Functional metagenomics for the investigation of ancient microbial antibiotic resistance in permafrost.

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Permafrost, or permanently frozen soil, is found in polar regions and underlies ~20% of the Earth's surface. Despite subzero temperatures, permafrost hosts a diversity of microbial life. Permafrost is an important study system because (1) thawing permafrost contributes to climate change and (2) permafrost is an analog for extraterrestrial subzero environments. Permafrost serves as a substrate in which to study survival strategies in extreme cryoenvironments. To understand microbial community survival, we focused on a particularly important aspect of community function--antibiotic resistance. Antibiotic resistance is ancient and occurs naturally among soil dwelling microbes. Antibiotic resistance genes are involved in community signaling, environmental sensing and play a role in competition and defense interactions. To identify antibiotic resistance genes in bacterial communities that have never been exposed to modern synthetic antibiotics and to determine their importance as a survival strategy, we employ functional metagenomics. Using this approach, we extract DNA directly from permafrost frozen for 19,000 to 33,000 years before present and clone it into a plasmid vector. A metagenomics library is thus constructed and expressed in an E. coli surrogate host. We screen for antibiotic resistance, pool resistant colonies, and sequence the antibiotic resistance genes using high-throughput next-generation sequencing. Results obtained from an analogous permafrost substrate demonstrated a robust metagenomic library size of 90.9 GB with 1 out of 15,000 clones expressing antibiotic resistance. Sequence data confirmed the transfer and identification of antibiotic resistance genes from both gram positive and gram negative microorganisms as well as novel functional resistance genes. These results expand our knowledge of functional resistance genes and reveal specific modes of action diverse microbes must use to survive in extreme environments.

Prospective Cohort Study Measuring the Impact of Antibiotics on the Fecal Microbiome and Resistome of Healthy Volunteers

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"Background: Over 67 million prescriptions for antibiotics are written each year in the US to treat acute respiratory conditions, over half of which are unnecessary (CDC). Previous antibiotic use is also an important risk factor for multi-drug resistant organisms. The goal of this study is to characterize the impact of antibiotics (ABX) used to treat community-acquired pneumonia (CAP) on the fecal microbiome and resistome in healthy volunteers (HV).
Methods: 20 HVs were randomized to receive 5 days of levofloxacin (LV), azithromycin (AZ), cefpodoxime (CF), or AZ+CF. Stool was collected before, during, and after ABX, then underwent microbiologic culture and shotgun sequencing. DNA was extracted, then sequenced using the Illumina NextSeq platform. Relative abundance of bacterial taxa was estimated by MetaPhlAn and antibiotic resistance gene (ARG) composition by ShortBRED. Analysis was in R.

Results: The mean HV age was 37 (range 24-59) and 10 were female. Species diversity measured via Shannon Index and richness were significantly lower in samples taken from all HVs 3 days post-ABX ($p<0.01$ for all). While non-metric multidimensional scaling (NDMS) ordination shows high interpatient dissimilarity (Bray-Curtis) for most samples, the post-ABX intra-patient dissimilarity varies by ABX. The AZ group exhibited chronic changes to taxonomic composition and the CF group had increases in dissimilarity immediately post-ABX. The CF+AZ group displayed both acute and persistent perturbations. There was a significant increase in overall ARG abundance across all samples ($p<0.003$). Within each ABX, there were unique changes in ARG abundance, and groups with CF had increases in ARG abundance.

Conclusion: ABX used to treat CAP can cause acute microbiome disruption, as evidenced by decreased microbiome diversity and richness, and an increase in ARG abundance post-ABX. The duration of this impact is variable. To prevent microbiome disruptions, measures to prevent inappropriate ABX use via ABX stewardship are necessary."

A Metagenomic Meta-Analysis Reveals Functional Signatures of Health and Disease in the Human Gut Microbiome

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While recent research indicates that human health depends, in part, upon the symbiotic relationship between gut microbes and their host, the specific interactions between a host and its microbiome that define health remain poorly resolved. Metagenomic clinical studies can reveal gut microbial functions that stratify healthy and diseased individuals. However, the typical single-disease focus of microbiome studies limits insight into which microbiome features robustly associate with health, indicate general deviations from health, or predict specific diseases. To improve our understanding of the association between the gut microbiome and health, we conducted the first integrative functional analysis of gut metagenomes by collecting all available clinical metagenomic data, which consists of about 2,000 samples obtained from eight clinical studies. Using a regression modeling approach, we robustly resolve functions in the microbiome that stratify diseased individuals from controls, and when possible control for study-specific effects. Functions that indicate multiple diseases occur at a greater rate than expected by chance, which bolsters the likelihood that these functions contribute to health. We also resolve indicator functions of specific diseases, which point to disease-specific etiologies. Many of these disease indicators also overlap with functions that stratify vertebrate microbiomes relative to free-living microbial communities, further supporting their potential importance to host health. Overall, these results clarify potential microbiome-mediated mechanisms of disease and reveal features of the microbiome that may be useful for the development of microbiome-based diagnostics.

Variations in local microbiome populations in normal colorectal tissue and adenomas

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Colorectal cancer (CRC) is the second leading cause of cancer deaths in the United States, with increasing rates in younger populations. While the composition of microbes in the gut has been associated with the development of CRC, prior studies have traditionally taken stool samples that reflect the microbial composition of the entire colon, with less focus on the local tumor environment. Furthermore, there are two main tumor types which arise through different pathways and may harbor distinct microbial communities: conventional adenomas are more common and easier to detect and treat, while sessile serrated adenomas (SSA) are less common but more dangerous, as they are flat and escape detection during colonoscopies. To understand the local microbial adaptations associated with the pre-cancer microenvironment, we aim to investigate the microbial composition of adenoma associated tissues, in addition to healthy adjacent tissue. To investigate the role of the microbiome in SSA versus conventional adenoma development, over 1,800 samples were collected by directly brushing or lavaging healthy and adenoma associated tissues from the colon of 141 different patients. Currently, we are characterizing the microbiome of these samples using whole genome shotgun metagenomic sequencing. Preliminary analysis has revealed no significant differences in the microbial composition between brush and lavage sample collection methods, with patient origin describing most of the variation. As we prepare to analyze more samples, we hypothesize that we will be able to distinguish tissue types based on their microbial signature. Our research seeks to identify microbial species uniquely missing or enriched in SSA and conventional adenoma tissues, thereby elucidating potential mechanisms and targets for screening, prevention, and diagnosis of CRC-associated neoplasms. This research was funded by the American Cancer Society institutional research grant #IRG-16-187-13.

Quorum-Sensing Modulates the Epibiotic-Parasitic Relationship between Actinomyces odontolyticus subspecies actinosynbacter strain (XH001) and its epibiont, a TM7 phylotype (TM7x)

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"Recent metagenomic analyses revealed a new lineage of bacteria, denoted Candidate Phyla Radiation (CPR), comprising upwards of >25% of the bacterial domain. TM7x, a Saccharibacteria/TM7 phylotype, was recently co-isolated with its bacterial host, Actinomyces odontolyticus subsp. Actinosynbacter Strain, XH001, as the only cultivated representative of the CPR group. Actinomyces species are among the early microbial colonizers in the oral cavity and the relationship of XH001 with TM7x may modulate the overall composition of the microbiota, potentially influencing oral disease. Furthermore, members of Actinomyces and TM7 are associated with multiple human systemic diseases, notably periodontitis. Metatranscriptomic analysis (RNA-seq) revealed a set of differentially regulated genes within XH001 when associated with TM7x as compared to parasite-free XH001. The most highly upregulated gene, a lsrB homologue, encodes a periplasmic binding protein for the auto inducer (AI) 2 signaling molecule. The focus of this study was to elucidate the role of AI-2 quorum sensing affecting the epibiotic-parasitic relationship between XH001 and TM7x as well as create a genetic system for XH001.

We created XH001ΔlsrB and XH001ΔluxS gene deletion mutants via homologous recombination and these mutants were subjected to phenotypic analyses, including growth kinetics, biofilm formation evaluated by confocal microscopy, and quantification of the AI-2 signaling molecule via GC-MS. lsrB upregulation was confirmed via quantitative real time PCR and a genetic modification system targeting the lsrB and luxS (encodes AI-2 synthase) homologues was validated. Phenotypic analyses provided data suggesting the association with TM7x enhances wildtype XH001's biofilm formation capability. Interestingly, confocal microscopy determined biofilm formation deficiencies in TM7x associated XH001ΔlsrB and XH001ΔluxS. XH001ΔluxS displayed abrogated AI-2 signal production compared to wildtype. We conclude that TM7x enhances XH001 biofilm formation by inducing its AI-2 quorum sensing system. Further phenotypic analyses will elucidate the mechanisms of quorum sensing in the epibiotic-parasitic relationship between XH001 and TM7x."

Novel diversity-generating retroelements encoded by bacteriophages

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Diversity-generating retroelements (DGRs) are genetic cassettes that create hypervariable proteins. This work surveyed bacteriophage genomes for novel DGRs and discovered 92 DGRs that were only found in phages exhibiting a temperate lifestyle. The majority of phage-encoded DGRs were identified as prophages in bacterial hosts from the phyla Bacteroidetes, Proteobacteria and Firmicutes. Sequence reads from these previously unidentified prophages were present in viral metagenomes (viromes), indicating these prophages can produce functional viruses. Five phages possessed hypervariable proteins homologous to the tail fiber of BPP-1, whereas the functions of the remaining DGR target proteins were unknown. A novel temperate phage that harbors a DGR cassette targeting a protein of unknown function was isolated from *Bacteroides dorei*. This phage, here named *Bacteroides dore*i ΦHankyphage, lysogenizes at least 13 different *Bacteroides spp*. and was present in 34% and 21% of whole-community metagenomes and human-associated viromes, respectively.

Phinch: An interactive, exploratory data visualization framework for -Omic datasets

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The sheer volume of data produced from high-throughput sequencing platforms (e.g. Illumina HiSeq/MiSeq) requires new paradigms for effective data analysis. Scientific visualization represents an innovative method towards tackling current bottlenecks; in addition to giving researchers a unique approach for exploring large datasets, it stands to empower biologists with the ability to conduct powerful analyses without requiring a deep level of computational knowledge. Phinch (http://phinch.org/) is an interactive, exploratory visualization framework that can be used to identify biological patterns in high-throughput environmental datasets (microbial ecology OTUs and metagenomes). Leveraging a close collaboration between UC Riverside and Pitch Interactive (a data visualization studio in Oakland, CA), this project takes advantage of standard file formats from computational pipelines in order to bridge the gap between biological software (e.g. QIIME) and existing data visualization capabilities (e.g. visualization-specific programming language such as D3.js). Phinch v2.0 represents a refactored framework released as an Electron desktop app, reducing previous reliance on cloud servers and improving support for larger file size imports. Other improvements include extended support for all BIOM file formats, extended export/sharing features, and a new suite of planned visualization tools and user interactions.

The effects of pathogen emergence on the genome of Mycobacterium abscessus

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"Mycobacterium abscessus is a rapid growing, environmental mycobacteria capable of causing lung infections. There have been multiple emergences of dominating circulating clones (DCCs) in both subsp. massiliense (MAM) and subsp. abscessus (MAA). These clones are globally distributed and provide insight into the emergence of environmental mycobacteria as human pathogens. The distribution of DCCs has been reported, but the genomic patterns associated with transitioning from environmental bacterium to human pathogen have not been characterized.

We de novo assembled and annotated the pangenomes of 120 MAA and 80 MAM clinical isolates from seven geographic regions. We characterized core genome patterns of lateral gene transfer (LGT) using Gubbins and compared recombinant tracts between DCCs and environmentally acquired isolates (EAIs). To infer LGT mechanism, we identified mobile genetic elements within the pangenomes. Additionally, we compared gene frequency and diversity between MAM and MAA, as well as DCCs and EAIs of each subspecies. We calculated □N/□S to compare selection pressures between DCCs and EAIs.

Both subspecies contain an extensive pangenome with a significant number of genes found at rare frequencies. Recombination has affected a larger proportion of MAM's core genome than MAA's. Preliminary results show the MAA DCC1 core genome appears more mature as the core genomes have diverged in gene content when compared to MAA EAI. The distribution of accessory gene □N/□S values are disparate in DCCs of both subspecies, likely the result of different selection pressures. Mobile genetic elements are likely the predominant LGT mechanism in M. abscessus.

Our results suggest the pathogenic emergence of environmental mycobacteria is associated with a decrease in the proportion of recombination affecting the core genome, an increase in gene pseudogenization, core gene content divergence, and a decrease in phage. These results further our understanding of the evolutionary pathway from the environmental reservoir to the human pathogenic niche."

The Role of the Gut Microbiome in Early Childhood Cognitive Development

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The development of the human gut microbiome begins at birth, and develops along with the growing infant. This development is complex and dynamic, and is shaped by numerous biological and environmental processes, and in turn the microbiome can alter fetal immune, metabolic, and cognitive development. To better understand the role of the microbiome on brain development, we analyzed whole shotgun metagenomic sequences from a pilot cohort of mother-child pairs, generating taxonomic and functional microbial profiles. Microbial features were tested for associations with a rich set of cognitive behavioral covariates as well as anatomical features measured with functional magnetic resonance imaging (fMRI). This group is part of the larger Environmental influences on Childhood Health Outcomes (ECHO) cohort of 1,100 mother-child pairs that are being longitudinally sampled over a seven year period, providing unprecedented incite into the complex interactions between the microbiome and human development.

Dynamics of gut microbiota-pathogen interaction and acquisition of antibiotic resistance during travel to high infectious burden region

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Over the past few decades, international tourism has experienced a continuous expansion. Every year about 100 million people travel to nations that are at high risk for diarrhea development, and ~40-60 million of them develop traveler's diarrhea (TD). Previous studies on multiple travelers population have shown that international travel to developing regions contribute significantly towards emergence and spread of antibiotic resistance (AR) worldwide. The inadequate water, sanitation, hygiene and lack of antibiotic stewardship in resource-poor settings in the developing world contribute to an increased incidence of diarrhea as well as spread of multi-drug resistant organisms (MDROs) worldwide. Existing studies on TD and AR in travelers have hitherto neglected the members of the gut microbiota which often accounts for >99% of gut bacterial taxa and may play a critical role in host susceptibility to GI infection due to permissive or protective architectures and their role in dissemination of AR genes to pathogens. Given the burden of diarrhea, the risks posed by antibiotic resistance, and the importance of the gut microbiota in maintaining host health, it is critical to understand how these three phenomena are interrelated and to apply this understanding to preventing the spread of diarrhea and antibiotic resistance in international travelers. To our knowledge, very few studies hitherto have focused on the impact of diarrhea on the host gut microbiome as well as the role of gut microbiota on the initiation and progression of diarrhea among international travelers.

In this proposal, we **propose to leverage a longitudinal study of diarrhea in international travelers to investigate the gut microbiota-pathogen interactions and subsequent carriage of MDR organisms (MDROs) that occurs following international travel to high infectious disease burden regions.** In this project, we aim to fundamentally improve understanding of:

- the effects of travel to resource-limited, developing settings on the gut microbiota in which US military personnel are likely to be deployed,
- the risk factors which promote diarrhea among travelers,
- the contribution of international travel, infectious diarrhea, and use of antibiotics to the global dispersal of AR bacteria,
- AR gene exchange networks between commensals and opportunistic pathogens, and
- Potential biomarkers that contribute to or restrict pathogen colonization and AR gene dissemination.

