorax paradoxus EPS

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Toxin-antitoxin (TA) systems are pairs of co-expressed genes found in many genomic contexts. They were originally identified as “addiction modules” in natural plasmids, but have subsequently been implicated in many microbial processes. This addiction is often based on the differential stability of the gene products, leading to post-segregational killing (PSK) in cells that lose the plasmid. We used the TASmania genome annotation for Variovorax paradoxus EPS to identify eight clear TA system loci in the genome. Gibson assembly was used to clone these loci into the broad host range vector pBBR8-GFPuv, replacing the fluorescent protein with the TA system structural genes under arabinose control. The pemIK genes from the plasmid pBBR5pemIKpBAD were used as a positive control for addiction phenotype. The plasmid sequences were verified using the whole plasmid sequencing service from Plasmidsaurus. Passage experiments with and without antibiotic selection were performed using arabinose to control expression of the TA system loci. After passaging, the cultures were plated on selective and non-selective media to determine the ratio of cells continuing to harbor the plasmid. Our hypothesis was that plasmid curing would be evident with no selection or induction, and that an active TA system would “addict” the cells to the plasmid, preventing loss. After 10d of passage in the stabilization assay we saw evidence for plasmid stabilization in the control construct as well as 2/8 Variovorax gene cassettes. We also saw evidence of toxicity in 3/8 constructs, indicating that the antitoxin efficacy as well as toxin function may be limited by host background. Samples from each culture were preserved for further analysis using Nanopore sequencing. Analysis of the variation in the plasmid population will shed further light on the selective process. This experimental scheme will provide a systematic strategy for identifying TA system genes in newly sequenced genomes.

Epidemiology of Plasmid Lineages Mediating the Spread of Extended-Spectrum Beta-Lactamases among Clinical Escherichia coli

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The prevalence of extended-spectrum beta-lactamases (ESBLs) among clinical isolates of Escherichia coli has been increasing, with this spread driven by ESBL-encoding plasmids. However, the epidemiology of ESBL-disseminating plasmids remains understudied, obscuring the roles of individual plasmid lineages in ESBL spread. To address this, we performed an in-depth genomic investigation of 149 clinical ESBL-like E. coli isolates from a tertiary care hospital. We obtained high-quality assemblies for 446 plasmids, revealing an extensive map of plasmid sharing that crosses time, space, and bacterial sequence type boundaries. We provide further support for plasmid-mediated spread of ESBLs but demonstrate that some ESBL genes rely on dissemination through plasmids more than the others. Through a sequence-based network, we identified specific plasmid lineages that are responsible for the dissemination of major ESBLs. Notably, we demonstrate that IncF plasmids separate into two distinct lineages that are enriched for different ESBLs and occupy distinct host ranges. Our work provides a detailed picture of plasmid-mediated spread of ESBLs, demonstrating the extensive sequence diversity within identified lineages, while highlighting the genetic elements that underlie the persistence of these plasmids within the clinical E. coli population.

Insights to Pathogenic Potential: Genomic Analysis of Escherichia coli from Asymptomatic and Symptomatic Patients

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Background: Urinary tract infections (UTI) afflict over 50% of women over the course of their lifetime. While uropathogenic Escherichia coli (UPEC) causes >75% of reported UTIs, UPEC lacks a specific molecular signature. In the clinical setting this poses a significant dilemma when patients present with asymptomatic bacteriuria (ASB), in which significant numbers of E. coli is found in the urine of patients, but without the associated symptoms of UTI. Both ASB and UTI-causing E. coli carry similar types of virulence factors, and – to date – no genomic signature exists to tell these two E. coli types apart.

Methods: In this study, we sequenced approximately 800 E. coli strains from the urine of symptomatic and asymptomatic patients. We utilized a custom bioinformatic pipeline to characterize strains outside of the traditional virulence factors, hypothesizing that a signature lies not in the carriage of distinct virulence genes, but rather in discrete polymorphisms that change tropism and pathogenic potential. Included in this analysis are applications of microbial Genome-Wide Association studies as well as machine learning methodology to detect potential loci associated with the pathotype in an unbiased manner.

Results: We show urine-isolated strains of E. coli maintain an open pangenome. These strains additionally carry a variety of virulence associated factors with no correlation of the development of symptoms. We queried mobile genetic elements, and found that plasmids, prophage, and other transposable elements also do not correlate with symptoms. We continue explore other more subtle genomic features that might constitute a defining feature of UPEC.

Discussion: Previous work has shown that strains causing UTI remain phenomenological, and there is no molecular signature to differentiate these strains from other extraintestinal E. coli strains. This work will more robustly define true uropathogenic strains with the aim of identifying a molecular signature to differentiate strains with pathogenic potential.

Taxonomic and Antibiotic Resistance Changes to Coastal Microbiomes in Response to Rainstorm Runoff

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Antibiotic resistance (AR) is a global healthcare issue driven by the overuse of antibiotics in clinical, agriculture, and aquaculture applications. Urban and agricultural runoff introduce antibiotic-resistant bacteria and antibiotic contamination to recipient environments. Antibiotics change microbial community compositions in favor of resistant species and can trigger the exchange of DNA carrying antibiotic resistance within a given community. Mapping the changes in microbial community composition and AR gene abundance in response to rainstorm runoff has yet to be elucidated. We therefore analyzed the taxonomic and AR gene abundance changes to coastal microbiomes throughout seasonal rainstorms. Sampling at the Batiquitos lagoon outlet in Carlsbad, California occurred over 14 days; before, during, and after the first two rainstorms of the 2019-2020 season. Coastal water was captured on-site on a 0.22 µm mixed cellulose ester (MCE) membrane filter. We performed total DNA isolation and shotgun library preparation on the isolated microbiomes followed by 2 x 150 base paired-end sequencing. Microbial composition and AR gene identification was performed on the resulting metagenomes, to determine a time course profile of relative microbial abundance and antibiotic resistance profiles. Additionally, we performed meta-SourceTracker analysis on the time course metagenomes to investigate proportions of exogenous and endogenous microbial community members throughout and following rainstorms, as well as to explore possible sources of exogenous taxa. We observed an overall bimodal increase in alpha diversity and AR gene counts in the 24-72-hour period following each rainstorm. Taxonomic changes are reflected by a relative depletion of Cyanobacteria and relative increase in Proteobacteria and Bacteroidetes. Increases in Proteobacteria appear to be predominantly marine eutrophication-associated microbes while Bacteroidetes increases were predominantly freshwater- and soil-associated microbes commonly implicated in fish and human disease. The microbial community profile returned to a pre-storm composition after approximately six days contrary to the three-day recovery time commonly referenced.