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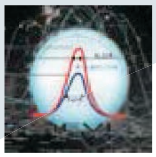

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Welcome!

Thank you for joining us at this inaugural conference on *Multi-omics for Microbiomes*. It's my hope that this will become a biannual event to bring together a cross-disciplinary group of scientists who apply multi-omics approaches to understand complex microbial communities, who develop tools to analyze and integrate omics data, and who develop omics technologies to increase our knowledge and understanding of the microbiome.

The importance of microbiome research is increasingly being recognized because of the critical roles that microbial communities play in the environment and human health. However, we are currently in the "discovery phase" of microbiome science and lack a mechanistic understanding of the roles that individual microbes and communities of microbes play in different environments and the impacts of perturbations on microbial community functions. The time is therefore ripe for us to exchange knowledge and expertise, and this conference is a significant step in doing so.

My thanks to all of you who are attending, and especially to our sponsors: Thermo Fisher Scientific, the new ASM mSystems journal, illumina, MO BIO Laboratories, Inc., and the TerraGenome RCN.

Janet Jansson
Conference Organizer
Director, Biological Sciences Division
Pacific Northwest National Laboratory

AGENDA

TIME	TOPIC	SPEAKER/PRESENTER(S)
	SUNDAY, SEPTEMBER 13, 2015	
5:00 – 7:00 pm	EARLY REGISTRATION Grand Ballroom A	
	MONDAY, SEPTEMBER 14, 2015	
7:30 – 8:30 am	REGISTRATION	
8:30 – 9:00 am	Welcome & Meeting Overview	Janet Jansson
Session Chair – Jack Gilbert		
9:00 – 9:30 am	Personalized Medicine: Multiomics Profiling During Periods of Health, Disease, and Weight Changes	Michael Snyder
9:30 – 10:00 am	Carbohydrate-binding Proteins as Microbial Detectors	Laura Kiessling
10:00 – 10:30 am	BREAK	
10:30 – 11:00 am	Differences in Susceptibility to Perturbation in the Gut Microbiota and Metabolome in a Genetically Divergent Mouse Population	Tom Metz
11:00 – 11:30 am	Microbes are STICKY: Large-scale Inference and Topological Analysis of Microbial Interaction Networks	Christian Müller
11:30 – 12:00 pm	Exploratory Data Analytics to Identify Signatures of Perturbations to the Soil Biogeochemical System	Lee Ann McCue
12:00 – 1:30 pm	Working Lunch – Poster Session Grand Ballroom B (odd numbers)	All
Session Chair – Joshua Adkins		
1:30 – 2:00 pm	Multi-omics of Cellulosic Biofuels Microbes	Timothy Donohue
2:00 – 2:30 pm	Mathematical Modeling of -omics Data for Biofuel Production	Hector Garcia Martin
2:30 – 3:00 pm	Impact of Plant Carbon Partitioning on the Rhizosphere Microbiome	Gary Stacey
3:00 – 3:30 pm	Networks of Exchanging Antibiotic Resistomes in Commensal, Environmental, and Pathogenic Microbes	Gautam Dantas
3:30 – 4:00 pm	BREAK	
4:00 – 4:30 pm	Stable Isotope Based Assessment of In Situ Metabolic Carbon Dynamics in a Phototrophic Microbial Mat	Mary Lipton
4:30 – 4:45 pm	Meta-omic Analysis Reveals Widespread Functionality in Antarctic Hypoliths from Two Dry Valley Systems	Thulani Makhalanyane

4:45 – 5:00 pm	Natural Occurrence of Beneficial Traits in the Rhizosphere Microbiome of Oilseed Rape Revealed by Linking Metagenomics with Single Strain Omics	Henry Müller
5:00 – 6:00 pm	Microbiome Roundtable	TBD
	Dinner on your own	
TUESDAY, SEPTEMBER 15, 2015		
Session Chair – Vanessa Bailey		
8:00– 8:15 am	Day Two Welcome	Vanessa Bailey
8:15 – 8:45 am	Microbial Diversity and Greenhouse Gas Dynamics in Coastal Wetlands	Susannah Tringe
8:45 – 9:15 am	Tracking Fracking: Constraints on Subsurface Life During Energy Extraction	Kelly Wrighton
9:15 – 9:45 am	How Electron Cryotomography is Opening a New Window into Microbial Cell Biology	Grant Jensen
9:45 – 10:15 am	BREAK	
10:15 – 10:45 am	Integration of Dynamic, Multi-omic Data for the Human Microbiome	Rob Knight
10:45 – 11:15 am	Digitizing the Chemistry of Microbes	Pieter Dorrestein
11:15 – 11:30 am	Understanding Childhood Asthma Through Multi-omics	Kathryn Holt
11:30 – 11:45 am	Comparative Systems Biology of Bacterial Metabolic Phenotypes	Germán Plata
11:45 – 1:30 pm	Working Lunch – Poster Session Grand Ballroom B (even numbers)	All
Session Chair – Lee Ann McCue		
1:30 – 2:00 pm	A Multi-scale Approach to the Microbial Community Ecology of Soil Carbon Cycling	Kirsten Hofmockel
2:00 – 2:30 pm	How Microbial Evolution Influences Environmental Ecosystem Services	Jack Gilbert
2:30 – 3:00 pm	Systems-based Approaches for Integrated Multi-omics Microbiome Data	Elhanan Borenstein
3:00 – 3:30 pm	BREAK	
3:30 – 4:00 pm	Towards Population-level Microbiome Monitoring: The Flemish Gut Flora Project	Jeroen Raes
4:00 – 4:30 pm	Cracking the Microbiome Through Advanced Mass Spectrometry	Lili Paša-Tolić
4:30 – 4:45 pm	Uncovering Microbial Dark Matter, Functional Stability, and Transcriptional Dynamics within the Oral Microbiome	Jeffrey McLean

4:45 – 5:00 pm	Functional Diversification in Microbial Symbiosis Supports Longevity under Extreme Conditions	Gabriele Berg
5:00 – 6:00 pm	Networking – Mixer	All
6:00 – 7:00 pm	***Keynote Address:*** Towards 4-Dimensional (Eco)Systems Biology in the Sea	Edward DeLong
7:00 – 8:30 pm	Dinner	All
	WEDNESDAY, SEPTEMBER 16, 2015	
Chemical Imaging Session – Steven Wiley		
8:00 – 8:15 am	Day Three Welcome	Steven Wiley
8:15 – 8:45 am	Structural Diversity among Fungal Hyphae: Insights into Cell Growth and Phylogeny	Robert Roberson
8:45 – 9:15 am	Chemical, Structural, and Dynamic Imaging Across Modalities and Scales	James Evans
9:15 – 9:45 am	BREAK	
9:45 – 10:15 am	Nano-enabled Imaging	Mitchel Doktycz
10:15 – 10:45 am	Infrared Spectromicroscopy for Live Cell Chemical Imaging	Hoi-Ying Holman
10:45 – 11:15 am	New Directions in Multi-omic Microbiome Measurements; Faster, Better, AND Cheaper	Richard Smith
11:15 – 11:45 pm	Closing Remarks	Janet Jansson
11:45 – 1:15 pm	Lunch and networking	All
	EMSL TOUR Participants: Check-in at Registration Desk*	
EMSL TOUR – OPTIONAL (Must Pre-register)		
1:15 – 1:30 pm	Board Bus for EMSL Tour *Near Main Entrance to Three Rivers Convention Center*	
1:30 pm	Bus Departure to EMSL Facility	
2:00 – 3:20 pm	EMSL TOUR • Quiet Wing (James Evans) • CISA Lab (Galya Orr) • NMR Labs (Karl Mueller) • Mass Spectrometry Lab (Lili Paša-Tolić)	
3:20 – 3:30 pm	Return to EMSL Lobby	
3:30 – 4:00 pm	Return to Three Rivers Convention Center	

***Closed-toe footwear is required.**

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Shannon Colson
Becky Ford
Lee Ann McCue
Ljiljana Paša-Tolić
Brian Thrall

Argonne National Laboratory

Jack Gilbert
Sarah O'Brien

INVITED SPEAKERS

Networks of Exchanging Antibiotic Resistomes in Commensal, Environmental, and Pathogenic Microbes