Genomic Insights into Geothermal Spring Community Members using a 16S Agnostic Single-Cell Approach

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With recent advances in DNA sequencing, rapid and affordable screening of single-cell genomes has become a reality. Single-cell sequencing is a multi-step process that takes advantage of any number of single-cell sorting techniques, whole genome amplification (WGA), and 16S rRNA gene based PCR screening to identify the microbes of interest prior to shotgun sequencing. However, the 16S PCR based screening step is costly and may lead to unanticipated losses of microbial diversity, as cells that do not produce a clean 16S amplicon are typically omitted from downstream shotgun sequencing. While many of the sorted cells that fail the 16S PCR step likely originate from poor quality amplified DNA, some of the cells with good WGA kinetics may instead represent bacteria or archaea with 16S genes that fail to amplify due to primer mis-matches or the presence of intervening sequences. Using cell material from Dewar Creek, a hot spring in British Columbia, we sequenced all sorted cells with good WGA kinetics irrespective of their 16S amplification success. We show that this high-throughput approach to single-cell sequencing (i) can reduce the overall cost of single-cell genome production, (ii) may lead to the discovery of previously unknown branches on the microbial tree of life, and (iii) may facilitate the analysis of populations when specific species level groups are highly represented.

Lineage calling can identify antibiotic resistant clones within minutes

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CCDD Harvard Chan School / DBMI Harvard Medical School

Surveillance of circulating drug resistant bacteria is essential for healthcare providers to develop empiric and effective prescribing practices. However, the results of surveillance are typically not available on a timescale where they could inform treatment of individual patients. Here we present a method for inferring characteristics of an unknown bacterial sample by identifying the presence of sequence variation across the genome that is linked to a phenotype of interest, in this case drug resistance. We demonstrate an implementation of this principle using sequence k-mer content, matched to a database of known genomes. We show this technique can run on data from an Oxford Nanopore device as it is generated and is capable of identifying the presence of a known resistant strain in 5 minutes, even from a complex metagenomic sample. This flexible approach has wide application in pathogen surveillance and may be used to greatly accelerate diagnoses of resistant infections.

Reconstructing the History of Genomic Island Insertions in Clades of Microbes Using xenoGI

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Horizontal transfer of genomic islands is a key process in microbial genome evolution. It is often desirable to reconstruct the history of this process in groups of organisms. Existing methods are most suited to recognizing islands that are present in one strain and absent in others. Thus they are not ideal for studying the history of genomic island insertions in an entire clade. Here we describe xenoGI, a software package that is able to reconstruct this history. Taking a set set of sequenced genomes and a phylogenetic tree as input, it identifies genomic islands and automatically determines the branch on which they inserted. To do this, the package creates gene families in a way that takes account of both the species tree and synteny information. It then identifies families whose members are adjacent and whose most recent common ancestor is shared, and merges them into islands reflecting a common origin. We demonstrate the capabilities of the package using simulations and a set of known genomic islands from the literature. We show that that xenoGI can accurately identify genomic islands and place them on a phylogenetic tree. xenoGI is available as a pip installable python package. There is also a web service available at: http://www.cs.hmc.edu/xgiWeb/.

The effect of captivity on chimpanzee and gorilla gut microbiota and their resistomes

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While the effects of captivity on the gut microbiota of monkeys and other mammals is well documented, little is understood about the effects on the gut microbiota of apes such as chimpanzees and gorillas. Additionally, the effects of antibiotic treatment on ape gut microbiota has never been described. We hypothesize that captivity and antibiotic treatment will result in lower microbial diversity and lead to an increased load of antibiotic resistant genes in the captive ape gut microbiome compared to wild apes. We also hypothesize that wild apes would show similar gut microbiomes to that of humans living nearby. We analyzed fecal samples from wild chimpanzees and gorillas in the Goualougo Triangle region of Nouabalé-Ndoki National Park and from humans living just outside the park in the Republic of the Congo. Samples were also collected from captive apes in the St. Louis Zoo and the Lincoln Park Zoo in Chicago. 16S metagenomic sequencing indicates that captivity increased the number of unique species found in the gut microbiome, with captive apes having more unique species than both wild apes and humans, without significantly altering the community evenness as measured by Shannon Diversity. Using shotgun metagenomics, we found that captivity and antibiotic treatment dramatically increased antibiotic resistance gene (ARG) diversity and abundance in chimpanzees with 3.6-fold more unique ARGs and 14.2-fold increased ARG abundance (RPKM). Gorillas showed slighter increases with captivity resulting in 1.6- and 1.7-fold increases in ARG diversity and abundance, respectively. Functional metagenomic selections identified DNA contigs including ARGs and mobile elements with high similarity from wild, captive, and human libraries indicating shared resistance genotypes and possible ARG transfer between hosts. These findings highlight the impact of captivity and antibiotic treatment on non-human primates and implicate the microbiomes of non-human primates as a reservoir of antibiotic resistance genes.

Development and Characterization of a Genetically Engineered E. coli Nissle for the Treatment of Phenylketonuria

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Phenylketonuria is a genetic disease characterized by the inability to metabolize phenylalanine (Phe), which can result in neurotoxicity. To provide a potential alternative to a protein-restrictive diet, we engineered a strain of Escherichia coli Nissle 1917 to express genes encoding two distinct Phe metabolizing enzymes, L-amino acid deaminase (LAAD) and phenylalanine ammonia lyase (PAL), with the latter under the control of an anaerobically-induced promoter to drive activity in the mammalian intestinal tract. To characterize the metabolic activity of the synthetic strain, SYNB1618, we developed an in vitro simulation (IVS) model to recapitulate physiological parameters of the human upper gastrointestinal tract. IVS studies of SYNB1618 revealed a temporal response to simulated upper gastrointestinal transit that included increased expression of amino acid and peptide transporters, suggesting a predisposition to metabolize luminal Phe. We also demonstrated that SYNB1618 consumes Phe over a period of several hours and determined that the products of Phe metabolized by SYNB1618 include phenylpyruvic acid (PPA; produced by LAAD), trans-cinnamic acid (TCA; produced by PAL), and additional minor degradation products. Administration of SYNB1618 in the context of a phenylketonuria mouse model (Pahenu2/enu2) reduced blood Phe concentration by 38% compared to administration of unmodified E. coli Nissle. In mice and primates, TCA was quantitatively metabolized to hippuric acid (HA) and excreted in the urine, enabling the use of HA as a predictive biomarker of strain activity. In healthy Cynomolgus monkeys, administration of SYNB1618 resulted in dose-dependent increases in urinary and serum HA, as well as other relevant serum metabolites. In addition, dosing Cynomolgus monkeys with SYNB1618 inhibited increases in serum Phe after an oral Phe challenge. These studies indicate that SYNB1618 can impact Phe metabolism in mice and monkeys, demonstrating potential for the treatment of metabolic disorders with genetically engineered bacteria.

Emergence of Soil Bacterial Ecotypes Along a Climate Gradient

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The high diversity of soil bacteria is attributed to the spatial complexity of soil systems, where habitat heterogeneity promotes niche partitioning among bacterial taxa. This premise remains challenging to test, however, as it requires quantifying the traits of closely-related soil bacteria and relating these traits to bacterial abundances and geographic distributions. Here, we sought to investigate whether the widespread soil taxon Curtobacterium consists of multiple coexisting ecotypes with differential geographic distributions. We isolated Curtobacterium strains from six sites along a climate gradient and assayed four functional traits that may contribute to niche partitioning in leaf litter, the top layer of soil. Our results revealed that cultured isolates separated into fine-scale genetic clusters that reflected distinct suites of phenotypic traits, denoting the existence of multiple ecotypes. We then quantified the distribution of Curtobacterium by analyzing metagenomic data collected across the gradient over 18 months. Multiple ecotypes were observed with differential abundances along the gradient, suggesting fine-scale niche partitioning. However, we could not clearly relate observed geographic distributions of ecotypes with their traits and the environmental variables. Thus, while we can resolve soil bacterial ecotypes, the traits delineating the ecological requirements to coexist in highly heterogeneous soil systems remains unclear.

Comparative genomic analysis of four probiotic Lactobacillus species, L. acidophilus, L. helveticus, L. rhamnosus, and L. casei

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Lactobacillus species are members of lactic acid bacteria and are widely used as a starter culture in the manufacture of fermented food products or probiotics for health improvement, because of its high metabolic function to produce various bioactive compounds. Here, we report the genome sequence of Lactobacillus helveticus LH5 and Lactobacillus rhamnosus LR5, each of which had been isolated from a healthy Korean adult. DNA sequencing was performed using the PacBio platform to assemble the genomes and Illumina MiSeq to improve the sequence accuracy. The genome sequences of L. helveticus LH5 and L. rhamnosus LR5 were compared with those of other strains in the species, along with those of Lactobacillus casei, Lactobacillus acidophilus. Using the genomic information, we underwent a comparative genomic analysis of factors that might be involved in adaptation to the host intestinal conditions, S-layer proteins, antioxidants, and bacteriocins. We found that strains possessing a high ratio of strain-specific genes tend to have more genes for adaptation in different environments. In the same context, S-layer gene clusters and antioxidant genes were more wildly distributed among habitat-versatile strains. A number of bacteriocin gene clusters were identified using a combination of in silico prediction tools.

A comparison of sequence-based predicted metagenome tools

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The use of predicted metagenomes since PICRUSt was developed in 2013 has expanded microbial ecology work with PICRUSt garnering over 1750 citations to date. Additional metagenome prediction tools have since been developed such as PAPRICA, Tax4Fun, and FaproTax. Including PICRUSt, most of these tools have been developed and released over three years ago with no update to the genomic database upon which they were built. Tax4Fun and FaproTax rely on taxonomic assignment which can skew results dependent upon classification technique and database. PICRUSt and PAPRICA utilize a 16S rRNA gene database that new sequences can be directly aligned to, removing uncertainty in taxonomic classification. However PICRUSt and PAPRICA have not been compared in terms of performance due to the use of separate genomic databases. This study prepares an updated version of PAPRICA using the KEGG database for comparison against PICRUSt. The new PAPRICA build was validated using k-fold validation (folds=10). PAPRICA was then compared to PICRUSt using the same k-fold (folds=10) validation approach. Practical evaluation of these tools was then compared using a 16S rRNA gene data set from the Western English Channel that analyzed seasonal changes in microbial function. PAPRICA validation shows improved R2 prediction of metabolic pathways for Archaea and Bacteria as 0.931 and 0.976 respectively. Comparison of the PAPRICA to PICRUSt using k-fold validation show better predictive power of a newly constructed PAPRICA over PICRUSt. Using the Western English Channel as a test ecological study revealed that the major limitation of PICRUSt was the aging database; including only 2,590 genomes compared to 4,181 and 6,885 unique KO versus 21,078. Predicted metagenomes will continue to be a helpful tool for microbial ecologists to understand perturbations in microbial functions as they respond to their environment, therefore it will be critical for these tools to access the most up-to-date databases.

Characterization of Tree Fruit Bacterial Communities during Harvest

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Recently, the tree fruit industry became required to validate sanitizing wash water systems on-site, but food safety issues limit using actual pathogens. Therefore, we hypothesize that the natural bacterial communities can be utilized as surrogates. However, data on tree fruit bacterial community composition throughout a growing season are limited. Therefore, we characterized the bacterial communities of three types of tree fruits, apples (pome), peaches (stone), and navel oranges (citrus) during different points in the growing season with the goal of establishing a bacterial index for on-site validation. Fruits were harvested during the early, middle or late part of the growing season and rinsed with a solution (0.15 M NaCl, 0.1% Tween 20) to remove bacteria. To differentiate between viable and total community composition, we treated half the rinsate with propidium monoazide, which removed 'relic' DNA from non-viable cells. The other half was untreated and represented the total community. DNA was extracted from both rinsate samples and 16S rRNA gene sequencing was performed using two different sets of barcoded primers, 799F – 1115R (chloroplast excluding) and 515F – 926R. Barcoded amplicons were sequenced on an Illumina MiSeq (v2, 300-cycle). The UPARSE pipeline (v8.1.1861) was used to pick operational taxonomic units (OTUs) and QIIME (v1.8) was used to calculate alpha and beta diversity metrics. Taxonomy was assigned using the RDP classifier against the GreenGenes database. Analysis of total DNA demonstrated that peaches have the highest amount of bacterial diversity followed by oranges, then apples. Similarly, community composition differed significantly between the different types of tree fruit. We also observed significant shifts in overall bacterial community composition during the growing season regardless of fruit type. Nevertheless, among the viable community, Planococcaceae, Sphingomonas sp., and Bacillus flexus, were consistently present on all fruits. These taxa represent potential surrogates for on-site validating tree fruit sanitizing systems.

Adaptive strategies of the candidate probiotic E. coli Nissle in the mammalian gut

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Probiotics are living microorganisms that are increasingly used as gastrointestinal therapeutics by virtue of their innate or engineered genetic function. Unlike abiotic therapeutics, probiotics can replicate in their intended site, subjecting their genomes and therapeutic properties to natural selection. By exposing the candidate probiotic E. coli Nissle (EcN) to the mouse gastrointestinal tract over several weeks, we uncovered the consequences of gut transit, inter-species competition, antibiotic pressure, and engineered genetic function on the processes under selective pressure during both within-genome and horizontal evolutionary modes. We then show the utility of EcN as a chassis for engineered function by achieving significant reduction in serum phenylalanine levels in a mouse model of phenylketonuria using an engineered probiotic. Collectively, we demonstrate a generalizable pipeline which can be applied to other probiotic strains to better understand their safety and engineering potential.

The benefit of staying together: Growth in spatial collectives allow bacterial cells to metabolize natural growth substrates

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In natural ecosystems, bacterial cells reside within spatial assemblages and often metabolize complex plant and animal derived nutrients like complex polysaccharides, in order to fuel essential cellular anabolic processes. As a consequence, growth and metabolism of cells brings about a transformation in the nutrient environment, which can strongly influence the behavior of cells residing in the same microenvironment. What bacterial growth behaviors manifest in the presence of complex resources like polysaccharides? And how do these growth behaviors influence cellular metabolism? Here we use microfluidics and time-lapse microscopy to study the spatial and behavioral dynamics associated with growth of the freshwater bacterium Caulobacter crescentus on xylan, a ubiquitous plant derived polysaccharide composed of recurring xylose units. In the presence of polymeric growth substrates, newly divided cells stay in close proximities of each, thus effective increasing size of a cell's neighborhood. In contrast, cells grown in presence of the monomeric growth substrate, xylose, largely lead solitary lives. We find that such collective behavior in the presence of xylan is beneficial for cells for two reasons: first, to avoid a diffusional loss and thus to potentially increase the availability of both, the exoenyzme and end product: i.e. xylanase and xylose. Second, collective growth results in cells which are born in a microcolony to progressively increase their growth rates, a possible benefit of being in the proximity of other polysaccharide degrading cells. Our results elucidate the advantages of growth in spatially structured environments and potentially shed light on a likely ubiquitous and adaptive metabolic behavior in microbial assemblages.

Multidrug resistant organism transmission dynamics on ICU surfaces in the United States and Pakistan

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"Background: Multidrug resistant organisms (MDROs) can survive for extended time on environmental surfaces. This is significant in hospital settings where healthcare associated infections can transmit via abiotic surfaces. We longitudinally sampled ICU surfaces in the US and Pakistan to identify reservoirs and understand transmission dynamics of MDROs.
Methods: We sampled 5 high contact surfaces using ESwabs in 4 rooms at tertiary care hospitals in St. Louis (USA) and Islamabad (Pakistan) for one year. We cultured MDROs from the ESwabs using selective and differential agars and identified bacteria using MALDI-TOF MS. Bacteria were then Illumina whole genome sequenced. We quality filtered and assembled reads and annotated assemblies for resistance genes. Core-genome phylogenies were used to build phylogenetic trees and SNPs were used to determine bacterial clonality. Clonal isolates were analyzed for spatiotemporal linkage by permutation testing.
Results: Six Acinetobacter baumannii and two Escherichia coli were isolated from US surfaces and 289 isolates from 31 species were isolated from Pakistani surfaces. A. baumannii was the most prevalent species in both countries. 89% (67/75) A. baumannii from Pakistan were extensively antibiotic resistant. 92% and 88% of A. baumannii isolates had the carbapenemases blaOXA-23 and blaOXA-66, respectively. E. faecium was the second most common bacteria from the Pakistani collections. These isolates were universally resistant to ampicillin and vancomycin, and many were resistant to nitrofurantoin, gentamycin, chloramphenicol, and doxycycline. Spatiotemporal analysis of clonal isolates indicated that clones had significant spatial and temporal linkage.
Conclusions: There was a high burden of MDROs on ICU surfaces in Pakistan, primarily by known nosocomial pathogens. Antibiotic resistance gene annotation of the isolated bacteria identified high prevalence of clinically important resistance genes. Bacteria likely exist close in space and time to other members of their clonal groups. These results may inform infection control protocols in hospitals."

Genomic Analysis of Lonepinella koalarum isolated from Koala (Phascolarctos cinereus) Fecal Material

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Koalas (Phascolarctos cinereus) are arboreal marsupials native to Australia that eat a specialized diet of almost exclusively eucalyptus leaves. Microbes in koala intestines are known to break down otherwise toxic compounds, such as tannins. Infections by Chlamydia, obligate intracellular bacterial pathogens, are highly prevalent in koala populations. If animals with Chlamydia infections are received by wildlife hospitals, a range of antibiotics can be used to treat them. However, previous studies suggested that koalas can suffer adverse side effects during antibiotic treatment. An analysis of 16S rRNA gene sequences derived from koala feces to characterize the intestinal microbiome of koalas throughout antibiotic treatment revealed an interesting finding: a bacterium identified as Lonepinella koalarum was strongly correlated with whether or not a koala survived antibiotic treatment. In this project, we sequenced the genome of L. koalarum due to it's identified importance in koala health and survival of antibiotic treatments. We used PacBio and Illumina technologies to sequence the genome of a L. koalarum isolate, using the Unicycler hybrid assembly pipeline to combine both sequencing outputs. We further analyzed the genome with several different bioinformatic approaches to learn about relevant genes associated with the detoxification of eucalyptus leaves in the koala host.

Determining spacer protection efficiency in Legionella pneumophila type I-F CRISPR-Cas systems

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In bacteria and archaea, several distinct types of CRISPR-Cas systems provide adaptive immunity through broadly similar mechanisms: short nucleic acid sequences derived from foreign DNA, known as spacers, engage in complementary base pairing against invasive genetic elements, setting the stage for nucleases to degrade the target DNA. A hallmark of type I CRISPR-Cas systems is their ability to acquire spacers in response to both new and previously encountered invaders, which are stored in a CRISPR array. Although computational studies suggest that CRISPR array length appears to be regulated by an unknown mechanism, the impact this has on spacer protection efficiency has not been well characterized. In this work, we have leveraged the power of Legionella pneumophila, a genetically tractable, gram-negative bacterium and the causative agent of Legionnaires disease, to examine spacer protection efficiency across CRISPR arrays. Using an established transformation efficiency assay, we showed that the type I-F system in L. pneumophila is a highly protective system, and provide evidence that newer spacers are more protective than older ones. Turning to a high-throughput approach, we used a pooled transformation efficiency assay and next-generation sequencing to systematically assess the protection efficiency of 135 spacers across three different CRISPR arrays. With these experimental data in hand, we will turn to mathematical modelling in order to investigate the impact that array length and CRISPR RNA transcript length has on protection efficiency. Our work provides the first systematic study of spacer protection from both an experimental and computational perspective, filling in key knowledge gaps in the CRISPR field and laying the groundwork for future work on CRISPR array dynamics.

Genomics and Lab Cultivation Suggest a Tungsten Requirement in Group 4 Aigarchaeota

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"Aigarchaeota is a deeply branching, physiologically diverse lineage whose members mainly inhabit geothermal systems worldwide including hot springs and marine sediments. The latest 16S rRNA phylogenetic analysis of available Aigarchaeota metagenomes hypothesize the existence of 9 genus-level groups. This work focuses on members of candidate genus-level Aigarchaeota Group 4 (AigG4). Stable laboratory cultures containing AigG4 were established using corn stover incubated in Great Boiling Spring (GBS), Nevada, as inoculum. One of these lab-cultivated enrichments was used to construct an AigG4 short read metagenome bin. This bin yielded an N50 of 14,086 bp, genome size of 1.48 Mb, 84.39% genome completeness, and 0.485% genomic contamination. After including long read data in the bin, genome contiguity improved to yield a higher N50 reading of 173,436 bp, a revised a size of 1.32 Mb, 84.95% genome completeness, and no detectable genomic contamination. At first, lab maintenance of AigG4 could not be maintained in synthetic media alone except when GBS spring water was included in the medium. Previous geochemical analyses of GBS water detected the presence of ~100 nM tungsten. In addition, currently available AigG4 metagenomic bins contain putative tungsten utilizing aldehyde:ferredoxin oxidoreductases and a putative ATP binding cassette (ABC) tungsten transporter suggesting that AigG4 may require tungsten. Consistent with this requirement, addition of 0.1-2 µM sodium tungstate facilitated AigG4 growth in synthetic medium. RT-PCR further supported these findings with the detection of all predicted transcribed genes coded for tungsten utilization. Performing fluorescent in situ hybridization (FISH)-NanoSIMS will help determine whether tungsten directly affects Aig G4 through incorporation in its cytoplasm or indirectly affects Aig G4 through sustained growth of an unknown tungsten utilizing Aig G4 symbiont."

Metagenomic Analysis of Long Read Sequences from Microbial Communities from Fermented Foods

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Fermented foods, such as cheese and kimchi, contain relatively simple microbial communities compared to environmental or human microbiomes. We examine microbial communities of eight fermented foods using long read DNA sequencing to test how well current tools assign taxonomy, functions, and mobile elements of long reads and their assembled contiguous sequences. Though most of these tools were designed for short read sequences or single organism analyses, many can be adapted for use with microbial communities by expanding databases and adjusting parameters while limiting computational cost. We present a two pipelines, one that may be completed on a laptop computer, and one that can take advantage of high performance computers to increase accuracy. The relatively low cost of owning a nanopore sequencer as well as standardized protocols can democratize the study of metagenomes and microbial communities while easing comparisons between these studies.

A systematic approach for the secretion of human cytokine IL-10 from Pediococcus pentosaceus SL4

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Besides the beneficial effects of probiotics for human health that have been studied, a number of research focused on using lactic acid bacteria for disease therapy such as intestinal cancer and inflammatory diseases. We attempted to engineer the generally-recognized-as-safe strain Pediococcus pentosaceus SL4 to secrete the therapeutic protein IL-10 that is an anti-inflammatory cytokine. A systematic approach to attain this goal comprises the following consecutive steps: in silico analysis and mining of signal sequences from the genome using SignalP 4.1, two-dimensional gel electrophoresis and peptide mass fingerprinting analyses of the secretome, selection of potential signal sequences and genetic fusion with the gene encoding IL-10, and then western blot and activity analyses of secreted IL-10. As a result, we have selected the signal peptide of LysM that is an N-acetylmuramidase, and expect that this signal peptide would be suitable for secretory expression of heterologous proteins in P. pentosaceus SL4.

Genomics-informed cultivation of a member of the candidate phylum Atribacteria (OP9)

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The Atribacteria (OP9) is a candidate bacterial phylum with no cultivated representatives, members of which are found in a variety of natural and man-made environments including marine sediments, petroleum reservoirs, anaerobic digesters, and geothermal springs. Nearly-complete genomes of two species of Atribacteria ("Candidatus Caldatribacterium") were previously obtained from sediments and in situ cellulosic enrichments from two Great Basin hot springs using single-cell genomics and metagenomics approaches. These genomic data, suggesting an anaerobic, fermentative, saccharolytic lifestyle, have been used to inform enrichment strategies targeting "Caldatribacterium" from Great Boiling Spring (GBS), Nevada. Initial anaerobic enrichment cultures using xyloglucan as a carbon source and inoculated with corn stover incubated in GBS allowed maintenance of "Caldatribacterium" at ~5% relative abundance based on FISH and qPCR. Further enrichments using various sugars as sole carbon sources, followed by several rounds of dilution-to-extinction using fucose yielded highly enriched cultures with "Caldatribacterium" present at ~95% relative abundance. Various approaches, including metagenomics and 16S rRNA gene tag sequencing, indicated that these enrichments were likely a co-culture of "Caldatribacterium" and a member of the genus Thermodesulfobacterium. Although attempts at isolation of "Caldatribacterium" were not successful, the Thermodesulfobacterium was isolated and characterized, and represents a new species in this genus; it is capable of using hydrogen, formate, and lactate as electron donors, which may enable syntrophic growth with "Caldatribacterium" when grown on fucose. This putative co-culture can serve as a basis for understanding the physiology and ecology of Atribacteria in hot springs and other environments.

Characterization of the mycobiome of the seagrass, Zostera marina, across time and space

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Seagrasses are globally distributed marine flowering plants that are foundation species in coastal ecosystems. Seagrass beds play essential roles as habitats and hatcheries, in nutrient cycling and in protecting the coastline from erosion. Although many studies have focused on seagrass ecology, only a limited number have investigated their associated fungi. In terrestrial systems, fungi can have beneficial and detrimental effects on plant fitness. However, not much is known about marine fungi and even less is known about seagrass associated fungi. Here we used culture-independent sequencing of the ITS region to survey the taxonomic diversity of fungi associated with the seagrass, Zostera marina, to tease apart the importance of these associations. To assess the diversity of fungi across spatial scales, we sampled from two seagrass beds in Bodega Bay, abbreviated WP and GP, and we dissected cores from WP to investigate intra-plant fungal diversity. Additionally, to assess diversity across time, we sampled from WP over three two week intervals. Preliminary analyses indicate that there are many fungal taxa for which a taxonomic assignment cannot be made living on and inside seagrass leaves, roots and rhizomes and that these plant tissues harbor distinct fungal communities. For example, the most prevalent amplicon sequence variant inside Z. marina leaves was classified as fungal, but was unable to be assigned to a phylum. We also found evidence of dark septate endophytes (DSE) living inside the leaves of Z. marina. DSE are known to colonize terrestrial plants, have been cultured from other seagrass species and are a morphological, not phylogenetic, group that is largely uncharacterized. DSE can be pathogens, but have also been shown to increase plant nutrient content and growth. This work highlights a need for further studies focusing on marine fungi and the potential importance of these understudied communities to the larger seagrass ecosystem.

A tale of two microbes and the role oxygen plays in their metabolic interactions

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The airways of people with Cystic Fibrosis become chronically infected with slow-growing, antibiotic-resistant opportunistic pathogens such as Pseudomonas aeruginosa. Anaerobes associated with the oral cavities have also been identified in patients' airway secretions, yet these bacteria are often overlooked in a clinical setting. While oral anaerobes are rarely destructive in healthy individuals with effective airway clearance, they can become dangerous in airway infections. For example, oral microbes produce volatile fermentation products that are both toxic to the patient and affect the physiology of opportunistic pathogens. One major environmental factor that drives bacterial fermentation and respiration is oxygen. Although CF sputum contains steep oxygen gradients, most CF microbiology studies disregard the effect of hypoxia on microbial interactions and physiology. We hypothesized that oxygen impacts the metabolic interactions between two CF isolates with different metabolic capabilities: P. aeruginosa and the oral facultative anaerobe, Rothia mucilaginosa. Using stable-isotope metabolomics, we found that P. aeruginosa utilized labeled substrates derived from R. mucilaginosa to generate different primary metabolites in low oxygen. We also used fluorescence lifetime imaging microscopy (FLIM) of NADH, a label-free method that measures NADH utilization, to track changes in sub-cellular metabolism of P. aeruginosa. Our FLIM results indicated a shift in bacterial central metabolism in different oxygen levels and during cross-feeding interactions. Taken together, our results indicated oxygen is an important driver of P. aeruginosa physiology and affects how P. aeruginosa utilizes R. mucilaginosa-derived metabolites. This work was supported as a pilot project from the UC Davis West Coast Metabolomics Center funded by NIH DK097154, and T.G. is supported through the BEST IGERT program funded by the National Science Foundation DGE-1144901.

Comparative genomics analysis of Salmonella Enteritidis clinical isolates from China

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Salmonella Enteritidis is a well-known pathogen causing food-borne disease in human with infected poultry as the most common reservoir. Genomic investigation of S. Enteritidis would provide important resources that help to understand its pathogenicity, tract its global spreading, and develop novel intervention strategies. The current study applied the whole genome shotgun sequencing (WGS) to analyze 20 clinical S. Enteritidis isolates that were collected from Shanghai, China, between 2010 and 2016. The WGS data of the clinical S. Enteritidis from the United States during the same period of time (covering 30 states) were downloaded from the Pathogen Detection database hosted by NCBI. k-mer-based single nucleotide variation (SVN) comparative results revealed a total of 15,845 SNV sites including 627 core SNVs and showed that China isolates were well separate from the US isolates except for two that formed subclonal cluster with three isolates from Minnesota and three isolates from NY State. The WGS data were further profiled against Resfinder database for antibiotic resistance gene identification. Among the isolates we studied, only 3 (including one from China and 2 from the US) were free of antibiotic resistant genes. Isolates from China apparently tended to be more multi-antibiotic resistant (75%, 15/20) when compared with the isolated from the US (12%, 27/220). One isolate from China even harbored 15 antibiotic resistance genes. In conclusion, the WGS analysis indicated that S. Enteritidis isolates between China and the US have evolved differently and present distinct antibiotic resistance characteristics.

Genomic Comparison of E. coli Genomes from the Bladders of Women with and without Urinary Tract Infections

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Escherichia coli is the most frequent cause of urinary tract infections (UTIs). Uropathogenic E. coli (UPEC) strains are both phenotypically and genotypically diverse. Previous analyses of E. coli genomes have not found a single gene indicative of uropathogenesis. Rather numerous virulence genes have been associated with UPEC strains. However, these virulence genes and pathogenicity islands also have been found within strains characterized as commensals. This raises the question: Are these commensal strains really UPEC strains? This would imply an impending infection or some barrier to infection. In an effort to unravel the genetic underpinnings of UTIs, we isolated 75 E. coli strains from catheterized urine samples of women. Forty-two of these samples were from women who had a UTI at the time of collection. Here, we present our genome sequencing and annotation results for these 75 strains. Genomes varied in size from 4.6 to 5.6 Mbp, with some strains harboring plasmids. Genome annotations uncovered various known virulence factors associated with uropathogenesis. From this study, we have identified genetic indicators of UPEC strains.

Persistent metagenomic signatures of early life antibiotic treatment in the infant gut microbiota and resistome

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Preterm infants are vulnerable to infection, with frequent and often prolonged exposures to antibiotic therapy during hospitalization. While others have described the acute effects of antibiotics on the preterm infant gut microbiota and resistome, the effects of early life antibiotic treatment that may persist following discharge from the neonatal intensive care unit remain unclear. Here, we use complementary metagenomic and culture based techniques to investigate the gut microbiota and resistome of extremely preterm infants both during their hospitalization and following discharge to home, as well as healthy infants sampled synchronously. We find evidence of microbiota immaturity, prolonged carriage of multidrug resistant Enterobacteriaceae, and distinct patterns of microbiota and resistome assembly in extremely preterm infants who receive early life antibiotic therapy. Our results reveal persistent collateral damage of early life antibiotic treatment and hospitalization in preterm infants and highlight the necessity for alternative strategies for infection management in highly vulnerable neonatal populations.