Gautam Dantas, Washington University School of Medicine

While the most acute effects of increasing antibiotic resistance in pathogens are observed in clinical settings, it is becoming increasingly clear that the evolution and transmission dynamics of resistance gene dissemination is an ecological problem. Indeed, steady use and abuse of antibiotics over the past century in food animals, humans, and the environment has provided substantial selective pressure for enrichment of resistance genotypes in each of their associated microbiomes. An over-reliance on culture-based methods, the standard in the study of clinical resistance, has vastly underestimated these reservoirs of resistance genes (or "resistomes"). To address this issue, we have recently developed high-throughput metagenomic functional selections, aided by next-generation sequencing, to characterize resistomes encoded by the microbiota of healthy human adults and children as well as diverse soils. By combining these analyses with 16S amplicon sequencing and deep shotgun sequencing, we model the impact of various anthropogenic perturbations on the transmission and evolutionary dynamics of microbial communities and their resistomes across time and habitats. Hundreds of resistance genes we identify from specific taxa in these different microbial communities are identical to resistance genes found in major human pathogens, indicating recent genetic exchange between these microbes. We also find thousands of functionally validated resistance genes which are genetically novel, but flanked by genes involved in horizontal gene transfer, including transposases and integrases. Together, these findings highlight the substantial antibiotic resistome encoded by microbes from diverse environments, which is available for exchange with pathogens, with the potential to severely exacerbate the problems with clinical resistance.

KEYNOTE ADDRESS: Towards 4-Dimensional (Eco)Systems Biology in the Sea

Edward DeLong, University of Hawaii

Microbial communities regulate the cycling of energy and matter in the marine environment, yet the variability of their activities in space and time and how they dynamically respond to both natural and anthropogenic environmental changes, are not well understood. Genome-enabled methodologies are now providing deeper perspective on the nature and the identity of microbial taxa, genes, and metabolic diversity in the marine environment. Yet one of the larger challenges remaining is defining the variability of these microbial taxa, genes, and processes on different spatial and temporal scales in the environment. Questions that need to be better addressed include: How do activities of different microbial species vary of the course of minutes, hours, days, and weeks? Over what spatial scales are temporal dynamics coherently predictable? How does variation in any specific population correlate corresponding environmental variation, and the variability of other taxa? Novel in situ robotic sampling strategies that capture transcriptomic temporal profiles of wild planktonic microbial populations have potential to provide a four-dimensional motion picture of microbial gene expression dynamics that can begin to address such questions. New results using such approaches show that individual coexisting eukaryote, bacterial and archaeal populations display remarkably similar, time-variable patterns of synchronous gene expression over extended periods of time. Furthermore, these patterns appear to be robust, and conserved in genetically related populations that span the Pacific Ocean. These results suggest that specific environmental cues may elicit cross-species coordination of gene expression among diverse microbial groups that potentially enable multispecies coupling of metabolic activity. These data are leading to specific, testable hypotheses about how microbial interspecies matter and energy exchange may influence the cycling of matter and energy in the ocean.

Nano-enabled Imaging

Mitchel Doktycz, Oak Ridge National Laboratory

Understanding how observable biological processes, carried out over wide-ranging temporal and spatial scales, arise from molecular scale events represents a grand challenge facing biological and environmental research. Underpinning this challenge is the need to monitor the flow of materials and information in response to environmental perturbations. To overcome this challenge, and to enable understanding of the connections across diverse scales, imaging platforms based on advanced micro- and nanofabrication techniques are being developed. Micro- and nanofabrication techniques allow for the construction of mechanical and fluidic devices that are ideal for manipulating materials at the molecular and cellular level. Further, such structures can facilitate sampling and analysis of small quantities. This presentation will highlight several uses of micro- and nanofabricated structures that are being created for studying different aspects of bacterial systems. A particular focus will be on the design and use of a multiscale fluidic environment. These structures have been built via the integration of micro- and nanofluidic technologies and can maintain and control the local cell culture environment while continuously probing and perturbing cells from the subcellular scale up through the community level. This platform technology allows for interactive imaging and enables the use of label free, chemically informative techniques, such as Raman and mass spectrometry, in living systems and at improved spatial resolution. The resulting capabilities will be useful for designing and implementing bioimaging experiments that reach across the hierarchies and dimensions of biological systems to provide understanding to a diverse array of biological and environmental processes. Progress related to the development and application of these technologies will be presented.

Multi-omics of Cellulosic Biofuels Microbes

Timothy Donohue, University of Wisconsin-Madison

In 2007, UW-Madison was named as home of the Great Lakes Bioenergy Research Center (GLBRC), a DOE-funded cellulosic biofuels research center that is directed by Dr. Donohue. Great Lakes Bioenergy conducts basic, genomics-enabled research to design microbial and plant systems that are needed to produce advanced biofuels and bioproducts from the lignocellulosic, non-edible, part of plant biomass. This presentation will focus on the use of acquisition and computational analysis of multi-omic data sets to make, test, and refine hypotheses for the design of plant and microbial systems with improved properties for production of advanced biofuels and bioproducts.

Digitizing the Chemistry of Microbes

Pieter Dorrestein, University of California, San Diego

Chemical, Structural, and Dynamic Imaging Across Modalities and Scales

James Evans, EMSL, Pacific Northwest National Laboratory

Biological organisms have evolved a number of spatially localized and highly orchestrated mechanisms for interacting with their environment. Therefore, there remains a need for multidimensional and multimodal imaging to fully understand biological phenomena and how chemical or structural changes at the molecular level impact the whole organism. We have been advancing new methods for both cryogenic and in-situ correlative analysis of biological samples using electron, ion, optical, and X-ray modalities on relevant spatial and temporal scales. In this talk, I will highlight recent results and outline our plans to expand the versatility of the platform to interrogate complex microbial interactions.

How Microbial Evolution Influences Environmental Ecosystem Services

Jack A. Gilbert, Argonne National Laboratory

Microbial ecology is the study of how these communities assemble and structure within ecosystems. This ecology does not occur in isolation, but is the result of manifold interactions and feedbacks between the biological, chemical, and physical components of the system. The extraordinary diversity of the microbial world has made it very difficult to elucidate the mechanism of interaction that underlies observed trends in structure and function. Advances in metagenomic sequencing and genome re-assembly provide access the individual functional potential of community members, and the ability to examine how strain level variation influences the properties of the ecosystem. The evolutionary processes that shape how bacteria respond to specific stressors, including horizontal gene transfer and synonymous/non-synonymous mutations, can help explain observed trends in the stability and resilience of community structure. Modeling the metabolism of individual bacteria can provide a framework in which to test hypotheses about potential metabolic interactions that shape ecosystem biogeochemistry. When we bring individual metabolic models together as a network of interacting components, we can extrapolate community scale observations to explain whole ecosystem properties. These can then be extrapolated to higher spatial and temporal scales to explain the interaction between microbial metabolism and climate-scale processes. Here I will highlight a number of published and ongoing studies that have started to quantify the impact of microbial evolution and natural selection on ecological dynamics. By specifically exploring bacterial communities at the strain-level we can determine how micro-evolutionary selection shapes the biochemical potential of an ecosystem, including gas flux and carbon/nitrogen metabolism.

A Multi-Scale Approach to the Microbial Community Ecology of Soil Carbon Cycling

Kirsten Hofmockel, EMSL, Pacific Northwest National Laboratory; Ryan Williams, Adina Howe, Elizabeth Bach, Iowa State University

Transforming molecular-scale information into ecosystem-scale knowledge is a formidable challenge that requires identifying keystone biogeochemical processes and characterizing the microorganisms involved at many scales. Here, I will present a research approach that combines metagenomic and biogeochemical data at multiple spatial scales to understand the ecology of cellulose degraders in agricultural soils. We address the composition and functional potential of C-cycling microbial communities using soil microhabitats (aggregate fractions) from reconstructed, fertilized prairies. Because soil C-cycling occurs as the concerted action of multiple, interacting microbial populations, we developed a novel co-occurrence workflow that links sequence and biogeochemical data to distinguish putative, community-scale networks. By applying this approach across small and large-scale experiments, we are developing a deeper understanding of the underlying microbial ecology that governs cellulose decomposition at micro and macro-system scales.