Comparative Metagenomic Analysis Identifies Conserved Genomic Diversity and Response to Perturbation in Vertebrates

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An expanding body of evidence indicates that human health is intricately associated with gut microbiome function. However, the mechanisms that define this relationship are unresolved. Identification of these mechanisms will transform our understanding of dysbiotic diseases and will aid in the development of microbiome-based therapeutics and diagnostics. High-throughput animal model systems, such as zebrafish, would greatly accelerate the ongoing effort to elucidate these mechanisms. However, limited knowledge of zebrafish gut microbiome functional diversity limits our ability to leverage this important model system. Therefore we quantified the functional diversity of the zebrafish gut microbiome. Specifically, we assembled ~600 million paired-end shotgun metagenomic reads generated from 29 adult zebrafish fecal samples. A total of 1.5 million non-redundant genes were identified in the resulting assembled contigs, many of which were assigned a functional homologue using publically available gene family databases. As microbial gene families that are consistently distributed in vertebrate gut microbiomes likely interact with host physiology, we then quantified shared functional microbiome diversity between our zebrafish gene catalogue and those of mice and humans. We found that the majority of zebrafish microbiome genes have human or mouse homologues despite little overlap in taxonomic composition of these communities. Fish and mice microbiomes also shared similarity in their response to consumption of different diets. For example, mice and fish fed zinc deficient diets both had reduction in the abundance of pathways associated with bile acid biosynthesis and an increased abundance in pathways involved in the production of antibiotics. Finally, we find that microbial gene family abundance differences associated with diet correlated with zebrafish intestinal gene expression. These results indicate that despite disparities in taxonomic composition vertebrate microbiomes possess similar functional diversity and microbiome operation. Moreover, the congruent responses to diet argue that discoveries in zebrafish may be translationally relevant in mammals.

Biogeography of the skin-associated microbiome across body regions of the Sierra Nevada yellow-legged frog (Rana sierrae)

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The Sierra Nevada yellow-legged frog, Rana sierrae, has been driven close to extinction in part by the amphibian fungal pathogen Batrachochytrium dendrobatidis (Bd). Skin-associated microbes can inhibit Bd infection, and several studies have shown that overall microbial community structure may result in distinct disease outcomes for the host. In addition, previous work has shown that Bd infection in frogs is primarily limited to the ventral abdomen, legs, and hind-feet. The central goal of our project was to assess whether the microbiome is heterogenous across distinct regions of the skin of an individual, and whether differences in community structure correspond to regions of the skin where Bd preferentially infects. We collected skin swabs from 10 distinct body regions of Rana sierrae individuals (n=13) at the San Francisco Zoo. We sampled Rana sierrae at the Zoo because they were reared there from tadpoles in order to reintroduce them in parts of the Sierra Nevada where they had previously gone extinct. We conducted Illumina sequencing of 16S rRNA genes that were amplified using "universal" bacterial primers. This targeted sequencing approach was used in order to look for differences in the taxonomic composition of microbiomes within individuals. We found that across most regions of the body, microbial community composition appeared relatively homogenous. However, the microbiome of the hind-feet had significantly different composition than other body regions. When we compared the microbial composition of the hind-feet, abdomen, and back, we saw that the relative abundance of the family Burkholderiaceae (Phylum Proteobacteria) on the hind-feet and abdomen were significantly higher than on the back. Some members of this family are known to inhibit Bd through production of the anti-fungal metabolite violacein. Their enrichment on the abdomen and feet supports our hypothesis that specialization of the microbiome corresponds to regions where Bd infects the skin.

Prebiotics Induced Oral Microbiota Changes Accompany Long-lasting Allergy Relief

Cliff Shunsheng Han*

Knoze Jr Corp

Finding the allergy reason that can be easily mitigated is the foundation to stop the allergy epidemic started about 50 years ago. The hygiene hypothesis has been proposed to explain the increase of allergy diseases and supported by numerous studies. However, attempts of using probiotics to cure or prevent allergy diseases have had limited effects. Saliva samples were collected from a subject for 3 years during which time the subject experienced yearlong allergy, seasonal allergy, and remission of allergy symptoms. Bacterial DNA was extracted and 16S rRNA genes were profiled with Illumina sequencing technology. Here I illustrate that the restructuring of oral microbiota may have led to lasting remissions of allergic rhinitis. I found that Veillonella and Streptococcus were less abundant when allergy symptoms were worse and more abundant in family members without allergies. A composition made to stimulate the growth of these bacteria can relieve allergy symptoms temporarily. A combination of substantial removal of the oral biofilm using physical means, including a local pyrotherapy, and usage of the composition lead to long-lasting remissions of allergy symptoms. These results indicate that one of the major causes of allergic rhinitis is likely the lack of metabolites from mutualism between Veillonella and Streptococcus, such as short chain fatty acids. Restoring the abundance of these bacteria may alleviate or even cure the disease. The easy destruction of oral biofilm by a moderate fever indicates a possible natural negative trigger that subsequently increases the efficacy of the immune system previously restrained by metabolites from microbiota. I name this mechanism as Theory of Negative Trigger. This mechanism could explain how fever increases immunity and helps the body fight infections and even cancers. This microbiota switch for the power of the immune system may be manipulated readily at will for clinical applications.

Comparative genomics of human associated Akkermansia muciniphila

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Akkermansia muciniphila is a mucin-degrading gut bacterium present in greater than 70% of humans and numerous recent studies have indicated that it may be a beneficial member of the human microbiome. However, the genomic content and metabolic diversity of human associated Akkermansia species is understudied as only one species has been formally described. To begin to fill this knowledge gap, we reconstructed 35 high-quality metagenome assembled genomes (MAGs) from fecal samples of 72 post-weaned children aged 2-9 years. Thirty-one of the 35 MAGs are >90% complete with <1% contamination. On average, each genome is approximately 2.9 Mbp in length with a coding density of 88.1%. To study the pangenome of human associated Akkermansia, we combined our MAGs with an additional 40 publically available genomes, 34 from adult humans and six from mice. Using this dataset, we identified at least 6,557 core genes, of which 2,780 (42.4%) were assigned COG functions. Based on average nucleotide identities (ANI), at least four phylogroups (AmI – IV) of human associated Akkermansia were identified. Each phylogroup contains lineage specific genes including an increased abundance of CAZymes in AmII, III, and IV compared to Am I, which includes the type strain A. muciniphila MucT. Other differentially present genes include various transporters and mobile genetic elements including plasmid and phage associated genes. Overall, these findings broaden our understanding of the genomic diversity of the Akkermansia lineage and provide clues of functional differences among phylogroups possibly delineating novel species.

An emerging human fungal pathogen, multidrug-resistant Candida auris

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"Fungi are becoming increasingly resistant to the few classes of antifungal drugs. Specifically, NIAID/NIH and CDC list azole resistant Candida spp. as urgent threats, as they account for an increasing proportion of bloodstream infections. This concern was heightened with the recent identification of a novel human pathogen, Candida auris, which has already developed resistance to all three classes of antifungals. C. auris bloodstream infections can be recalcitrant to treatment, exacerbated by the organism's high antimicrobial resistance. Further complicating infection control, C. auris colonizes a patient's skin and persists on environmental surfaces, enabling its spread in healthcare settings.

We aim to characterize the fungal and bacterial communities on the skin of patients colonized with C. auris. Sequencing CDC samples taken in focal outbreaks of long term acute care and skilled nursing facilities, we observed fungal and bacterial dysbiosis on the skin of these patients. While the skin of healthy volunteers is dominated by Malassezia spp., many of the patients' skin fungal communities were dominated by Candida spp., C. auris, C. albicans and others. The bacterial communities on these same patients were dominated by Proteobacteria spp. more commonly considered as healthcare-associated pathogens, including Acinetobacter, Klebsiella and Pseudomonas spp., compared to the skin of healthy volunteers, which is dominated by Corynebacterium, Propionibacterium and Staphylococcus spp.

Directed by the clinical data, we established a murine C. auris colonization model to assess risk factors for colonization. We tested wild-type, diabetic and immune deficient mouse models for colonization and identified a population of immune cells whose absence promotes long term C. auris colonization. This translational research program starting from clinical data to mouse models has the potential to inform clinical practices for patients with a higher risk of C. auris colonization."

Discovery and Biosynthesis of Bacterially Produced Inflammatory Plasmalogens from the Human Gut Microbiota

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The human microbiota is comprised of a diverse group of microbes living within our bodies and has been linked to many diseases including type 1 and type 2 diabetes, Crohn's disease, metabolic syndrome, and autism. The microbiota plays a key role in maintaining health, largely by producing molecules that can be sensed by the host. We set out to identify new molecular signals produced by gut microbes that may be important for regulating host-microbe interactions. We created a fractionated extract library of metabolites from disease-associated microbes and conducted mechanism-informing immune-based bioassays. Using this strategy, we identified a new family of inflammatory plasmalogen glycolipids produced by the human gut microbe Collinsella aerofaciens. We then developed a color-based assay to detect plasmalogens directly in bacterial colonies and performed a transposon screen to identify genes involved in bacterial plasmalogen biosynthesis. To better understand the role of plasmalogens in bacteria, we subjected plasmalogen-deficient mutants to various stresses and observed effects related to salt tolerance and oxidative stress. In summary, this work describes a strategy to discover bioactive molecules from the human microbiota and highlights progress towards understanding the biosynthesis and function of bacterially produced plasmalogens in the context of host-microbe interactions.

Linking viruses to hosts from across the tree of life using more than 2000 single-cell genomes

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Viruses are abundant, ubiquitous drivers of microbial ecology and evolution, but our knowledge of them remains biased towards certain microbial taxa and habitats. Much progress has been made towards the challenge of viral sequence detection in metagenomes utilizing hallmark genes and common genomic features, but assigning hosts to viral sequences is often difficult. Here we leverage a unique dataset of 2266 single amplified genomes (SAGs), including many candidate phyla, to directly associate viruses with their hosts. Four different established viral annotation pipelines were used in combination to identify putative viral contigs and prophages within the SAGs. Over 70% of all viral contigs found were predicted by only a single method, and very few were predicted by all four methods. Putative viral contigs were detected in 32% of SAGs, and were most prevalent within Aquificae, Candidate division GAL15, and Firmicutes, but the proportion of SAGs containing viral contigs varied widely between phyla. Potentially novel viruses without a close match in the IMG/VR database were found in 657 SAGs, most abundantly in Microgenomates, Aquificae, and Omnitrophica. Potentially novel viruses were also abundant in SAGs that could not be classified, some of which may represent lytic viral infections. For select phyla, we also examined the prevalence of viruses and the assortment of viral types across multiple sampling sites and habitat types. Further investigation into possible co-infection of single host cells by multiple viruses, and metabolic genes within viral contigs is ongoing. This dataset offers insights into viruses infecting uncultivated and poorly understood microbial lineages, and how they may shape the ecology and evolution of their hosts.

A glimpse into the genomic plasticity of multidrug-resistant Acinetobacter baumannii isolates collected from a single institution

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Acinetobacter baumannii is one of the most important multidrug-resistant (MDR) bacterial pathogens, causing nosocomial infections worldwide. Herein, we sequenced and analyzed the genomes of 98 MDR A. baumannii strains collected from a single institution in Seoul, Korea, during 2009□2015. Core gene phylogenetic analysis and overall genome-related index-based clustering grouped the strains into five clades, the largest of which was ST191 (n = 60). Interestingly, 94.7% of strains possess alternative gdhB alleles (#189) at nonhomologous loci (~150 kb apart from each other), implying frequent chromosome rearrangement and/or recombination. Despite using the Illumina short-read sequencing platform, 115 complete plasmid sequences were successfully reconstructed using Unicycler assembly of paired reads of plasmid origin, recruited from reads mapped to reference plasmid sequences identified by Plasmid Profiler. Plasmids were clustered into six groups similar in terms of sequence (rep genes and entire sequences) and length, and two groups were invariably carried by nearly all strains. Unexpectedly, we did not detect any resistance genes from plasmid sequences or very short AbaR-like resistance islands, despite the widespread distribution of resistance genes throughout genome assemblies. In summary, genome plasticity caused by chromosomal recombination and dynamic plasmid assortment appears to be the major factor shaping A. baumannii genomes disseminated following antimicrobial treatment.

Revealing potential antimicrobial resistance gene mobility trends using >15000 replicons

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Advances in genomics has transformed infectious disease surveillance and assessment of public health risks associated with antimicrobial resistance (AMR). Nevertheless, limitations still exist in understanding the mobility of resistance genes, which is needed for better and more focused risk assessment of AMR spread in pathogens of both human health and agri-foods interest. To date, some AMR genes have been empirically observed to be highly mobile, contributing to clinically-relevant resistance that appears to circulate between pathogens. Despite these observations, no large-scale study has answered the question of whether AMR genes are indeed strongly associated with mobile elements, including plasmids and genomic islands comprising phage, integrons, transposons etc. Here, we present the first comprehensive examination of AMR association with mobile elements across all NCBI refseq bacterial isolates sequenced to date, totalling ~16600 bacterial replicons. AMR profiles for these replicons were predicted using the Resistance Gene Identifier and mobile chromosomal elements or genomic islands (GIs) were predicted using IslandViewer 4. Statistical tests were performed of the association between AMR genes and predicted non-mobile chromosome sequences, GIs and/or plasmids – using the full dataset and datasets with sampling bias reduction. This large-scale analysis reveals that AMR genes, collectively, are disproportionately found in mobile regions of the genome. However, classification of AMR genes into higher-level categories (e.g. resistance mechanisms) using the Antibiotic Resistance Ontology, identifies certain drug classes and resistance mechanisms that are significantly more associated with mobile or non-mobile sequences. Notably, AMR resistance mechanisms that are specialized in functions tend to be more mobile. Using these data, we propose the beginnings of an evolutionary model that would predict which AMR genes may be more likely laterally transmitted, with the future goal of potentially aiding risk assessment of AMR transmission and prioritization of policies for different antimicrobial classes regarding appropriate antimicrobial use.

Identifying reservoirs of antimicrobial resistance in faecal microbiota using high resolution chromosome conformation capture

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Bacterial infectious diseases contribute to more than 54% of global infectious disease and the ever-increasing multitude of antimicrobial resistant (AMR) pathogens is of global concern for animal and public health. Acquired resistance traits are often located on mobile genetic elements (MGEs). Next generation sequencing (NGS) has substantially increased our understanding of the microbiome, but current NGS analysis fails to accurately identify the location of specific genes of interest within any single host genome of a mixed community. Chromosome conformation capture and its derivative Hi-C are powerful techniques that allow the physical crosslinking of prokaryotic genomes and the associated MGEs. This study aims to demonstrate AMR gene identification within complex microbial communities, and localize mobile genetic elements to their host bacteria, without the need to culture, using next generation sequencing and bioinformatics. De novo genomic assemblies were constructed from shotgun metagenomic NGS data. After mapping Hi-C fragments to the contigs, pairwise inter-contig connections were used to define core-communities representing one prokaryotic genome or a group of very similar genomes. We used Ariba for AMR gene identification in raw reads and BLAST to find those genes in our final core-communities. Our final core communities mapped to published genomes and MGEs with high identity (>98%) and coverage (>95%). Within these communities, we have identified all known AMR genes and were able to associate 22/23 o them to the known host bacteria. Our technique allows for culture-free identification and tracking of MGEs (potentially containing AMR genes). This technique will allow us to study the effects of antibiotic use on microbial communities, and the acquisition of resistance at a population level in hosts, ultimately leading to better treatment regimes.

ITS Demonstrated Protocol and BaseSpace™ Analysis for Fungal Metagenomics Application

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DNA barcoding of fungi is an effective method for characterizing the mycobiome and fungal biota within complex microbial communities. The internal transcribed spacer (ITS) has emerged as the primary barcode of choice for fungal studies due to ease of amplification and accurate classification. Illumina sequencing technology enables such fungal metagenomics studies, allowing for sensitive discrimination and multiplexing of amplicon libraries. One caveat, however, is taxonomic bias during PCR as some taxa have less unique sequences than others. Several studies have addressed this issue with in silico analyses to design primer sequences that have more taxonomic coverage. However, it is difficult to amplify all members of the vast and diverse Fungal kingdom with a single primer pair. Furthermore, while Illumina currently offers a user-friendly BaseSpace™ Sequencing Hub (BSSH) analysis solution for bacterial amplicon sequencing via the 16S Metagenomics Application, a similar solution for fungal sequencing has yet to be offered. Here we present a demonstrated 2-step multiplex PCR (mPCR) workflow for ITS1 amplification, sequencing, and analysis. This includes a primer set designed to address gaps in taxonomic coverage and a user-friendly BSSH analysis solution. The newly designed primer set was generated from an in silico PCR analysis on the SILVA and RDP Warcup Training set databases to optimize taxonomic coverage. The newly released ITS Metagenomics Application on BSSH utilizes the RDP classifier to classify samples against the curated UNITE ITS database. Analysis reports include: taxa read counts, diversity statistics, and visualization tools for characterization of samples. To demonstrate feasibility with several sample types, this workflow was tested with microbial community standards, fungal isolates, and real-world environmental samples. This simple workflow enables fungal metagenomics studies by providing an optimized primer pool and a supported, easy-to-use data analysis and storage solution.

Ancient metabolic evolution: atomic fossils and their synergy with molecular clocks

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Microbial metabolic innovation has driven dramatic changes in Earth's atmosphere and ocean since the proliferation of life. For example, the products of methanogenesis (CH4 – greenhouse gas), oxygenic photosynthesis (O2), and denitrification (N2 and N2O – greenhouse gas) have regulated climate and the cycling of bioessential elements (C.H.O.N.P.S) since their respective geneses. The timing of these metabolic inventions and so also the timing of their global influences, however, remains poorly constrained. The atomic fossil record – isotopic ratios of various elements indicative of specific metabolisms – provides firm minimum ages for the evolution of certain metabolisms. However, poor preservation yields an Archean fossil record that is notably sparse, preventing high temporal resolution. Molecular clocks, with the rapid accumulation of genetic sequence data and advances in Bayesian molecular clock dating methodology, have become useful tools that work in concert with the fossil record to elucidate early metabolic innovation. This synergy stems from molecular clock reliance on fossil and/or geologic calibrations to prescribe absolute geologic dates for microbial evolution. Here, I review the current methods of exploring the atomic fossil record through isotope biogeochemistry. As a case study, I discuss recent findings of the oldest robust evidence for nitrification and denitrification 2.66 billion years ago, and evidence for oxygenic photosynthesis over 300 million years before the Great Oxidation Event. More recent analytical techniques that measure atomic fossils on the micron scale, such as elemental mapping coupled with secondary ion mass spectrometry, will help characterize ancient metabolic diversity, leading to better calibrations for molecular clocks. Ultimately, multiple independent biogeochemical proxies can provide firm minimum and soft maximum ages for metabolic evolution, that when used as calibrations for molecular clocks can better constrain the timing of metabolic innovation and environmental change.

Genomic and Transcriptomic Reconstruction of a Marine Flavobacterium with Microbial Rhodopsins

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Microbial rhodopsins are commonly found in marine prokaryotes and function as light-driven ion pump to allow microbes to use light as an energy source and survive harsh environments. Proteorhodopsin-containing marine microbes such as those in the class Flavobacteria play a pivotal role in the biogeochemical cycle of the euphotic zone. The genome sequence analysis of marine flavobacterium Nonlabens (Donghaeana) dokdonensis DSW-6 uncovered a gene encoding an unexpected type of microbial rhodopsin containing a unique motif in addition to a typical proteorhodopsin. This novel rhodopsin turned out to be a sodium ion pump. To infer the biological role of these sodium- and proton-pumping rhodopsins in DSW-6, the gene expression levels under various environmental conditions were examined. The gene expression of the novel rhodopsin increases with increasing NaCl concentration, as well as in the presence of light and the absence of nutrients. Metagenomic surveys demonstrate the diversity of the novel rhodopsins in nature and the prevalent occurrence of the encoding genes among microbial communities inhabiting hypersaline niches, suggesting its involvement in sodium metabolism and the sodium-adapted lifestyle. We also survey transcriptional responses of DSW-6 during the growth under nutrient limitation and salinity stress in the light and the dark. It helps understanding the physiological features that allow survival in marine oligotrophic environments as a marine photoheterotroph, and estimating the ecological impact of the microbial rhodpsins.

Superbugs of a different sort: phenotypic diversity of toxin tolerance in a methylotrophic microbe

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"While microbiologists often make the simplifying assumption that genotype determines phenotype, it is becoming increasingly apparent that phenotypic diversity (in which one genotype generates multiple phenotypes simultaneously) is common in many microbial populations. Non-genetic diversity is often hypothesized to be a beneficial strategy for survival in unpredictably fluctuating environments, and has frequently been observed in stressful conditions such as toxin exposure (or shifts in carbon substrate availability. Here, we describe a novel instance of phenotypic diversity in which central carbon metabolism and toxin resistance interact.

Methylobacterium extorquens is a ubiquitous plant epibiont, capable of growing on the methanol periodically released from plant leaves. The first intermediate in methanol metabolism is formaldehyde, a potent cellular toxin that is lethal in high concentrations. Yet we have found that at moderate concentrations, formaldehyde tolerance in M. extorquens is heterogeneous, with individual cells ranging between 2 mM and >5 mM in their maximum tolerance levels. This heterogeneity is both continuous (in the range of maximum tolerances possible) and discrete (at a given formaldehyde concentration, cells either grow normally or die, with no intermediate phenotype). And it has population-level consequences: growth of a few tolerant individuals can rescue a population. We present results from bulk liquid culture experiments, flow cytometry, time-lapse microscopy, and genome resequencing, which show that this diversity is not due to genetic mutation, and that cells can shift their tolerance phenotypes in response to growth conditions. Finally, we use mathematical modeling with partial differential equations to better understand the processes by which cells change phenotype. While the molecular mechanism for phenotypic formaldehyde tolerance is still unknown, we propose that it may be an adaptation to the unpredictable conditions of life on the plant leaf, and presents a unique system in which to study the role of non-genetic diversity in environmental microbial populations."

A novel culture-independent functional assay for identifying, sorting, and sequencing antibiotic resistant microbes from environmental samples

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Bioinformatic analyses and traditional functional metagenomic screens can uncover antibiotic resistance genes, but both methods have limitations. Informatic approaches cannot easily predict the complete range of antibiotic compounds and concentrations for which resistance genes are effective, while functional metagenomics (e.g. fosmid/BAC libraries) only reveals genes that are compatible in heterologous expression hosts. Here we present a novel functional assay that overcomes these limitations and enables screening and recovery of uncultured, antibiotic resistant cells from environmental samples using fluorescence activated cell sorting (FACS). Briefly, environmental samples are incubated with antibiotics that inhibit transcription or translation. Next, cells that are still synthesizing protein are fluorescently labeled using BioOrthognal Non-Canonical Amino acid Tagging (BONCAT). Antibiotic resistant cells labeled by BONCAT are then sorted using FACS, and their genomes recovered using single cell genomics and metagenomics. In a proof of concept focusing on a freshwater pond, we found the proportion of total cells synthesizing protein changed dramatically depending upon the concentration of antibiotic added to each sample. For example, only ~1% of cells were synthesizing protein after a 2hr incubation with either 25ug/mL chloramphenicol or 50ug/mL—concentrations typically used in selective agar plates—compared to ~75% cells when grown without antibiotics. Sequencing of flow sorted cells revealed massive enrichments of antibiotic resistance genes in BONCAT-labeled cells compared to the whole community. Thus, BONCAT+FACS enable a novel functional assay to identify and sequence unculturable, antibiotic resistant bacteria from the environment. This method bypasses limitations of traditional functional metagenomics which requires compatibility with heterologous expression hosts, and it provides insights into the function of antibiotic resistance genes (e.g. the specific compounds and concentrations to which they provide resistance) that cannot be gleaned from bioinformatic analysis alone.

The soil microbiome and toxicology: from traditional tests to transcriptomes

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Soil is an important natural resource that underpins many ecosystems on Earth. Our lab conventionally uses toxicity endpoints derived from a battery of tests to assess the effects of contaminants on soil microbial health. Recently, high-throughput sequencing has opened the door to affordable, high-quality metagenomic and metatranscriptomic techniques for characterizing nucleic acids in soils. Genomics-based methods are often less laborious than traditional endpoints, require less starting material, and can provide detailed data on community structure and function. We aimed to compare the correlation of metagenomics-based endpoints with our established soil microbial health endpoints. We spiked a healthy sandy loam soil (Vulcan, Alberta, Canada) with 0, 60, 145, 833, and 2000 mg/kg of silver nanoparticles (AgNPs). Assessments performed using traditional tests showed the toxicity of AgNPs on soil microbial processes (Samarajeewa et al., 2017, Environ. Pollut. 220:504). We expanded this analysis by using 16S/ITS amplicon sequencing, whole-metagenome DNA sequencing, and whole-metatranscriptome sequencing to determine if taxonomic and gene expression changes in microbial communities correlate with known indicators of soil health. Sequences were analyzed using DADA2/Phyloseq, MG-RAST, and edgeR. Ordination analyses revealed that microbial communities cluster strongly by AgNP treatment within amplicon, shotgun, and metatranscriptome sequence datasets, marked by the broad-spectrum toxicity of AgNPs to multiple soil bacteria as well as the emergence of the silver-tolerant genus Rhodanobacter. In addition to identifying taxa that are influenced by AgNPs, we found that genes involved in heavy metal efflux were highly upregulated in the presence of silver (e.g., czcA, log2 fold-change 3.5-4.1, FDR<0.01). Various genes involved in heavy metal resistance and toxicity response were also upregulated in AgNP-treated soils. Future studies could employ curated gene lists targeting specific endpoints for in silico analysis. These results pave the way for the use of genomics-based technologies in soil microbial health assessment.

Cultivation, genomics, and FISH-based detection of an Aigarchaeota Group 1 lineage

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Aigarchaeota, a deeply branching lineage in the domain Archaea with no cultivated representatives, includes both thermophilic and hyperthermophilic microorganisms that reside in terrestrial and marine geothermal environments. The Aigarchaeota consists of at least nine proposed genus-level groups that have been confirmed via 16S rRNA sequencing, with Group 1 Aigarchaeota (AigG1) being the focus of this study. Based on cultivation-independent genomic data available from several AigG1 members in Great Boiling Spring (GBS), NV, and Yellowstone National Park, 22 different types of growth media were designed and tested for their ability to support growth of AigG1. These media were inoculated in the field with freshly collected GBS sediment, incubated at 80°C in the laboratory, and transferred (1/100 vol.) after 3-4 weeks of growth. Of the 22 growth conditions, only two were found to support significant growth of AigG1 based on 16S rRNA gene tag sequencing. These growth conditions included hot spring mat extract with 2% oxygen and casamino acids with 10% oxygen. AigG1 ranged from 1-20% of the total population of microorganisms, reaching densities up to 107 16S rRNA gene copies/mL. Metagenome sequencing from one of these laboratory cultures, using both Illumina and Oxford Nanopore platforms, yielded a ~1.43 Mb AigG1 bin with an N50 of 542,176 that is ~97% complete, a significant improvement on previous AigG1 bins and single-cell genomes. An AigG1 probe was developed and tested using Clone-FISH and CARD-FISH techniques, with probe signal being significantly stronger than the only previously published AigG1 probe. This work will facilitate use of FISH coupled with nano-scale stable isotope mass spectrometry (nano-SIMS) to track the uptake of 13C labeled compounds by AigG1 in future studies of their catabolic capabilities. It will also help enable morphology-based single-cell sorting of AigG1 to obtain pure or further-enriched cultures.

Hi-C reconstructs genomes and plasmid-host dynamics in a wastewater community

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Phase Genomics

Horizontal transfer of plasmid-borne antibiotic resistance genes (ARG) is an important contributor to the evolution of multi-drug resistant bacteria. A wide range of environments have been pointed out as reservoirs of the ARG we find in today's pathogens. However, limited information is available to identify their hosts and the plasmids that carry them in these ecosystems and may spread them to pathogens. This limitation is due to our poor ability to link metagenomic sequences to each other or to specific organisms. Chromosomal conformation capture approaches, such as Hi-C, have been recently introduced as methods to deconvolute bacterial communities from metagenomic data by physically linking fragments of DNA that occupy the same cells within a mixed community.

Here we hypothesize that by linking ARG, plasmids, and bacterial hosts with the Hi-C method, we can determine the natural reservoirs of antibiotic resistance genes and how they are spread by plasmids.We applied the Hi-C method to a real microbial community known to be a reservoir for ARG and plasmids, i.e., municipal wastewater. A sample of raw wastewater from the municipal wastewater treatment plant of Moscow, Idaho (USA) was used to prepare the Hi-C and shotgun libraries and sequenced. A total of 36 marker sequences for plasmids (PlasmidFinder) and 167 ARG sequences (MEGARes) were identified from the de novo shotgun metagenome assembly. In parallel, the metagenome assembly was deconvoluted with ProxiMeta, which uses the intra-cellular proximity signal captured by Hi-C reads as a direct indicator of which sequences originated from the same cell. The deconvolution produced 1307 genome clusters composed of contigs that likely originated from the same cells.

Whole genome sequencing and assembly of three high G+C environmental isolates using the Oxford Nanopore MinION

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"Three bacterial genome sequences were obtained using the Oxford Nanopore MinION system. A Variovorax paradoxus CSUSB strain that was previously sequenced using Illumina short read data only has been augmented with 3rd generation long read data to construct a hybrid assembly. The two long-read only genomes come from an additional strain of Variovorax paradoxus (VAIC) and a putative strain of Pseudomonas alkylphenolica. The V. paradoxus CSUSB strain and P. alkylphenolica were cultured on the CSUSB campus by undergraduate microbiology students and the VIAC strain was previously identified from a farm in Iowa as a quorum sensing signal degrader. Genomic DNA was first isolated using the Quick and Loman ultra-long read protocol followed by needle shearing. The DNA was then sequenced using the Oxford Nanopore 3rd generation sequencer (Flowcell MIN-106, Barcoding kit RBK-004). The CSUSB strain genome was assembled using a hybrid method combining Illumina MiSeq platform (500-600 Library fragment size using Nextera XT kit, 2x250 reads) and Oxford Nanopore long read data in Unicycler without references. VAIC and Pseudomonas were assembled using just long read sequences in a de novo assembly in Unicycler without a reference. The VAIC strain gave a single contig with 5,480,175 bp (G+C). The CSUSB strain gave a single contig with 5,446,423 bp (G+C), and when examining the 16s rRNA gene it showed >99% identity to other Variovorax paradoxus species, along with 5032 protein coding sequences. Unlike some other strains of V. paradoxus neither one of these sequences contain a plasmid or a second chromosome. The Pseudomonas strain was assembled into six contigs totaling 5,602,903 bp (G+C) with the closest identity on NCBI being at 89% (Pseudomonas alkylphenolica). The multiple contigs on the P. alkyphenolica suggest the present of mobile elements such as plasmids."

Lend me your sugar, I am your neighbor: Vaginal microbiome clusters drive metabolic profiles during healthy pregnancy

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Early life exposure to microbes is vital for the establishment of healthy microbiomes, yet still poorly understood. One moment of exposure comes during pregnancy and childbirth, when a baby may be exposed to vaginal microbes and metabolic products. Vaginal microbial communities are typically dominated by one or a few microbes, which are able to survive in a narrow pH range. Interestingly, vaginal communities depend on human-derived mucins and related nutrients more than human microbial communities occupying other body sites. In this study, we characterized the vaginal microbiomes and metabolomes of 18 women throughout three trimesters of pregnancy. Specifically we examined which microbes are present during healthy pregnancy and whether there are specific metabolic biomarkers associated with the microbial communities. 16S amplicon sequencing revealed simple communities dominated by Lactobacillus sp. and more diverse communities characterized by a high abundance of Gardnerella. Integrating GCMS and LCMS metabolomic data with amplicon sequencing, we found metabolites that distinctly associate with particular communities. For example, mannitol and indole-3-lactate are highly correlated with the presence of Lactobacillus crispatus. Metabolites that are distinct to a particular community type may indicate orthogonal approaches a community uses to metabolize the available carbon sources. Additional sequence data from shotgun metagenomics allowed us to better define the taxonomy established by amplicon sequencing, and to search for functional genes required for biocatalysis of the metabolites. Genomic analysis confirmed that many identified metabolic biomarkers could be processed or produced by the microbial communities present. Lastly, we used the metagenomic data to compare carbon utilization potential, and found differential potential for the dominant communities to break down mucin. Using several –omics approaches, we characterize the vaginal microbiome during healthy pregnancy, providing indicators of the various approaches these microbes use to survive in this unique environment.