Infrared Spectromicroscopy for Live Cell Chemical Imaging

Hoi-Ying Holman, Lawrence Berkeley National Laboratory

The ability to modify metabolic processes induced by either genetic alterations or environmental stimuli is critical for maintaining an efficient functional cell. Much of our qualitative and quantitative understandings of cellular pathways at a molecular level have come from traditional biochemistry experiments with time constraints that are averaged over large populations as well as performed in vitro with purified biomolecules. Although these approaches have clarified many detailed mechanisms, they have difficulty elucidating the phenotypic differences that exist even within a genetically homogeneous population. A pervasive key challenge ranging across microbial ecology, bioenergy, and biomedicine is to identify role-defining cells within a large population and to monitor their real-time biochemistry in situ.

In this presentation, we highlight technological advances that now allow infrared spectromicroscopy to address these tasks and present application examples. Central to this approach is the use of the synchrotron light source,

which enables high-throughput noninvasive spectroscopic microanalysis. This capability can precisely target subpopulations with a diffraction-limited spatial resolution while tracking their unlabeled chemical reactions with molecular specificity—with currently acquirable timescales associated with a number of important biological processes.

Acknowledgment

The Berkeley Synchrotron Infrared Structural Biology Program at the Advanced Light Source is a user facility supported by the U.S. Department of Energy, Office of Science, and Office of Biological and Environmental Research

How Electron Cryotomography is Opening a New Window into Microbial Cell Biology

Grant Jensen, Caltech, Howard Hughes Medical Institute

In the last ten years electron cryotomography has made it possible to visualize large macromolecular assemblies inside intact cells in a near-native, "frozen-hydrated" state in 3-D to a few nanometers resolution. Increasingly, atomic models of individual proteins and smaller complexes obtained by X-ray crystallography, NMR spectroscopy, or other methods can be fit into cryotomograms to reveal how the various pieces work together inside cells. A few good pictures are therefore sometimes all that is really needed to distinguish between competing models. To illustrate these points, I will show how we used electron cryotomography to dissect the molecular architecture of the *Myxococcus xanthus* type IV pilus, and then how this led to a nearly complete pseudo-atomic model of the machine.

Carbohydrate-Binding Proteins as Microbial Detectors

Laura Kiessling, University of Wisconsin-Madison

Humans must fight off microbes that they encounter in the environment and therefore require means of distinguishing host from guest. One defining difference between microbial and mammalian cell surfaces is the carbohydrates they display. In principle, these differences in glycosylation could be exploited to combat microbial infections or to promote a healthy microbiome. We employed glycan arrays to examine the recognition properties of a human lectin, human intelectin-1 (hIntL-1), a lectin found in the intestine and lung that genetic data link to diabetes, Crohn's Disease, and asthma. This lectin fails to bind known human glycan epitopes but rather interacts with multiple, distinct glycan epitopes found exclusively on microbes. We determined its structure complexed with galactofuranose group, and the structure reveals how this X-type lectin can coordinate a functional group common to many microbial glycans but yet avoid interaction with human glycans. The selectivity of hIntL-1 suggests it functions in microbial surveillance, and that its recognition properties might be harnessed to yield new strategies for diagnostic or delivery agents that exploit its selectively for microbial glycans.

Integration of Dynamic, Multi-omic Data for the Human Microbiome

Rob Knight, University of Colorado-Boulder

The human microbiome is often studied in a static context, yet temporal patterning is increasingly important as a key factor governing microbial communities. Extending studies beyond lists of taxa is also critical for understanding, although moving from a list of taxon changes that can't be interpreted to a list of gene, transcript, or metabolite changes that can't be interpreted either is a pyrrhic victory. Integration of multi-omics data therefore requires a range of approaches integrating statistical, biological, and computational knowledge. However, the potential for integration of these approaches is immense, and the payoff will be the ability to understand and control complex microbial communities on scales ranging from our bodies to our planet.

Multi-analytic-based Determination of Substrate Fate From in situ Stable Isotope Labeled Exposures of Natural Microbial Mats

Mary S. Lipton, Amy A. Boaro, Eric L. Huang, Thomas O. Metz, Marina Gritsenko, Alexandra Cory, Krystin Riha, Jennifer Mobberley, William Nelson, Young-Mo Kim, James J. Moran, Pacific Northwest National Laboratory

Metabolic interactions between organisms in microbial communities are predicted to be the mechanism by which higher-order emergent abilities of the community are established. To understand how communities develop these abilities, we must understand the fate of substrates as they are shared between organisms. Stable isotope probing (SIP) is a standard approach to reveal inter-organism flow of metabolites, but each individual approach is limited in the breadth of the analyses. Integrated SIP analytics applied to a photosynthetic microbial mat revealed different metabolic fates and organism-specific uptake for three carbon-based substrates: glucose, acetate, and HCO_3^-. When compared to acetate, glucose and HCO_3^- showed a greater incorporation into extracellular material, while acetate had a greater conversion to intracellular fatty acids. This suggests that HCO_3^- and glucose are more readily shared as a community currency than acetate. All substrates were converted to amino acids and proteins, but while glucose and HCO_3^- demonstrated considerable incorporation into proteins produced by heterotrophic community members, the conversion of acetate to proteins in the organisms was minimal, implying that acetate-derived intermediates are not a currency of metabolic exchange in the community. HCO_3^- incubations resulted in predominant labeling of photosynthetic proteins, while glucose and acetate incubations labeled mainly transporter proteins, potentially indicating the repression of photosynthetic activity in the presence of an alternative carbon source. Investigations of this nature directly measure resource partitioning and metabolic activities of populations within a community and reveal the nature and mechanism of interactions that could explain the origin of higher-order community properties.

Mathematical Modeling of -omics Data for Biofuel Production

Hector Garcia Martin, Joint BioEnergy Institute

The genomic revolution has afforded us easily available genomes. Furthermore, while genomic information provides an inventory of what an organism can do, functional genomics in the form of -omics data (transcriptomics, proteomics, metabolomics, fluxomics) provides a picture of what the organism is actually doing. However, an standing problem is how to efficiently use such a wealth of data to produce actionable items; e.g., which treatment to use to alleviate an illness, or which gene to modify to improve biofuel production? In this talk, we will present an example on how to use metabolomics data to measure metabolic fluxes for genome-scale models and how to use these fluxes to improve biofuel production. We will also present an example of using proteomics data and statistical techniques to improve jet biofuel production. Finally, we will introduce some of the tools we had to develop in order to mathematically model metabolism to obtain these results.

Exploratory Data Analytics to Identify Signatures of Perturbations to the Soil Biogeochemical System

Lee Ann McCue, Pacific Northwest National Laboratory

Computational methods to efficiently explore and accurately analyze multi-omics datasets have lagged behind our ability to generate these data. For example, the existing computational tools to process complex metagenome sequence data from natural microbial communities have considerable computational requirements and often result in gene or transcript abundance tables for which the appropriate statistical models for analysis are unclear. Similarly, as the resolution of mass spectrometry technologies improves, the computational tools and analytical methods to align and differentiate spectra need to be refined. We are developing tools to explore these high-throughput datasets to identify relationships among explanatory variables and assess the relationships between explanatory and outcome variables. We are also mining data sources such as KEGG to link geochemical features identified by high-resolution

mass spectrometry with metagenomic features to infer relationships between the metagenomic capability and changes in the soil organic matter in arid lands soils subjected to perturbation. Our tools provide interactive visualization, and the flexibility to view and interact with the data in various ways to aid interpretation.

Differences in Susceptibility to Perturbation in the Gut Microbiota and Metabolome in a Genetically Divergent Mouse Population

Thomas O. Metz, Young-Mo Kim, Colin J. Brislawn, Erika M. Zink, Sarah J. Fansler, and Janet K. Jansson, Pacific Northwest National Laboratory; Antoine M. Snijders Sasha A. Langley, Yurong Huang, and Jian-Hua Mao, Lawrence Berkeley National Laboratory; Sarah E. Cates, Nashiya Robinson, Ginger D. Shaw, and Darla R. Miller, University of North Carolina at Chapel Hill

Microorganisms are increasingly recognized for their roles in ecosystem health. However, the mechanisms by which microorganisms communicate and interact at the molecular level with each other, with plant and animal hosts, and with their environments are largely unknown. For example, understanding the respective interplay between the host and the gut microbiome is complicated due to their respective variability. One approach to characterize the relative influence of the host genetic background and environment on the microbiome composition and function is to simultaneously monitor both in a panel of lines from the Collaborative Cross (CC) mouse resource, a population of recombinant-inbred lines generated by randomizing the genetic diversity of 8 founder lines. In this study, we characterized the gut microbiomes and metabolomes of samples collected from 24 CC lines at the University of North Carolina and Lawrence Berkeley National Laboratory. Analysis of 6.4 million 16S rRNA sequences characterized 3,786 operational taxonomic units, and metabolomics analyses quantified 232 metabolites in the samples. While distinct microbiome and metabolite profiles were observed for different mouse lines, the largest influence on the profiles was not host genetics. Our results demonstrate the importance of including metabolomics in studies of microbial communities and also illuminate new considerations when tackling the gut microbiome in particular.