Temperate prophage elements increase bacterial sensitivity to antibiotics

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"A chemical stress that is commonly faced by clinically-relevant bacteria is exposure to antibiotics which kill or inhibit cell growth. Like antibiotics, bacterial viruses (bacteriophages) are a pervasive selective force acting on the evolution of bacterial genomes. Temperate bacteriophages can integrate into bacterial genomes (where they are termed ‘prophages’), and are ubiquitous in the genomes of bacterial pathogens.

A number of antibiotics have been shown to induce the SOS response, a bacterial DNA-damage response pathway which also frequently activates prophage lytic replication (and phage-mediated cell death) of many prophages. However, the interaction between prophages and antibiotic exposure at an evolutionary level has not been studied.

Here I present data suggesting that the removal of prophage elements from the genomes of Salmonella strains increases the minimum inhibitory concentration of Ciprofloxacin necessary to inhibit growth. The data are consistent with a model where the presence of inducible prophage elements increase the sensitivity of bacteria to antibiotics.

This poster explores the physiological and evolutionary mechanisms by which prophages and antibiotics may interact."

Microbial origins of volatile molecules from cystic fibrosis sputum

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Background: Cystic fibrosis is a genetic disease that is characterized by impaired lung function due to a chronic polymicrobial infection. Patients experience periods of worsening lung function called pulmonary exacerbation events or CFPE that may be related to changes in polymicrobial community composition or physiology. To predict CFPEs and disease progression, one approach is to detect metabolites that are differentially produced by microbes during times of exacerbation. Specifically, we are interested in the production of volatile metabolites and their microbial origin. Volatile compounds, small molecules that can travel large distances, have been shown to affect the physiology of bacteria in biofilm development, antibiotic susceptibility, and motility. However, the molecular or microbial origin of volatile molecules has often been overlooked. Approach: To detect volatiles actively produced by microbes in CF sputum samples, we add 13C labeled glucose and media to a sputum sample, extract volatiles with a technique called vacuum assisted sorbent extraction, and detect the labeled metabolites by gas chromatography mass spectrometry. To predict the microbial origin of the labeled volatile molecules, we mined sputum metagenomes for pathways specific for the production of those metabolites. Results: We identified active microbial production of acetone, 2,3-butanedione, acetic acid, and other volatile molecules from sputum cultures. We are still in the process if analyzing the metabolomics data and mining metagenomes for pathways. Conclusions: Identifying volatile biomarkers of disease progression for CF and the producers of the volatile molecules would not only aid in treatment for patients, but would also elucidate microbial community interactions in CF.

Mechanisms of Bacterial-Fungal Interactions in a Model Microbiome

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In their native environments, microbes grow in close association not only with other members of the same species but frequently with many species. Microbes in these communities have evolved complex interaction systems. Bacterial-fungal interactions, in particular, are key factors in determining the composition and function of many microbiomes, and are relevant to medicine, soil health, and the texture, taste, and smell of fermented foods. Understanding bacterial-fungal interactions will be vital to our ability to predict responses of these microbial communities to disturbances or to push these communities toward desired outcomes. In this work, we use RB-TnSeq (a variation of traditional TnSeq) to identify genes important for bacterial-fungal interactions in a model microbiome (cheese). Pairwise assays have been performed by growing a bacterial barcoded transposon mutant library either alone or with one of 8 fungal members of the cheese ecosystem on in vitro cheese medium for seven days. The fitness effect of each mutation was determined and compared across conditions to identify the genes and pathways important for species interactions. These assays have been performed with a barcoded mutant E. coli library as well as a library created in the cheese-associated Pseudomonas psychrophila. By using a diverse set of interaction partners (molds and yeasts, five different fungal genera) with multiple bacterial mutant libraries, it is possible to determine the relative conservation or specificity of genetic mechanisms of bacterial-fungal interactions. We identified both general and specific microbial interaction mechanisms across diverse interaction combinations, which include responses to both nutritional and toxic stresses.

Population structure, antibiotic resistance, and uropathogenicity of Klebsiella variicola

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"Klebsiella variicola is a member of the Klebsiella pneumoniae subspecies complex along with the eponymous K. pneumoniae and Klebsiella quasipneumoniae. The importance of Klebsiella pneumoniae as a human pathogen has been studied extensively, however, a dearth of knowledge exists on K. variicola. We hypothesize that due to their genetic identity, features related to population structure, antibiotic resistance, and uropathogenicity will be similar between K. pneumoniae and K. variicola. We performed Illumina whole genome sequencing on 55 K. variicola isolates. We obtained 90 K. variicola genomes from NBCI. We determined the core-genome phylogeny, identified acquired antibiotic resistance genes and virulence genes for the 145 K. variicola genomes. We developed a urinary tract infection model for K. variicola pathogenesis, and infected 5 strains along with the model K. pnuemoniae TOP52 isolate.
K. variicola population structure has 2 distantly related lineages composed of 2 and 143 genomes, respectively. The second lineage has 26 deep-branching clusters with only 1.3% of the genome recombinant between strains. 6.9% (10/145) of K. variicola genomes had carbapenem resistance genes. One K. variicola strains had higher bladder colony forming units than K. pneumoniae TOP52 after 24 hours infection. Type 1 pilus production and activity were higher in 4/5 K. variicola strains compared to TOP52. 9 newly reported types of pili genes were discovered in the K. variicola pan-genome, including the first P-pilus.
Importantly, we demonstrate that high-risk ARGs and VGs are present in K. variicola genomes from a variety of geographies. This may have important clinical ramifications as we demonstrate that K. variicola clinical isolates can be superior uropathogens compared to K. pneumoniae. Therefore, due to the potential of multidrug resistance and pathogenic efficacy, identification of K. variicola and K. pneumoniae to a species level should be performed to optimally improve patient outcomes during infection."

High-Throughput Discovery of Mobile and Intrinsic Resistance Factors in Enterococcus faecalis using TnSeq

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Enterococci are nearly ubiquitous among the gut microbiota of land animals, having first emerged as commensal inhabitants of early terrestrial life after diverging from an aquatic ancestor. Enterococcus faecium and Enterococcus faecalis are also among leading causes of nosocomial infections, likely due to their intrinsic resistance to many antibiotics, antiseptics, and surface disinfectants. While many genes fostering high-level resistance to a variety of antibiotics have been identified, the underlying mechanisms of these species' intrinsic hardiness remain largely unknown, hampering efforts to prevent their rise and spread within hospital settings. Using the TnSeq (Transposon Sequencing) high-throughput sequence-based screening approach in the hospital-adapted, mobilome rich E. faecalis MMH594 strain, we have identified genes that contribute to both intrinsic and acquired resistance of Enterococcus to various (n = 11) antibiotics. Specifically, we identified more than 100 candidate genes required for growth in the presence of sub-inhibitory levels (avoiding stochasticity and ensuring high reproducibility) of one or more of the tested compounds. Further experimental work on top candidates, including genes coding for proteins of unknown function, largely validated their role in resistance. Interestingly, when placed within a phylogenomic framework along with other hospital and non-hospital associated strains of E. faecalis and other species from the Enterococcus genus, we observed two main patterns for these newly identified resistance genes: i) association with mobile elements enriched in hospital-adapted lineages and ii) ubiquity to all enterococci including commensal species rarely associated with humans (e.g. E. columbae in birds). The former suggests recent adaptation to the hospital environment through horizontal gene transfer, while the latter leads us to raise the hypothesize that ancestral, intrinsic enterococcal genes, that were instrumental in enabling the emergence of the genus from marine associated microbes and adaptation to life on land, are now also fortuitously advantageous in the modern hospital.

The antibiotic resistome of Akkermansia muciniphila, a mucin-degrading gut specialist

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The human gut is colonized by a diversity of symbiotic microorganisms including the mucin-degrading specialist Akkermansia muciniphila. Antibiotics are known to disrupt the composition of these gut communities yet little is known how some community members like A. muciniphila responds to different antibiotics. The primary objective of this project is to identify antibiotic sensitivity and resistance in A. muciniphila in vitro. Using the Comprehensive Antimicrobial Resistant Database (CARD), we first predicted the resistome of 36 Akkermansia genomes including the type strain A. muciniphila MucT. For this analysis, we focused on genes predicted to inactivate antibiotics and thus confer resistance. Although the majority of our hits were classified as ‘lose’, all genomes possessed genes putatively coding for resistance to a diversity of antibiotics. The analysis of these genes and their abundance in our genomes suggest at least three prominent phylogroups of Akkermansia among these 36 genomes agreeing with our other genomic analysis. Using the CARD results as a guide, we next selected 8 antibiotics and incubated representatives of two of the three phylogroups with the antibiotics across 10 concentrations to determine the minimum inhibitory concentration (MIC) of each antibiotic. After a 24-hour anaerobic incubation, results showed that the type strain A. muciniphila MucT is resistant to kanamycin and pristinamycin and sensitive to ampicillin and meropenem at all concentrations tested. MIC values were determined for cefotaxime (1 µg/ml), chloramphenicol (1 µg/ml), rifamycin (2 µg/ml), and tylosin (32 µg/ml). Experiments with other strains are ongoing. Ultimately, understanding how Akkermansia reacts to antibiotics will broaden our understanding of the functional diversity of this enigmatic lineage and how they influence human health.

Bacterial genome-wide association study to identify genetic variants linked to complex in vitro phenotypes

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"Clostridium difficile is the most common cause of hospital-acquired infection in the United States but the genetic determinants of its clinical success, particularly variation within epidemic lineages, are both nuanced and poorly described. This knowledge gap is partly due to the genetic intractability of many C. difficile strains. We have applied genome-wide association studies (GWAS), first pioneered in humans, to C. difficile to identify genetic variants associated with in vitro phenotypes without requiring traditional genetic manipulation experiments.

Thus far bacterial GWAS have primarily identified risk variants associated with discrete traits assumed to be under strong selection, specifically antibiotic resistance. In our study we extend GWAS to several complex phenotypes (toxin activity, growth rate, spore viability, and germination efficiency) that may be relevant to the clinical success of C. difficile. Transitioning from discrete traits typically encoded by few loci to continuous traits controlled by many loci required both modification of previous methods and the testing of evolutionary assumptions. In our cohort of over 100 clinical samples the phenotypes of distantly related isolates were more similar than expected under a Brownian motion evolutionary model, suggesting that horizontal gene transfer and/or convergence have an outsized impact on these phenotypes. The rejection of a Brownian motion evolutionary model supports the use of GWAS methods that either explicitly identify cases of convergence (PhyC adapted for continuous data) or transform the tree into a more star-like topology (generalized estimating equations with appropriate phylogenetic correlation structures). We used these GWAS methods in parallel to identify the single nucleotide variants, indels, large structural variants, and accessory genes associated with the in vitro phenotypes. We next propose functional validation of the most promising hits. The identification of novel and functionally relevant genetic markers of pathogenicity or fitness could allow for targeted, sequence-based clinical diagnostics that identify C. difficile isolates of greatest concern."

Optimized Analysis of the Lung Allograft Microbiota from Bronchoalveolar Lavage Fluid

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"Introduction: The study of microbial communities within the lung, once regarded as sterile, has now been revolutionized with the use of culture-independent techniques. However, the use of low density bronchoalveolar lavage fluid for analysis of the lung microbiota poses specific challenges due to its variability in bacterial density and are highly susceptible to artefacts. Methods/Results: We used two synthetic microbial communities, representing a range of densities (102 - 107 CFU/ml) to assess sequencing accuracy and precision of our 16S rRNA gene sequencing assay. We found that the degree of dissimilarity of the different densities to the highest concentration, or reference (= 107 CFU/ml), measured using the Bray-Curtis index (BCI), is low (<0.25) for all concentrations above 105 CFU/ml. BCI is between 0.25 and 0.5 for input densities between 103 CFU/ml and 105 CFU/ml. Below 103 CFU/ml, sequencing accuracy is low, with a BCI above 0.5 and over 50% of the observed signal is due to contaminants. We measured sequencing precision by measuring the BCI between two replicates and found out that reproducibility is low (BCI > 0.5) for densities below 103 CFU/ml. We also tested the impact of various pre- and post-sequencing approaches and their effect on sequencing accuracy and precision. Concentrating samples increased the bacterial density and the proportion of mock community taxa (signal) to contaminants (noise) in samples greater than 103 CFU/mL. DNase treatment decreased density and the signal/noise ratio in samples less than 105 CFU/mL.
Conclusions: These results suggest that low density samples are highly susceptible to contamination originating from different experimental steps. We recommend the use of positive (mock community) and negative (reagents) controls at every step to identify contaminants present during sequencing. This study has significant implications for the interpretation of microbiota in BALF samples, and possibly other sample types with low-bacterial load."

Comparative Genomics of Tandem Filamentous Prophages in Uropathogenic E. coli

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Filamentous bacteriophages are threadlike viruses that establish chronic infections in bacteria. These phages often contribute directly to the virulence of bacterial pathogens, including Vibrio cholerae, Yersinia pestis, and Ralstonia solanacearum. Recently, we discovered a novel filamentous phage integrated in triplicate in a clinical isolate of uropathogenic E. coli (UPEC). The closest known relative of this virus is the phage I2-2, which is not known to integrate into the host chromosome and which has not been associated with bacterial virulence. Here, we describe the diversity of this novel phage across bacterial genomes available in sequence repositories. Though primarily found in UPEC strains, we also identified homologous phages infecting strains of Salmonella, Klebsiella, Citrobacter, and Yersinia. Many of these bacteria were also from clinical urine samples. Like other integrated filamentous phages, all of these viruses integrate at their host's dif site, and most are present as tandem repeats. Further, most variation in the phages occurs in a glycine-rich repeat region within the phage attachment protein, suggesting that these phages are ecologically active. We have recently induced four of these viruses from clinical UPEC isolates and are working to establish an in vitro system for maintaining the phage in culture. It remains unclear what role, if any, this group of viruses plays in UPEC and other urine-associated microbes.

Comparative genomics predicts widespread crossfeeding of cobamide cofactors and their biosynthetic precursors

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Cobamides, cofactors including vitamin B12, are important for many microbial metabolisms, such as methionine synthesis, acetogenesis, and reductive dehalogenation. However, not all microbes that use cobamides are able to produce them, suggesting that cobamide crossfeeding is required by some microbes. To better predict cobamide crossfeeding in bacteria, we used comparative genomics to analyze the extent and distribution of cobamide production and use across 11,000 bacterial species. We find that 86% of bacteria in this data set have at least one of 15 cobamide-dependent enzyme families, yet only 37% are predicted to synthesize cobamides de novo, so we hypothesize that there is widespread sharing of cobamides in microbial communities. The distribution of cobamide biosynthesis varies at the phylum level, with 57% of Actinobacteria, 45% of Proteobacteria, 30% of Firmicutes, and less than 1% of Bacteroidetes containing the complete biosynthetic pathway. Cobamides contain variable upper and lower ligands coordinated to the central cobalt atom. The upper ligand depends on the reaction catalyzed, but why the lower ligand varies is unknown, though bacteria cannot use cobamides with different lower ligands equally well. Cobamide structure could be predicted for 58% of cobamide-producing species. Our predictions also revealed that 17% of bacteria have partial biosynthetic pathways. These include a newly defined category of bacteria lacking the first step to make the precursor 5-aminolevulinic acid (ALA), suggesting that an exogenous source of ALA is needed for cobamide biosynthesis. We experimentally verified the ALA salvaging phenotype in four bacteria: Treponema primitia, Clostridium sporogenes, Clostridium scindens, and Clostridium difficile. As C. difficile is an important human pathogen with multiple cobamide-dependent pathways, we investigated its cobamide biosynthesis, structure, and metabolism further. The combination of genomics and microbial physiology show the variation of cobamide biosynthesis and salvaging capabilities among bacteria, furthering the understanding of nutrient crossfeeding relationships.

The demographic history of Mycobacterium tuberculosis introduced from Europe through colonial migrations

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"Mycobacterium tuberculosis (M.tb), the etiological agent of tuberculosis, is an urgent global public health threat. M.tb strains circulating in the indigenous populations of Canada and New Zealand (NZ) present a unique framework to examine the demographic history of M.tb. Previous studies have demonstrated M.tb was introduced to Canadian indigenous populations from Europeans via the fur trade. More recently, an analysis of a globally distributed sample of M.tb lineage 4 (L4) revealed isolates from indigenous NZ populations nest within the diversity of isolates of sub-lineage L4.4.1.1 from indigenous Canadian populations. The dispersal of M.tb L4.4.1.1 resulting in its current, and curious, distribution remains an open question.

Here, we sought to characterize the migratory history of M.tb L4.4.1.1 using demographic models implemented in BEAST2. We obtained the alignment of L4.4.1.1 (n=117) previously described and estimated the evolutionary rate and divergence times using the GTR model of nucleotide substitution, a strict molecular clock and Bayesian skyline plot model. Additionally, we performed ancestral reconstruction, modelling country of origin (n=14) for each isolate as a discrete trait. We inferred migration rates from a maximum clade credibility tree, defining migration events as a change in the most probable reconstructed state from parent to child node.

We infer a Canadian ancestor for the NZ-Canadian clade and estimate the clade diverged mid-17th century. This is consistent with the previously described introductions of European M.tb lineages to Canada. We infer two introductions of M.tb to NZ indigenous populations in the 19th and 20th centuries. These may be the result of highly similar European M.tb lineages introduced via the European whaling industry and accelerated migration to the South Pacific.

Full reconstruction of M.tb's demographic history in Canadian and NZ populations can further our understanding of this pathogen's epidemiology and elucidate drivers of its spread, ultimately informing control and eradication strategies."

BioLockJ: A modular, open-source, scalable, flexible, extensible pipeline for reproducibly executing complex analysis paths from a single configuration file

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The ability to consistently generate reproducible results is a core challenge in modern genomics research. Bioinformatics pipelines inevitably generate large numbers of iteratively developed scripts with complex interdependencies, the proliferation of which can be difficult to manage even with diligent use of source control applications. For example, a typical informatics pipeline in metagenomics might in a UNIX environment filter sequences for quality control and execute a taxonomic classification algorithm, build statistical models to identify significant taxa in R, and publish summary reports and data visualizations online in JavaScript. Accurate reproducibility requires the execution of the correct version of each custom script in each environment with the same software version of each application dependency, which all must be executed in the same order and in the same runtime environments used to produce the original analysis. These complex inter-dependencies are very difficult to capture and document. To address these problems in our lab, we are developing a unified Java-based pipeline BioLockJ (https://github.com/mikesioda/BioLockJ_Dev/wiki) whose goal is to capture in a single configuration file all the steps of a metagenomics analysis from sequence pre-processing to statistical modeling and generation of interactive visualizations. Users can choose whether to run jobs locally on a single computer, in parallel on a suitably configured cluster, or within Docker containers, which simplifies installation and will enable deployment on cloud services in the future. Critically, pipeline analyses can be fully reproduced at any time on any platform by rerunning the application on the original sequence files by using the same BioLockJ configuration file. As analyses continue to move to the cloud, this strategy for abstraction and the organic documentation of pipeline processes will become an increasingly appealing method to facilitate the wide dissemination of highly flexible and scalable, yet reproducible, research practices.

Bacillus thuringiensis as a potential reservoir and vector of antibiotic resistance.

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"Bacillus thuringiensis (Bt) is the most widely used biopesticide in the world. While considered safe for human consumption, Bt has been documented as carrier of multiple plasmids and has demonstrated resistance to antibiotics, raising the possibility it may be a reservoir/vector of antibiotic resistance.

We accidentally detected a high prevalence of multi-drug Bt in waste water when attempting to sequence MRSA. We tested commercial Bt products and found them to be also multi-drug resistant. We then used bioinformatic analyses to determine the presence of antibiotic resistance genes in 489 published B. thuringiensis genomes. We compiled a database of complete, whole genome sequences from NCBI and split the nucleotide sequences by location (plasmids and chromosomes). To determine if antibiotic resistance genes are present in B. thuringiensis, we searched all sequences for antibiotic resistance genes using the Comprehensive Antibiotic Resistance Database (CARD).

We found 295 occurrences of 15 unique antibiotic resistance genes. Chromosomally-encoded antibiotic resistance genes accounted for 287 of the sequences, with all genomes containing a core set of three antibiotic resistance genes (BLA1, BLA2 and fosB). Additional well-represented genes included those encoding resistance to vancomycin (95% of genomes), clindamycin (81% of genomes). Of the eight antibiotic resistance genes located on plasmid sequences, six were located on one single plasmid, encoding resistance to tetracycline and vancomycin.

Use of Bt as method of pest control may be unintentionally facilitating the transfer of antibiotic resistance genes in the environment. This investigation of B. thuringiensis genomes indicated that the presence of antibiotic resistance genes have been integrated into these bacterial genomes. Integrated antibiotic resistance genes may be more likely to be maintained by the host and potentially spread to other organisms through horizontal gene transfer."

GWAS of delta toxin production in S. aureus

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"The delta toxin of Staphylococcus aureus is the only hemolysin shown to cause mast cell degranulation and been linked to atopic dermatitis, a chronic inflammatory skin disease. Currently, other than the agr quorum sensing system, it is unknown which factors modulate delta toxin production. Traditional genome wide association studies (GWAS) have focused on largely binary phenotypes such as antibiotic resistance. The present study aims to characterize delta toxin production and elucidate the genetic features that regulate delta toxin in diverse set of S. aureus strains.

106 S. aureus representing 18 clonal complexes (CC) were sequenced by Illumina and subjected to HPLC to measure delta toxin production. Reads were assembled de novo using SPAdes, and SNPs were called by the GATK pipeline using S. aureus N315 as a reference. Delta toxin production varied widely between strains (range 0-97k) with evidence of both lineage and strain specific variation. CC30, underrepresented in atopic dermatitis cases, and CC45, overrepresented in atopic dermatitis, had lower and higher than average delta toxin production respectively. In addition, MSSA (methicillin sensitive) strains had higher delta toxin production than MRSA (methicillin resistant) strains.

Several GWAS methods (treeWAS-binary data/phylogenetic population correction, SEER-kmer data/principal component population correction, bugwas-kmer and binary data/PC population correction, DBGWAS-de Brujin graphs/PC population correction) were employed and compared. Results varied between methods but indicate a complex regulation intimately linked to the metabolic state of the cell. No single locus contributes significantly to the phenotype. Instead, we believe many low loci affect a complex gene regulation network. Machine learning approaches can be used to quantify the contributions of the genes identified by GWAS with the ultimate goal of building a predictive model."

Characterization of a large Chinese cohort reveals clustering of the gut microbial community by province and urbanization.

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Urbanization is associated with an increased risk for a number of diseases, including obesity, diabetes, and cancer, which all also show associations with the microbiome. In previous work, we reported on differences in the microbiota and metabolome between rural and urban samples within Hunan province in China. Here we extended this work to two large cohorts respectively including 2,164 subjects from 15 provinces and 1,242 subjects from 4 of these 15 provinces across a wide geographic reach of China as part of the China Health and Nutrition Survey (http://www.cpc.unc.edu/projects/china). With 16S sequencing analysis, we found profound differences in the microbiome by province, presumably reflecting large variations in culture and diet over different regions of China. Consistent with our previous observations, both 16S and metabolite profiles revealed substantial differences between rural and urban samples. Utilizing non-parametric tests, we found a large number of statistically significant correlations between microbial community, metadata, dietary variables and metabolites. This analysis revealed associations between the microbiota and dietary consumption, including calories and fat from animal sources, diet diversity (the number of different food groups consumed) and vitamin intake levels. Associations between the microbiota and the metabolome suggest differences in the activity of xenobiotics within the metabolome are linked to microbiome differences in province and urbanization. These associations suggest novel hypotheses about how diet and the microbiome may interact to contribute to different health outcomes associated with the wide range of lifestyles within rural and urban China.

Metagenomics insights into the intestinal resistomes of cattle, swine, chickens and turkeys

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FDA

The intestinal microbiome is an important reservoir of antibiotic resistance (AR) genes, yet the true diversity of intestinal resistomes in food producing animals is poorly understood. We employed a shotgun metagenomics approach to catalogue the full complement of intestinal resistomes in the four major food animals (cattle, swine, chickens, and turkeys) monitored by the National Antimicrobial Resistance Monitoring System. A total of 658 cecal samples collected from cattle (177), swine (165), chicken (214) and turkey (102) in 2017 were included in the study. The resistomes were characterized and quantified by identifying antibiotic resistance genes annotated in the ResFinder Database from the metagenomes of each sample using ShortBRED. We used LEfSe to determine the resistance genes most likely to explain differences between sources. We identified over 190 resistance genes representing 12 antibiotic resistance classes. Tetracycline and macrolide resistance genes were the most widespread AR genes in the animal intestinal microbiome, with at least one resistance gene from the two resistance classes being identified in 100% of animals. The ten most frequently identified antibiotic resistance genes were lnuC (100%), tetQ (99.5%), tetW (99.5%), tet40 (98.2%), tetO (93.7%), aph3'-III (85.7%), ant6-Ia (76.6%), ermB (67.2%), ermG (60.9%) and fosA (54.3%). The distribution and relative abundance of AR gene observed varied by source. The aph3'-III, ant6-Ia, dfrA25, mphC, and aac6-Isa genes were differentially abundant among turkey metagenome samples while qnrB41, ermB, tetX, blaOXA-85, mefB and qnrS1 were abundant among swine. The lnuC, aac6-Im, aph2-Ib, ermG, aph2-Id and npmA were differentially abundant among chicken metagenome samples. Tetracycline resistance genes (tetQ, tet40, tetL tetO, and tet32), cfxA6, blaOXA-51, ermE, cmx and aadD were consistently higher among cattle metagenome samples. Our study provides insights into food animal intestinal resistomes and helps assess the impact of antimicrobial use intervention efforts. In addition, by indexing the animal resistomes in the food chain, metagenomics promises to greatly augment antimicrobial resistance surveillance.

Exposure to a Healthy Gut Microbiome Improves Reproductive and Metabolic Phenotypes in a Mouse Model of Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting ~10% of women worldwide. Diagnosis requires two of the following: hyperandrogenemia, polycystic ovaries, anovulation. In addition to infertility, many women with PCOS have metabolic dysfunction that increases their risk of developing type 2 diabetes but the mechanisms involved are unknown. Recent studies have revealed an association between the gut microbiome and PCOS in both women and mouse models. However, it is unknown if the gut microbiome plays a causal role in the PCOS metabolic phenotype and whether manipulation of the gut microbiome could be a therapeutic option for treatment of PCOS. I hypothesized that exposure to a healthy microbiome would restore the PCOS gut microbiome and improve the PCOS phenotype. A cohousing study was performed using a letrozole-induced PCOS mouse model that recapitulates the PCOS metabolic and reproductive phenotype. Since mice are coprophagic, cohousing results in repeated gut microbial inoculation among cohoused animals. Over the course of the 5-week experiment (3 groups, 2 mice/cage: cohoused letrozole, cohoused placebo and cohoused letrozole with placebo), letrozole mice cohoused with placebo showed a significant improvement in metabolic and reproductive phenotypes compared to letrozole mice cohoused together. This included a significant reduction in weight gain, abdominal adiposity and restored insulin sensitivity when compared to letrozole mice cohoused together. Letrozole-treated mice cohoused with placebo-treated mice also showed a significant reduction in testosterone levels and improvement in ovarian phenotype. Letrozole induced PCOS mice, like some PCOS women, show acyclicity, cystic ovaries and lack ovulation; this was improved when cohoused with placebo mice. Using 16S rRNA gene sequencing, we saw that cohousing letrozole-treated mice with placebo-treated mice resulted in changes in beta diversity and specific bacterial genera that may be candidates for probiotic therapies. Our findings suggest that manipulation of the gut microbiome may be a potential treatment option for PCOS.

Endosymbiont in endosymbiont: A Rickettsia-like bacterium within mitochondria of the amoeba Vannella sp.

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Bacterial endosymbionts are usually localized in the host cytoplasm or vacuoles derived from phagosomes. Less frequently, endosymbiotic bacteria have been found to colonize nuclei and very rarely mitochondria, an organelle which itself evolved from an ancient symbiosis. The only intramitochondrial bacterium identified to date is the ticks' symbiont Midichloria mitochondrii (Alphaproteobacteria, Rickettsiales). We here report another symbiont of intramitochondrial lifestyle tentatively termed Mitorickettsia vannellae that is harbored in a free-living amoeba of the Vannella genus (Amoebozoa, Discosea). We used transmission electron microscopy to morphologically examine and describe this association. Although the endosymbiont was lytic to mitochondria, both partners can be kept in a stable monoxenic culture. To gain insight into the genomic features and metabolic potential underlying the intramitochondrial lifestyle, we used flow-cytometry to sort individual endosymbiont cells obtained from lysed amoeba and sequenced the symbiont's genome. With a size of more than 1.2 Mbs and a GC content of 34 % GC the Mitorickettsia vannellae genome is well within the typical range of genomes of bacterial endosymbionts of protists. The phylogeny inferred from conserved marker proteins, places the endosymbiont as a member of the Rickettsiaceae family (Alphaproteobacteria, Rickettsiales) branching basally to the genus Rickettsia. A more detailed 16S rRNA gene phylogeny revealed its position in a clade of marine rickettsiae forming a sister clade to Megaira polyxenophila, an endosymbiont able to infect various freshwater ciliates and green algae. The availability of Mitorickettsia vannellae in stable co-culture with its amoeba host provides a unique experimentally accessible model system. Its study will reveal evolutionary and functional mechanisms underlying the exploitation of mitochondria as intracellular niche for bacterial replication with broader implications for our understanding of intra-compartmental symbiosis, host-symbiont co-evolution and organelle formation.

Learning the Language of Promoters: Global Analysis of Promoter Architecture in E. coli

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While modern DNA sequencing technologies have provided us with an incredible number of genomic sequences, we are in desperate need of high-throughput and computational methods to explore and annotate functional elements within this ever-growing sequence space. In prokaryotic organisms, promoters are the key drivers of gene expression and are largely responsible for the regulation of cellular responses to time and environment. Promoters encode their impressive dynamic range of expression and environmental plasticity through unique combinations of regulatory sequence motifs. However, to encode this incredible flexibility, promoter sequence space is incredibly complex, rendering us largely unable to quantitatively predict the function of a promoter given its sequence. Here we demonstrate an experimental platform to functionally characterize the promoter activity of hundreds of thousands of genomic sequences, reveal the promoter landscape, and dissect sequence features that define promoters in E. coli. We use a genomically-integrated massively parallel reporter assay (MPRA) to measure expression of 17,798 reported promoters and find that a majority of reported promoters are likely sources of transcriptional noise, rather than productive transcription. Furthermore, we use this MPRA to measure promoter activity of nearly 450,000 DNA fragments spanning the entire E. coli genome with 11.7x coverage, allowing us to definitively identify the breadth of endogenous sequences that contribute to expression with single-nucleotide resolution. We relate promoter landscape to local transcription profiles and show that antisense transcription is a common means of negative genetic regulation, especially amongst cryptic operons. Lastly, we use this rich dataset to train support vector machines and convolutional neural networks to distinguish promoter from non-promoter regions and reveal the sequence features necessary for transcription in E. coli.