Microbes are STICKY: Large-scale Inference and Topological Analysis of Microbial Interaction Networks

Christian Müller, Simons Foundation

In recent years, 16S-rRNA and other environmental sequencing measurements of microbial communities have revealed phylogeny diversity and abundances of microbial populations across many ecosystems. While changes in microbial community composition are demonstrably associated with certain environmental conditions (from metabolic and immunological health in mammals to ecological stability in soils and oceans), the identification of interaction patterns among microbes is largely elusive and requires new statistical tools. We here present an application of our recently introduced SPIEC-EASI (SParse InversE Covariance Estimation for Ecological Association Inference) framework that combines compositional data transformations with algorithms for sparse neighborhood and inverse covariance selection to robustly and reproducibly infer microbial interaction networks from 16S rRNA data.

We investigated a large number of publicly available data sets across many ecosystems, ranging from gut and freshwater habitats to urban environments. We used SPIEC-EASI to robustly learn and analyze the topology of the microbial interaction networks using concepts from graph theory and statistics. Surprisingly, we find general topological features emerging within and across habitats that pertain to the ecological stability and interaction patterns of microbial communities. We also observe that the STICKY random graph model (where hub species are assumed to highly interact with each other) describes microbial networks accurately across multiple habitats. We believe that these findings shed a new light on the organization principles of microbial communities and present a promising step toward the unification of ecological theory and data-driven systems microbiology.

Cracking the Microbiome Through Advanced Mass Spectrometry

Ljiljana Paša-Tolić, Malak M. Tfaily, Nikola Tolić, Jared B. Shaw, Tzu-Yung Lin, Aleksey V. Tolmachev, Christopher R. Anderton, Errol W. Robinson, David W. Koppenaal, and Nancy J. Hess. EMSL, Pacific Northwest National Laboratory

Given that over 90% of microbial community members cannot be cultivated (and thus have unknown functions and identities), together with exceptional diversity and lack of representative assembled genomic databases, various omics data is extremely challenging to acquire, analyze and interpret, particularly with temporal and spatial resolution needed to decipher functional roles within the microbiome. Additionally, a limited understanding of the molecular composition and dynamics of natural organic matter (e.g., soil or dissolved organic matter) within microbial habitats limits our ability to routinely decipher (bio)chemical processes within complex matrixes (e.g., soil). Here, we will describe advanced mass spectrometry (MS) capabilities available at EMSL, a DOE national scientific user facility located at PNNL, to address the key knowledge gaps in functional understanding of how complex microbiomes influence and are influenced by their environment and present selected applications to oceanic, freshwater, and soil microbiome field studies. Special emphasis will be on the novel high-magnetic-field (i.e., 21T) Fourier transform ion cyclotron resonance (FTICR) mass spectrometer recently brought online at EMSL. Because all key measures of FTICR MS performance improve with increased magnetic field strength, a high-field FTICR spectrometer will arguably provide that next level of performance needed to understand, e.g., chemistry and dynamics of the belowground carbon cycle. Identification and quantitation of intact molecular structures derived from higher plants and soil microbiome within their native 3D inorganic soil matrix are essential for advancing molecular-scale mechanistic understanding of the role of physical, geochemical, and biological processes belowground. The 21T FTICR platform will dramatically increase spectral acquisition rate and attainable sensitivity, and thus enable characterization of intact molecular structures in soil (and other complex matrixes) while addressing spatial relationships and heterogeneity, particularly in combination with other imaging modalities; e.g., FISH and/or nanoSIMS.

Towards Population-level Microbiome Monitoring: the Flemish Gut Flora Project

Jeroen Raes, Vlaam Institute for Biotechnology

Alterations in the gut microbiota have been linked to various pathologies, ranging from inflammatory bowel disease and diabetes to cancer. Although large numbers of clinical studies aiming at microbiome-based disease markers are currently being performed, our basic knowledge about the normal variability of the human intestinal microbiota and the factors that determine this still remain limited. Here, I will present a large-scale study of the gut microbiome variation in a geographically confined region (Flanders, Belgium). A cohort of >5000 individuals from the normal population is sampled for microbiome analysis and extensive metadata covering demographic, health- and lifestyle-related parameters is collected. Based on this cohort, a large-scale cross-sectional study of microbiome variability in relation to health as well as parameters associated to microbiome composition is being performed. In this presentation, I will discuss our experiences in large-scale microbiome monitoring, show how the development of dedicated computational approaches can assist in microbiome analysis and interpretation, and present first results coming out of this effort.

Structural Diversity among Fungal Hyphae: Insights into Cell Growth and Phylogeny

Robert Roberson, Arizona State University

The defining feature of filamentous fungi is the hypha; a tube-shaped cell that grows at its tip. The mode of hyphal growth is a complex process that has allowed the fungi to successfully utilize a wide range of ecological habitats and develop multiple lifestyles. The hyphal cytoplasm contains many of the organelles and sub-cellular inclusions found in other heterotrophic eukaryotic organisms. However, because of their mode of growth and

diverse interactions with the ecosystem, hyphae also maintain a cytoplasmic structure and contain elements that are unique to the eukaryotes. Some cytoplasmic features show significant structural plasticity within the fungi themselves or are present in only certain fungal groups. These elements (e.g., Spitzenkörper) are of particular importance in understanding aspects of hyphal growth and are being used as indicators of fungal evolution. In this presentation, light and electron microscopy are used to review cytoplasmic features of hyphae from diverse taxonomic groups. Characteristics of cytoplasmic order, behavior, and selected organelles that are relevant to understanding hyphal growth and fungal phylogeny will be presented.

New Directions in Multi-omic Microbiome Measurements: Faster, Better, AND Cheaper

Richard D. Smith, Pacific Northwest National Laboratory

Mass spectrometry (MS)-based proteomics, metabolomics and other MS-based 'pan-omics' microbiome measurements are having profound impacts on broad areas of biological research. Increasingly, advances in the quality, resolution, and the speed of, e.g., polypeptide and protein separations are arguably as important as mass spectrometric developments in improving the sensitivity and coverage of measurements. Both liquid phase, e.g., using liquid chromatography, and increasingly gas phase ion mobility separations, respectively, provide a basis for increasing the quality of proteomics measurements, such as the completeness of protein coverage. While these capabilities are challenged by very small sample sizes, the recent development of more efficient nanoelectrospray ion sources and MS interfaces has helped enable ultra-sensitive measurements. Increasingly advances involve gas phase ion manipulations that are conducted between the ion source and m/z analyzer. These manipulations include: ion transport through regions of elevated pressure, trapping, reactions (both ion-molecule and ion-ion), and mobility-based separations. This presentation will discuss the utility of ion mobility separations for biological applications, and describe new developments based upon long path-length Structures for Lossless Ion Manipulations (SLIM) that enable very fast high resolution separations, as well as the use of other gas phase ion manipulation approaches having broad utility for facilitating MS analysis capabilities. The SLIM developments will be discussed with regard to their sensitivity, measurement throughput, cost and quality. The presentation will conclude with consideration of pending developments enabled by the combination of SLIM with massively parallel MS platforms.