Transposon Mutagenesis to Evaluate Degradation of Staphylococcus aureus Biofilms by Variovorax paradoxus EPS

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Variovorax paradoxus is a common soil bacterium in the family Comamonadaceae that produces an antimicrobial molecule with anti-staphylococcal activity in both liquid culture and plate based killing assays, under certain conditions. A library of Tn5 mutants was generated in a previous directed insertion mutant of V. paradoxus EPS (del4519::Kan) to examine genes affecting antibiotic production on agar plates. The parent strain has a defect in surfactant production, which we hoped would reduce variability in inhibition zone size. Mutants for further evaluation were selected from mixed culture plates and verified using a plate-based zone of inhibition assay. From the set of mutants identified in this manner, 16 were selected for biofilm screening. Equal numbers of mutants were selected with enhanced and inhibited inhibitory characteristics. Initial experiments showed that both wild-type and del4519 V. paradoxus EPS inhibited the formation of S. aureus AH1710 (RN4220 + pCM29, constitutive GFP expression) biofilms in 6 well mixed cultures. Wild type and mutant biofilm co-cultures in 6 well plates were evaluated by crystal violet staining of 3 day biofilms. The stained biofilms were imaged to identify cocci and bacilli in the biofilm, and the total biofilm was dissolved in 95% ethanol and quantitated spectrophotometrically. The mixed culture fluid was also collected and filtered to evaluate direct anti-staphylococcal activity. Statistical analysis by comparison to S. aureus AH1710 monoculture biofilms and a mixed culture of S. aureus and V. paradoxus mutant 35 was performed using the Student's unpaired t-test. Biofilm inhibition activity was present in all mutants which may suggest a difference in expression of the antimicrobial molecule in liquid culture. Filtered culture fluid was tested for killing activity in a plate-based inhibition assay. Plate imaging from these experiments showed potential killing activity in the culture fluid, which may allow direct biochemical characterization of the active compound.

The good, the bad and the protist: characterization of beneficial and parasitic bacteria associated with a giant ciliate host

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Chemoautotrophic systems are hypothesized to have played an important role in the evolution of life, and their study might reveal coding potential and biochemistry in primordial environments. Zoothamnium niveum, a marine colonial ciliate, together with its ectosymbiont Cand. Thiobios zoothamnicoli, represents one of the few chemosynthetic symbiotic model systems that can be manipulated and studied in the lab. While the role of the bacterial symbiont is partially known, the mutualistic relationship is not characterized. To better understand symbiont-host interaction, in particular the role of the symbiont in fixing inorganic carbon and its uptake by ciliate host, we performed 13C bicarbonate incubations under varying environmental conditions, followed by NanoSIMS and transmission electron microscopy to trace the fate of labeled carbon. Furthermore, we were able to identify missing partners in this relationship through a metagenomics approach and assessed their contribution to the biochemistry of the ciliate host. Our results showed that in the presence of sulfide, ectosymbionts fix substantial amounts of inorganic carbon, part of which is transferred to the ciliate. Interestingly, genomic analysis and microscopy studies revealed the presence of a second bacterium within the ciliate cells, which we tentatively refer to as Candidatus Janabacterium zoothamnicoli for now. Janabacterium belongs to the Dependientae (TM6) candidate phylum. With 0.71Mb (26% GC content) its genome is highly reduced in size, with conspicuous nutritional deficiencies and many ATP and other nucleotide transporters. We also observed signs of pathogenicity in infected ciliate cells, similar detrimental effects on the host has been described for other members of the Dependientae phylum. Taken together, we shed light on a unique system in which a ciliate hosts a beneficial ectosymbiont and a likely parasitic endosymbiont. We also underscore the need for a combination of approaches to fully characterize mutualistic relationships.

Linking Microbial Community Composition and Carbon Cycling Functions in a California Salt Marsh Through Space and Time

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Salt marshes are sites of active carbon cycling mediated by the tight coupling of photosynthetic primary productivity with microbial decomposition processes. Salt marshes are, however, complex, heterogeneous environments likely resulting in differences in carbon turnover rates through space and time. Spatially, distinct environments within a marsh such as tidal creek sediments, hypersaline pools, and photosynthetic mats vary in their physicochemical conditions resulting in contrasting microbial communities. Temporally, physicochemical conditions fluctuate daily with tidal cycles and seasonally with precipitation and runoff. The goal of this study is to link microbial community composition with carbon cycling functions through space and time within a local salt marsh. Using amplicon sequencing of microbial marker genes and fluorescent extracellular enzyme assays to measure carbon mineralization, we found that microbial community composition and carbon degrading capabilities varied spatially and temporally. Most notably, the photosynthetic mat exhibited the highest enzymatic rates, yet was among the least diverse communities surveyed. Enzymatic rates generally peaked in winter across the marsh but also decreased as a function of depth. Dominant phyla that were positively correlated with enzyme activity across all environments were Proteobacteria and Bacteroidetes. Unlike other environments, Cyanobacteria were positively correlated with most of the enzymes in the photosynthetic mats only, suggesting that the dominant carbon-degraders varied through space. Overall, results suggest that spatial differences in microbial community composition lead to functional differences in carbon mineralization rates through time. These differences ultimately influence the overall carbon budget of salt marsh ecosystems because while marshes are generally regarded as carbon sinks, there are local hot spots that may act as sources.

Predictable molecular adaptation of coevolving Enterococcus faecium and a widespread lytic phage

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Bacteriophages in the human microbiome are highly abundant and diverse, yet their interactions with resident bacteria are understudied. Phage predation can drive bacteria to evolve resistance to phage infection, which can then drive reciprocal phage evolution to overcome that resistance. Such coevolutionary dynamics have not been extensively studied in human gut bacteria and are important for understanding and eventually manipulating the human gut microbiome. We performed experimental evolution of Enterococcus isolates from healthy human stool in the absence and presence of bacteriophages isolated from sewage. Genome sequencing revealed that Enterococcus evolved resistance to phage through mutations in exopolysaccharide biogenesis and export genes, and mutations in the RNA polymerase β' subunit. Phages acquired mutations in structural genes as well as varying numbers of large tandem duplications within the long tail fiber gene. This study showed that genomic analysis of experimental coevolution can quickly illuminate the evolutionary arms race between phage and bacteria.

Non-genetic paternal effects on early trout development

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In most species with an external breeding system and no parental care it is generally assumed that males only provide genes to the next generation. Recent studies however demonstrated that offspring can also inherit non-genetic traits, such as epigenetic effects or bacteria. Here we characterized symbiont bacterial communities in milt of brown trout (Salmo trutta) and rainbow trout (Oncorhynchus mykiss) and tested whether bacteria are transferred from the father vertically to the next generation. We used a full-factorial breeding design and in vitro fertilizations in order to separate genetic and environmental effects on offspring performance. For the fertilizations we either washed milt with antimicrobial compounds or we left it untreated. We found a high diversity of bacteria in the milt of different sires and we monitored bacterial communities on developing embryos until one day after hatching. Offspring that originated from washed milt hatched later and were smaller than their natural fullsiblings. This difference in size persisted until ten days after hatching (the end of our experiment). Our results are discussed in the light of anthropogenic influences (i.e., micropollutants in freshwater systems) and the coevolution of hosts and their microbiomes.

Single Chromosomal Genome Assemblies on the Sequel System with Circulomics High Molecular Weight DNA Extraction for Microbes

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"The Nanobind technology from Circulomics provides an elegant HMW DNA extraction solution for genome sequencing of Gram-positive and -negative microbes. Nanobind is a nanostructured magnetic disk that can be used for rapid extraction of HMW DNA from diverse sample types including cultured cells, blood, plant nuclei, and bacteria. Processing can be completed in <1 hour for most sample types and can be performed manually or automated with common instruments.

We have validated several critical steps for generating high-quality microbial genome assemblies in a streamlined microbial multiplexing workflow. This new workflow enables high-volume, cost-effective sequencing of up to 16 microbes totaling 30 Mb in genome size >7 kb repeats. Fragment size was increased to ~14 kb, with some fragments >30 kb.

Here we present a demonstration of these capabilities using isolates relevant to high-throughput sequencing applications, including Shigella, common foodborne pathogens (Listeria, Salmonella), and species often seen in hospital settings (Klebsiella, Staphylococcus). For nearly all microbes, including the difficult class 3 microbes, we achieved complete de novo microbial assemblies of ≤5 chromosomal contigs with minimum quality scores of 40 (99.99% accuracy) using data from multiplexed SMRTbell libraries. Each library was sequenced on a single SMRT Cell 1M with the PacBio Sequel System and analyzed with streamlined SMRT Analysis assembly methods.

In conclusion, we achieved high-quality, closed microbial genomes using a combination of Circulomics Nanobind extraction and PacBio SMRT Sequencing, along with a newly streamlined workflow that includes automated demultiplexing and push-button assembly."

Dissecting microbial genomes from their own homologous sequences

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"Homologous sequences are accumulated, fragmented and structured in the evolution of a genome through duplication, conversion, recombination and transposition events.
We have developed a computational system for genome analysis, called Genome Dynamics Analyzer. First, the system takes the whole sequence of a single genome, conducts self-alignment to collect all homologous sequences on the genome, assembles them into continuous domains, hierarchically classifies the domains into families (HDFs), and visualizes the domains on the genome. Next, for multiple different genomes, the system analyzes homologies among their HDFs, bundles those containing homologous sequences as a group (HDFGs) and visualizes the relationship of the strains sharing HDFGs as a network.
Using this system, five different strains of Escherichia coli, including O7:K1, O104:H4, O157:H7, O83:H1 and K12 were first analyzed. A total of 309 HDFGs was obtained, of which 205 were local to a single strain, 104 were shared by multiple strains. Out of 35 HDFGs shared among the first four pathological strains only, 22 were related with virulence and self-defense genes, mostly of phage origin. Detoxification genes appeared uniquely in the non-pathological K12 strain, while evolutionary core genes were shared among all strains.
Similarly, 76 strains of 36 genera (including E. coli) in the order Enterobacterales were analyzed. Out of total 3904 HDFGs, 366 were shared by multiple strains. In the visualized network, O157:H7 and O104:H4 were separated from O7:K1, O83:H1 and K12; Salmonella appeared in-between and Shigella near the latter. Overall, pathological or symbiotic strains, including O157:H7, O104:H4, Pluralibacter, Sodalis, Serratia and Xenorhabdus, showed up as outliers.
While conventional phylogenetic approaches focus on vertical gene transfer of orthologs, our approach that extracts plastic domains of parlogs has potential to bring out horizontally transferred genes and mobile elements. It is useful for elucidating the architecture, evolution, pathogenicity and self-defense mechanisms of microbial genomes."

Novel giant virus genomes from deep sea sediments expand the ocean megavirome and support independent origins of viral gigantism

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The Nucleocytoplasmic Large DNA Viruses (NCLDV) of eukaryotes (proposed order ”Megavirales”) include the families Poxviridae, Asfarviridae, Iridoviridae, Ascoviridae, Phycodnaviridae, Marseilleviridae, Pithoviridae, and Mimiviridae, as well as still unclassified Pandoraviruses, Molliviruses and Faustoviruses. Several of these virus groups include giant viruses, with genome and particle sizes exceeding those of many bacteria and archaea. The discovery of giant viruses has changed the perception of viral complexity and the entire concept of a virus. We explored the presence and diversity of the NCLDV in deep-sea sediments from the Loki’s Castle hydrothermal vent area. Using a metagenomic approach, we reconstructed 23 high quality genomic bins of novel NCLDV, 15 of which are related to Pithoviruses, 5 to Marseilleviruses, 1 to Iridoviruses, and 2 to Klosneuviruses. Some of the identified Pitho-like and Marseille-like genomes belong to deep branches in the phylogenetic tree of core NCLDV genes, substantially expanding the diversity and phylogenetic depth of the respective groups. The discovered viruses have a broad range of apparent genome sizes including putative giant members of the family Marseilleviridae, in agreement with multiple, independent origins of gigantism in different branches of the NCLDV. Phylogenomic analysis reaffirms the monophyly of the Pitho-Irido-Marseille branch of NCLDV. Similarly to previously analyzed giant viruses, the Pitho-like viruses from Loki’s Castle encode translation systems components. Phylogenetic analysis of these genes indicates a greater bacterial contribution than detected previously. Finally, genome comparison suggests extensive gene exchange between members of the families Pithoviridae and Mimiviridae. Further characterization of the genomic repertoire of the discovered viruses and exploration of the genomic diversity of ”Megavirales” in additional sediment samples is expected to yield new insights into the evolution of giant viruses and the composition of the ocean megavirome.

Constraints on horizontal gene acquisition in bacteria: restriction-modification system involvement

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"Bacteria assemble genome islands, which code for niche-adaptive functions, by RecA-independent mechanisms that remain obscure and play a major role in horizontal gene transfer (HGT). Of particular interest are regions not mobilized by site-specific recombinases or known transposases, such as the Immigration Control Region (ICR). This region is highly enriched for variable sequence-specific restriction-modification systems involved in protection against exogenous DNA entrance. In addition, this region was suggested to be involved in site-specific HGT. Therefore, the ICR is a relevant locus of study of lateral gene transfer between two species.

To study HGT mechanisms, we developed a conjugal transfer system of chromosomal DNA to characterize basal and enzyme-stimulated RecA-independent gene transfer of the ICR between laboratory descendants of one natural isolate of E. coli. The basal intralineage events move very large (60 kb-2 Mb) segments, replacing recipient DNA with donor sequence. Interestingly, in our preliminary results in crosses with a restriction-deficient Salmonella enterica sv Typhimurium strain, we identified two distinct properties: an increased fraction of events are additions, and shorter segments predominate.

As part of the groundwork for the intergeneric experiments, we determined the sequences of Salmonella enterica sv Typhimurium LT7, an isolate of the model organism S. typhimurium LT2 obtained from the Segall laboratory, and the multiply restriction-deficient hybrid strain often used for molecular genetic constructions (LB5000). Much of the early work characterizing restriction activity in Salmonella was carried out in LT7 and mutations later moved into LT2. Comparison of the LT2 genes that determine the restriction activities SenLT2I (LT, StyLT in the early literature), SenLT2II (SA, StySA) and SenLT2III (SB, StySB) with those of LT7 and the restrictionless host permitted us to identify the mutations that result in restriction-deficiency in the hybrid. Variation contributed by the four inducible prophages of LT2 will also be outlined."

Staphylococcus aureus bacteriophage suppresses LPS-induced inflammation in MAC-T bovine mammary epithelial cells.

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Several previous studies have shown that bacteriophages can significantly affect the production of various cytokines. The aim of the current study is to investigate the inflammatory effects and mechanisms of bacteriophage vB_SauM_JS25 in stimulated MAC-T bovine mammary epithelial cells by Real-time PCR and Western blotting. Experiments show that vB_SauM_JS25 reduces *Staphylococcus aureus* (*S. aureus*)- or lipopolysaccharide (LPS)-induced TNF-α, IL-1β, IL-6, IL-8, IL-10 and RANTES mRNA levels in MAC-T cells, which should be unrelated to its antibacterial action. Moreover, *S. aureus* bacteriophage vB_SauM_JS25 suppresses the LPS-induced phosphorylation of NF-κB p65, which may represent an important mechanism mediating these effects. A carefully regulated balance between activation and inhibition by phages must be kept avoiding inappropriate inflammatory responses. This ability of vB_SauM_JS25 to influence immune response indicates the potential development and application of bacteriophage-based therapies and may represent a novel anti-inflammatory therapeutic strategy.