Personalized Medicine: Multiomics Profiling During Periods of Health, Disease, and Weight Changes

Michael Snyder, Kevin Contrepois, Brian Piening, Wenyu Zhou, Dalia Perelman, Gucci Gu, Denis Salins, Shana Leopold, Jessica Sibal, Tejas Mishra, Liang Liang, Varsha Rao, Nastaran Heidari, Reza Sailani, Lihua Jiang, Colleen Craig, Candice Allistar, Erica Weinstock, Justin Sonnenburg, George Weinstock, and Tracy MacLaughlin, Stanford University

Understanding health and disease requires a detailed analysis of both our DNA and the molecular events that determine human physiology. We performed an integrated Personal Omics Profiling (iPOP) on 70 healthy and prediabetic human subjects over periods of viral infection as well as during controlled weight gain and loss. Our iPOP integrates multiomics information from the host (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) and from the gut microbiome. For the metabolomics we optimized a LC-MS platform for detailed analysis of blood and urine. Longitudinal multiomics profiling reveals extensive dynamic biomolecular changes occur during times of perturbation, and the different perturbations have distinct effects on different biomolecules in terms of the levels and duration of changes that occur. Overall, our results demonstrate a global and system-wide level of biochemical and cellular changes occur during environment exposures.

Plant Mutants Affecting Root Carbon Allocation Alter the Root Microbiome

Ljiljana Paša-Tolić[1], Charles K. Ansong[1], Heather Brewer[1], Alice Dohnalkova[1], Richard Ferrieri[2], Susannah Green Tringe[3], Michael Sadowsky[4], Chan Lan Chun[4], Christopher Staley[4], Lihui Song[5], Yaya Cui[5], Vania C. S. Pankievicz[5], Fernanda P. do Amaral[5], Karina Freire d'Eça Nogueira Santos[5], Angela D Norbeck[1], Abigail Ferrieri[1], Emanuel M de Souza[6], Fabio Pedrosa[6], Gary Stacey[5]

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The plant rhizosphere contains a complex microbial community that directly impacts plant growth, health, and development. This microbial community, located chiefly in the rhizosphere, is largely supported by the release of nutrients from the plant root with carbon and nitrogen sources being among the most important. Climate change factors, such as elevated CO_2 and soil temperature, are likely to impact the rhizosphere community with perhaps detrimental effects for plant health. In this study, we utilized well-characterized *Arabidopsis* mutants defective in carbon allocation to study changes in the rhizosphere microbial community.

We compared rhizospheric soil of wild-type *A. thaliana* and mutants defective in shoot-root carbon partitioning to directly analyze the role of plant carbon allocation and root exudation in the control of rhizosphere microbial populations. Roots were isolated from plants harvested at first light in the morning and just after the lights were turned off in the evening. The rhizosphere and rhizoplane communities were extracted by either gentle washing or sonication. We used a variety of analytical tools to view the interactions of plant roots with the soil microbial population including: i) 11C-labeling to measure carbon allocation from shoot to roots; ii) characterization of rhizosphere populations by metagenomic and 16S rDNA amplicon sequencing; iii) imaging the rhizosphere using scanning electron microscopy (SEM); and iv) quantitative metaproteomic and metabolomic measurements.

The data indicated that there are significant differences in the rhizosphere microbiome diversity and activity depending on mutant genotype and time of harvest. These results provide a baseline for further studies to examine the specific impact of different amounts and quality of carbon partitioning on microbial rhizophere communities.

Microbial Diversity and Greenhouse Gas Dynamics in Coastal Wetlands

Susannah Tringe, Lawrence Berkley National Laboratory

Wetland restoration efforts in San Francisco Bay have the potential to reverse land subsidence and sequester atmospheric carbon dioxide. However, the benefits of carbon sequestration may be negated by increased methane production in newly constructed wetlands, shifting the balance between greenhouse gas (GHG) sink and source. Increased salinity due to sea level rise will likely also affect GHG cycling in these sensitive ecosystems. We investigated the effects of salinity and wetland restoration status on belowground microbial communities responsible for GHG cycling in a suite of historic and restored wetlands across the San Francisco Bay/Delta. Using DNA and RNA sequencing, coupled with real-time GHG monitoring, we profiled the diversity and metabolic potential of wetland soil microbial communities. We observe large differences in GHG production between freshwater and saline wetlands, as well as between historic and restored, that can be linked to microbial community composition.

Tracking Fracking: Constraints on Subsurface Life During Energy Extraction

Kelly Wrighton, Ohio State University

CONTRIBUTED TALKS

Meta-omic Analysis Reveals Widespread Functionality in Antarctic Hypoliths from Two Dry Valley Systems

Thulani Makhalanyane, Surendra Vikram, Leandro Guerrero, and D.A. Cowan, University of Pretoria; Janet K. Jansson, Carrie D. Nicora, Samuel Purvine, Sarah J. Fansler, Young-Mo Kim, and Thomas O. Metz, Pacific Northwest National Laboratory; S. Craig Cary, University of Waikato

Antarctica is covered by an expansive ice-sheet, with less than 4% of the continent being ice-free. These regions are mostly hyperarid and potentially store a vast amount of terrestrial carbon. The microbial communities in these soils, and associated cryptic niche habitats, are relatively underexplored and it remains completely unknown how they are likely to respond to changing climatic regimes. The studies undertaken in these terrestrial systems have been primarily DNA based, which could represent historical gene signatures. To decipher the relationship between community structure and function, we applied metagenomics, metaproteomics, and metabolomics to hypoliths and soils from two Antarctic Dry Valleys. Targeted analysis of 16S rRNA gene amplicons from hypoliths and soils indicated broadly similar microbial phylogenetic composition. However, results from both metaproteomics and metabolomics clearly indicated highly distinct functional microbial communities and biogeochemical cycling processes, which were largely driven by hypolithic communities (niche effect), with very little activity in soils. These results provide the first evidence of microbial functionality (c.f. to functional capacity) using integrated 'omic' analysis in depauperate Antarctic systems and allow us to better predict how soil communities may respond to changing climates.

Natural Occurrence of Beneficial Traits in the Rhizosphere Microbiome of Oilseed Rape Revealed by Linking Metagenomics with Single Strain Omics

Henry Müller and Gabriele Berg, Graz University of Technology

We analyzed the microbial metagenome of the oilseed rape rhizosphere in respect of bacterial genes that were identified to be essentially involved in plant-microbe interaction of two beneficial plant-associated bacteria. By studying the genomes and transcriptomes of the rhizospheric bacteria *Stenotrophomonas rhizophila* SPA P69 and *Serratia plymuthica* 3Rp8, we pinpointed genetic factors featuring colonization competence, antagonism, and plant growth promotion. The genome of *S. rhizophila* SPA P69 comprises genes that are important for ecological fitness and intimate interaction with plants. By analyzing the transcriptome in response to root exudates, we identified functional genes that explain the colonization competence as well as the ability to enhance plant growth and stress tolerance. This includes genes for host cell attachment, biofilm formation, and synthesis of protective compounds. Spermidine, described as a plant growth regulator, was newly identified as a protector against stress. The isolate *S. plymuthica* 3Rp8 possesses a broad antagonistic activity and is applied to seeds as biocontrol agent to suppress soilborne fungal pathogens. A cursory search of the genome sequence revealed the presence of gene clusters putatively involved in antagonistic activity including genes for synthesis and exudation of hydrolytic enzymes and antifungal compounds. Genes encoding particular functions in both beneficial bacteria were selected for determining their occurrence in the rhizosphere microbiome of two oilseed rape cultivars at two field sites compared to bulk soil. In metagenomes obtained from the rhizospheres, we detected several genetic elements in higher abundances with increased diversity revealing the natural potential of plants to acquire favorable microorganisms.

Understanding Childhood Asthma Through Multi-omics

Kathryn Holt, Shu Mei Teo, Howard Tang, Kym Pham, Michael Walker, and Michael Inouye, University of Melbourne; Danny Mok, Merci Kusel, Michael Serralha, Niamh Troy, Barbara Holt, Belinda Hales, Elysia Hollams, and Patrick Holt, The University of Western Australia; Yury Bochkov, Kristine Grindle, and James Gern, University of Wisconsin School of Medicine and Public Health; Sebastian Johnston, Imperial College London; Peter Sly, The University of Queensland

Myriad factors contribute to the development of childhood asthma, including host genetics, viral respiratory infections during infancy, sensitization to aeroallergens, and early bacterial colonization of the respiratory and gastrointestinal tracts. To investigate how these factors interact during early childhood to cause asthma in school-age children we performed multi-omic analyses in a birth cohort (n=234) at high risk of allergy that has completed a 10-year follow-up for asthma, including respiratory, physiological, and immune phenotypes. Nasopharyngeal samples were collected by the study clinician up to age 5, including regularly during periods of respiratory health and during every parent-reported episode or respiratory illness. These were subjected to viral profiling and bacterial profiling (by and 16S deep sequencing), and all subjects were genome-wide genotyped.

The nasopharyngeal microbiome was relatively simple in structure, with most children initially colonized (~2 months of age) by *Staphylococcus*, *Corynebacterium* or *Streptococcus*; then becoming colonized by *Moraxella* or *Alloiococcus* by 1-2 years of age and increasing in bacterial diversity up to age 5. Early asymptomatic colonization with *Streptococcus* (~2 months) was significantly associated with the development of allergic asthma by age 5.

Lower respiratory tract illness during infancy (first year of life), particularly febrile episodes, were significantly associated with asthma at age 5. Symptomatic respiratory illnesses were characterised by detection of well-known viruses (HRV, RSV) but also *Streptococcus*, *Haemophilus*, or *Moraxella*. Interestingly, during infancy but not later, these genera were also associated with symptom severity including progression to lower respiratory tract and presence of fever.

Host genome-wide association scans of microbial phenotypes in the critical window of infancy yielded surprisingly strong signals given the cohort size, including loci for specific viral infections, wheeze during infection, and early *Streptococcus* colonization. These data shed new light on the diverse interactions involved in asthma pathogenesis.

Comparative Systems Biology of Bacterial Metabolic Phenotypes

Germán Plata and Dennis Vitkup, Columbia University

The range of environments in which a bacterium thrives is a major component of its ecology and natural history. Bacterial metabolic networks connect the set of extracellular nutrients present in such environments to growth, synthesis of secondary metabolites, and potential inter-species interactions. While measuring these transformations in vivo can prove challenging, metabolic phenotypes can be readily predicted using genome-based metabolic network models. We used hundreds of such models and constraints-based analyses to generate nutrient utilization, gene essentiality, and gene-pair synthetic lethality profiles of bacteria, spanning billions of years of evolutionary divergence. Comparison of these profiles revealed a striking regularity in the process of long-term bacterial phenotypic evolution. The analysis showed substantial phenotypic diversification within individual species followed by an exponential-like decay of phenotypic similarity with divergence time. The generality of the observed trends was validated using phenotypic microarray and gene essentiality data for independent sets of species. Comparison of different phenotypic properties showed that gene essentiality and the ability to grow on different nutrients are generally more conserved between distant pairs of species than synthetic lethal interactions, which are therefore less likely to be selected by the environment. Our comparative approach highlights the typical divergence rates of phenotypic properties across sequenced bacterial species; this provides a reference to map phenotypic similarities between species with different lifestyles or taxonomy, which is useful for the interpretation of the processes leading to bacterial adaptation.

Uncovering Microbial Dark Matter, Functional Stability, and Transcriptional Dynamics within the Oral Microbiome

Jeffrey McLean, University of Washington

Although many model species found associated with health or diseased conditions have been characterized in vitro, we do not know if the physiology in pure cultures are maintained in vivo in the presence of a mixed microbial community. Understanding the physiology of bacteria within complex communities including currently uncultured species has become the limiting step in studying the ecology of polymicrobial communities. We have applied next-generation sequencing approaches such as single cell genomics to push this further and enable rare bacteria to be sequenced to gain insight into their functions and also enable cultivation efforts. This has led to the cultivation and sequencing of the first rare Candidate Phylum TM7 member. TM7 has an extremely small genome and physical size (200–300 nm) and has a distinctive lifestyle not previously observed in human-associated microbes. It lives as an obligate epibiont of another bacterium yet also has a parasitic phase, thereby killing its host. In addition, knowledge on the metabolic functions in the microbiome is also restricted due to the lack of tools to study the dynamics of metabolism in real-time. A hallmark of supragingival oral microbial communities is the ability to rapidly metabolize carbohydrates, which dramatically reduces the pH. The subsequent recovery of pH is a homeostatic mechanism and a key aspect of a host-beneficial microbiome that maintains oral health yet is poorly understood. Combining metatranscriptomics and global metabolomics in a model oral biofilm system, we have gained Insights from the community to the gene level using a unique in vitro biofilm model system that maintains hundreds of bacterial species. Temporal meta-omics analyses allowed differential expression profiles of community functions as well as full transcriptomes of active species that show oral community functions occur in succession. Importantly, expression of key pathways involved in homeostasis within the oral microbiome community was revealed.

Functional Diversification in Microbial Symbiosis Supports Longevity under Extreme Conditions

Gabriele Berg, Tomislav Cernava, Anastasia Bragina, Jung Soh, and Christoph W. Sensen, Graz University of Technology; Ines Aschenbrenner and Martin Grube, University of Graz; Katharina Riedel, University of Greifswald

Symbiosis represents a frequent and successful lifestyle on Earth. Recently, bacterial communities were identified as stable, specific, and structurally integrated partners of the classic lichen symbiosis [1]. Metagenomic, -proteomic, and metabolomic data were comparatively assessed to understand the role of bacteria and archaea within the symbiosis using the lung lichen *Lobaria pulmonaria* as a model. We have found that more than 800 prokaryotic species have the ability to contribute multiple aspects to the symbiotic system such as (i) nutrient supply, (ii) resistance against biotic stress factors, (iii) resistance against abiotic factors, (iv) support of photosynthesis by provision of vitamin B12, (v) fungal and algal growth support by provision of hormones, (vi) detoxification of metabolites, and (vii) degradation of older parts of the thallus [2]. The results were confirmed by bridging multi-omics with culture studies [3]. Vertical transmission of a core microbiome was identified [4]. Our findings showed the potential of lichen-associated bacteria to interact with the fungal as well as algal partner to support health, growth and fitness of their hosts. We developed a model of the symbiosis depicting the functional multi-player network of the participants, and argue that the strategy of functional diversification supports the longevity and persistence of lichens under extreme and changing ecological conditions. Our model can be confirmed for Sphagnum mosses and their symbiotic microbiota [5, 6]. In addition, we exploited our results for biotechnological applications; e.g., bioactive volatiles produced by lichen-associated are able to suppress multi-resistant human pathogens [7].

[1] Grube *et al.* ISME J (2009)

[2] Grube *et al.* ISME J (2015)

[3] Cernava *et al.* Frontiers in Microbiology (2015a)

[4] Aschenbrenner *et al.* Environmental Microbiology (2015)

[5] Bragina *et al.* ISME J (2012)

POSTERS

1. Coupling High-resolution Soil Carbon Metabolomics with Microbial Profiling Techniques in a Permafrost Transition

Carolyn Anderson, James C. Stegen, and Malak M. Tfaily, Pacific Northwest National Laboratory

Permafrost thaw has complex effects on the carbon cycle in northern high latitudes. Because permafrost soils harbor twice the amount of C as contained within Earth's atmosphere, thaw may cause net loss of C to the atmosphere in sufficient quantities to result in a positive feedback, whereby warming drives the loss of C, which drives further warming. The response of C cycling in permafrost zones to ongoing warming will therefore have a major influence over future climate. There is limited understanding, however, of the interplay among soil-atmosphere C fluxes, microbial communities, and soil organic matter (SOM) chemical composition. To address this knowledge gap, we leveraged the distinct spatial transitions in permafrost-affected soils at the Caribou Poker Creek Research Watershed, a 104-km^2 boreal watershed ~50 km north of Fairbanks, AK. We couple field C flux and vegetation structure measurements with soil analyses, including metabolomics (ultrahigh resolution SOM chemical composition from Fourier-transform ion cyclotron resonance mass spectrometry, FT-ICR) and microbial community composition (16S rDNA sequencing). Structural equation modeling suggests linkages among abiotic and biotic variables that control microbial community richness, including novel contributions of high-resolution SOM data. Specifically, we find that while some compounds in SOM directly govern microbial diversity levels (e.g., CHO and CHOSP compounds), others indirectly control diversity levels through cell density (e.g., amino sugars). Additionally, the model suggests that total soil C levels strongly mediate cell density, which in turn controls microbial diversity levels. Coupling high-resolution SOM metabolomics and microbial profiling techniques, in tandem with soil physicochemical characterization and ecosystem structure and process measurements, enables a novel analysis of the connections between complex microbial communities, the soil environment, and C cycling in a permafrost system. This work will ultimately provide key insights needed to improve the representation of interactions among SOM, microbial communities, and C cycling across permafrost transition zones, thereby helping to reduce uncertainty in global climate predictions.

2. Probe-Enabled Live Cell Discovery of Microbial Vitamin Transport and Enzyme-Cofactor Interactions

Lindsey N. Anderson, Phillip K. Koech, Andrew E. Plymale, Allan Konopka, Mary S. Lipton, Margaret F. Romine, and Aaron T. Wright, Pacific Northwest National Laboratory; Elizabeth V. Landorf and Frank R. Collart, Argonne National Laboratory

Extracellular salvage of vital nutrients, in addition to the intracellular utilization and metabolic exchange of these nutrients as protein cofactors, is critical to the growth, resilience, and resistance to perturbation of microbes and microbial communities. Nutritional limitations have resulted in a widespread pattern of microbial auxotrophy and opportunism for B-type vitamins. Microbial auxotrophy and opportunism for vitamins is facilitated by a transport domain acting as the cellular interface with the surrounding environment for the selective uptake of vital cofactors and enzymatic intermediates involved in both salvage and *de novo* biosynthetic pathways. Vitamin auxotrophy has the potential to create interdependence between organisms, therefore influencing the balance of microbial membership, spatiotemporal functions, and the metabolic mutualism found within thriving ecosystems. However, the functional and mechanical diversity of membrane-embedded transport systems prove challenging to assign function and specificity to a wide range of experimentally unidentified/predicted proteins by comparative genomic analyses alone. Herein, using newly developed chemical probes derived from B-type vitamins, we identify and experimentally characterize B1-, B2-, and B7-specific transporters and selective intracellular protein-protein associations through live cell probe labeling of *Chloroflexus aurantiacus*. *C. aurantiacus*, unique in its ability to grow photolithoautotrophically (anoxic conditions in the light), is one of the most metabolically versatile members of the phylum *Chloroflexi*, thus serving as a phototrophic model for understanding the functional origin and evolution behind key autotrophic/heterotrophic interactions under metabolic flux in diverse environments. Given the ever-expanding collection of microbial genomes, probes such as those described here will become evermore important in accurately characterizing nutrient/cofactor-specific uptake and metabolic exchange patterns, which will inform us on how microbes in the environment respond to change and alter metabolic outputs.

3. The Combined Application of Liquid Chromatography, Ion Mobility Spectrometry, and Mass Spectrometry Enhances Kansas Soil Multi-omic Analyses

Erin Baker, Kristin E. Burnum-Johnson, Jennifer E. Kyle, Xing Zhang, Bobbie-Jo M. Webb-Robertson, Matthew E. Monroe, Samuel O. Purvine, Carrie D. Nicora, Sarah J. Fansler, Richard A. White III, Eric M. Bottos, Taniya Roy Chowdhury, Lee Ann McCue, Richard D. Smith, and Janet K. Jansson, Pacific Northwest National Laboratory

Multi-omic analyses of complex environmental samples present numerous analytical challenges, such as inadequate measurement throughput and high concentrations of organics. To address these challenges, ion mobility spectrometry (IMS) measurements can be easily integrated between liquid chromatography and mass spectrometry (LC-MS) analyses. Interfacing LC-IMS-MS measurements greatly increases the overall peak capacity of each measurement since you are able to analyze 3-dimensions simultaneously: LC (hydrophobicity), IMS (structure) and MS (size). The ability to perform these multi-dimensional analyses is very important for multi-omic studies since many different types of molecules must be detected. LC-IMS-MS measurements for biological samples have shown reduced false discovery identifications due to the additional discrimination afforded by the third dimension, while also having higher sensitivity and throughput than LC-MS measurements alone. LC-IMS-MS has also been instrumental in the analysis of complex environmental water, soil, and plant matter samples. In these samples the three dimensions are particularly useful for separating high concentrations of organics (e.g., humic acid substances in soil), natural contaminants (e.g., abundant salts or polymers), and detergents in samples where removal through additional sample processing can result in prohibitive losses. In this presentation, LC-IMS-MS results for proteomic and lipidomic analyses of Kansas soil samples before and after glycine treatment will be presented. In the proteomic analyses, IMS was able to separate humic acids from the peptides of interest, providing three times as many highly confident peptide hits as the LC-MS analyses with a Q-Exactive. The three-analysis dimensions also greatly reduced the false discovery rate of the peptides detected. The LC-IMS-MS lipidomic analyses were able to detect new lipid species that have not previously been observed in any of our other sample types. Statistically significant protein and lipid changes were seen when glycine was added to the soil, and these results will be illustrated in the presentation.

4. Tracking the Movement of ^{13}C Labeled Substrates into and Through a Hypersaline Phototrophic Microbial Mat Using SIPPER

Amy Boaro, Eric Huang, Jennifer Mobberley, Alexandra Cory, William Nelson, James Moran, and Mary Lipton, Pacific Northwest National Laboratory

Microbial autotroph and heterotroph interactions are integral to global biogeochemical cycles. Cyanobacterial mats are stratified arrangements of photoautotrophic cyanobacteria and associated heterotrophic microorganisms that form at the interface between water and sediments. We hypothesized that the chemical nature of a substrate applied to a mat system will affect its eventual fate. To evaluate this hypothesis, we performed stable isotope probing using bicarbonate, glucose, and acetate to track carbon flow into and through a cyanobacterial mat from hypersaline Hot Lake (Oroville, WA). We used the Stable Isotope Probing Protein Extraction Resources (SIPPER) program and MSGFdb to evaluate the incorporation of ^{13}C substrates into peptides and to identify them at a phylogenetic level. All substrates were taken up preferentially by cyanobacteria, with glucose showing more heterotrophic uptake than other substrates. Over 50 percent of labeled proteins were unique between the bicarbonate and glucose treatment, indicating differential partitioning of substrates. Label from the bicarbonate treatment was present in more proteins relevant to amino acid transport and metabolism, as well as translation, ribosomal structure, and biogenesis. Label from the glucose treatment was more prevalent in proteins responsible for carbohydrate transport and metabolism, and inorganic ion transport and metabolism.

5. Multi-omic Responses to Glycine Addition in Complex Soil Communities

Eric M. Bottos, Taniya RoyChowdhury, Richard A. White III, Colin Brislawn, Young-Mo Kim, Thomas O. Metz, Lee Ann McCue, and Janet K. Jansson, Pacific Northwest National Laboratory

Sequencing technologies are rapidly expanding our abilities to unravel aspects of microbial community structure and function; however, characterizing highly diverse communities, like those in soils, is problematic, due primarily to challenges in data analysis. To address this problem, we aimed to constrain microbial diversity in a soil community by enriching for particular functional groups through addition of "trigger substrates". Trigger substrates are characteristically low molecular weight, readily soluble and diffusible in soil solution, representative of soil organic matter derivatives, and rapidly degradable. Addition of these substrates to a soil community would presumably select for a cohort of microorganisms in a state of metabolic alertness and with the energetics and cellular machinery for their utilization. Glycine is a free amino acid known to have short turnover times (in the range of hours) in soil: amino acids are a source of nitrogen, easily degradable, and can

serve as building blocks for proteins and other biomass components. We hypothesized that the addition of glycine as a trigger substrate will decrease microbial diversity and evenness, as taxa capable of rapidly metabolizing it are enriched in relation to those that are not. We tested this hypothesis by incubating three Kansas native prairie soils with glycine for 24 hours, and measured community level responses by 16S rRNA gene sequencing, metagenomics, metatranscriptomics, and metabolomics. Preliminary evaluation of 16S rRNA gene sequences revealed only minor changes in bacterial community composition in response to glycine addition, while analyses of functional gene abundance and expression are ongoing. Metabolomic analysis revealed significantly ($p<0.05$) higher concentrations of threonine and serine in glycine treated samples, which will be useful in targeting abundance and expression level analyses of genes involved in these pathways of glycine metabolism. The results of this research will inform sequencing strategies aimed at dissecting and deciphering complex microbial communities.

6. Signatures of Climate Change: Implementation of High-throughput Sequence Data Analysis Methods

Lisa Bramer and Lee Ann McCue, Pacific Northwest National Laboratory

Analyzing high-throughput sequencing data is particularly challenging due to the nature of the data collected. Before analysis can be done across samples, normalization must be performed to account for differences in sequencing depths and consequently make samples comparable. Additionally, traditional statistical tests for differential expression are not appropriate with the count data that is produced by sequencing. While numerous normalization and differential abundance algorithms for sequencing data exist, there is no clear optimal method in either case. This research focuses on comparing and contrasting normalization and differential testing methods and the resulting biological inferences. Here we focus on the methods of DESeq and edgeR for pairwise tests and use big data tools developed by the Tessera project to do exploratory data analysis and examine cases where the two tests differ.

7. High-throughput Structure Determination of Essential Proteins from Pathogenic Agents Exposed to the Human Microbiome

Garry W. Buchko, Pacific Northwest National Laboratory; Bart L. Staker, Center for Infectious Disease Research; Gabriele Varani and Wesley C. Van Voorhis, University of Washington; Tom E. Edwards, Beryllium; Peter J. Myler, Center for Infectious Disease Research and University of Washington

Among the many functions of the human microbiome is the provision of colonization resistance from pathogenic species. To establish a viable niche, pathogenic species counter in a number of ways via the functions orchestrated by their expressed proteins. To better understand the molecular basis of the struggle and to assist the development of new infectious disease therapies, the National Institute of Allergy and Infectious Diseases (NIAID) has funded two state-of-the-art, high-throughput, structural genomics centers to determine the structures of Category A-C infectious disease species proteins. One of these centers is the Seattle Structural Genomics Center for Infectious Diseases (SSGCID, www.ssgcid.org), a well-polished interactive machine composed of four centers performing specialized tasks: the Center for Infectious Disease Research (coordination and target selection), the University of Washington (cloning, expression testing, protein production, and NMR spectroscopy), Beryllium (X-ray crystallography), and Pacific Northwest National Laboratory (NMR spectroscopy). Since inception in 2007, SSGCID has purified protein from >3,000 targets and deposited almost 800 protein structures into the Protein Data Bank, including >300 structures from targets requested by the scientific community. While most of the structures are determined using X-ray crystallography, some proteins are recalcitrant to forming well-diffracting crystals. In these cases the fleet of medium- and high-field NMR spectrometers at PNNL, the largest assortment at any National Laboratory, is used to collect NMR data and solve protein structures in the solution-state. Almost 30 SSGCID targets have been "rescued" by NMR-based methods at PNNL. The structures determined by SSGCID and our sister center, CSGID, help scientists understand the molecular functions performed by these proteins and provide a blueprint for structure-based drug design. An overview of the SSGCID mandate will be provided with a few examples of recent publications that include structures determined at PNNL.

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8. Multi-omic Characterization of Plant Polymer Degradation in Leaf-cutter Ant Fungus Gardens

Kristin Burnum-Johnson, Eric L. Huang, Richard A. White, Carrie D. Nicora, Young-Mo Kim, Bobbie-Jo M. Webb-Robertson, Thomas O. Metz, Erin S. Baker, Mary S. Lipton, and Richard D. Smith, Pacific Northwest National Laboratory; Frank O. Aylward, Lily Khadempour, and Cameron R. Currie, University of Wisconsin-Madison

Leaf-cutter ants are hallmark examples of insect herbivores that gain access to nutrients in plant material through symbioses with microbes. Through the cultivation of fungus-bacterium "gardens" on fresh foliar material, these ants are able to access nutrients in plant biomass that would otherwise be unavailable. Here using proteomic, metabolomic and lipidomic analyses we investigate lignocellulose degradation in the gardens of laboratory-kept *Atta cephalotes* and *Acromyrmex echinatior* leaf-cutter ants. We show that *Leucoagaricus gongylophorus* produces a diversity of lignocellulases in ant gardens and is likely the primary driver of plant biomass degradation in these ecosystems while the bacterial community plays a dominate role in nutrient cycling by converting this nitrogen-poor forage into B-vitamins, amino acids and other cellular components. We show that the fungus produces distinct sets of lignocellulases throughout the different stages of biomass degradation; many cellulases that likely play an important role in lignocellulose degradation localize to the bottom strata near the end of the degradation process which also shows an accumulation of cellobiose and lignin derivative metabolites. Many metabolites and lipids were found to decrease in abundance from top to bottom strata; this is consistent with the idea that readily accessible nutrients from plant material are consumed first. Using proteomic measurements we investigate changes in lignocellulose expression due to substrate availability in *A. cephalotes* colonies fed leaves, flowers, oats or a mix of all three. In these analyses we show that the fungal cultivar produces enzymes to break crystalline cellulose when lignocellulose rich food sources, such as leaves and flowers are available. These discoveries provide new enzyme targets that could be tested for their biochemical properties and application in bioethanol production.

9. The CCMEE (Culture Collection of Microorganisms from Extreme Environments), a Unique Public Resource for Fundamental and Applied Extremophile Research at the Pacific Northwest National Laboratory

Sherry L. Cady, Jessica K. Cole, Jory T. Brookreson, Pacific Northwest National Laboratory; Igor Broun, Beijing University of Chemical Technology; Richard W. Castenholz, University of Oregon

The Culture Collection of Microorganisms from Extreme Environments (CCMEE) is a unique public resource established for teaching and not-for-profit research purposes. In its current configuration, the CCMEE contains more than 2,000 samples collected from hot and cold deserts, hypersaline and acidic waters, hot springs, permafrost, and habitats exposed to high solar irradiance. This diverse and irreplaceable culture collection was obtained from extreme environments on all seven continents over the course of decades of research by Drs. Richard Castenholz, E. Imre Friedmann, and Igor Broun. CCMEE samples include psychrophiles (grown at $T \geq 4°C$), mesophiles, and thermophiles (grown at $T \leq 58$ °C). Major taxonomic groups in the collection include *Cyanobacteria* (e.g., *Chroococcidiopsis*), *Chlorophyta* (e.g., *Chlorococcales*), *Rhodophyta* (e.g., *Cyanidium*), *Stereophyta* (e.g., *Klebsormidium*), and *Stramenophiles* (e.g., *Heterococcus*). As such, the CCMEE harbors a wide swath of biodiversity from a range of habitats and represents a rich source of genetically and biochemically diverse material for research in microbial physiology, ecology, evolution, (poly)extremophily, synthetic biology, biofuels, biochemistry, bioprocessing, bioremediation, and astrobiology. Current projects with collaborators include identification of the evolutionary relationships of the cyanobacteria in the collection based on their 16S rRNA phylogeny; isolation of novel strains of cyanobacteria and red algae; comparison of phototrophic and heterotrophic interactions of different extremophile communities; identification of cyanobacteria capable of intracellular biomineralization; and characterization of microbial biosignatures of geobiological interest. CCMEE sample requests should be sent to Dr. Sherry Cady at EMSL, a DOE Office of Science User Facility sponsored by the DOE Office of Biological and Environmental Research and located at the Pacific Northwest National Laboratory.

10. Parasail: High Performance Vectorized C Library for Global, Semi-global, and Local Pairwise Sequence Alignments

Jeff Daily, Pacific Northwest National Laboratory

Parasail is a high performance C library implementing the Smith-Waterman (local), Needleman-Wunsch (global), and ends free (semi-global) pairwise sequence alignment algorithms. The high performance is achieved by using Single-Instruction Multiple-Data (SIMD) vector instructions for today's most advanced CPU instruction sets including SSE2, SSE41, and AVX2. The resulting performance of parasail's optimality-guaranteeing implementations are comparable to heuristic-based methods such as BLAST. The parasail library provides an application programming interface (API) that can be used as a component of C/C++/Python software to perform optimal protein or genome sequence alignment. Though the library does not provide a full alignment traceback, it does return the score and alignment locations as well as optional alignment statistics such as the number of exact matches, similarities, and length. Parasail also provides a sample application that can do protein and genome alignment directly while also optionally filtering out alignments using arbitrarily long exact-matching seeds. As a result, parasail is readily available to be integrated into larger projects or useful as a stand-alone application.

11. The Microbial Ecology of Leafy Greens: Understanding the Microbiome for Plant Health and Food Safety

Armin Erlacher and Gabriele Berg, Graz University of Technology; Massimiliano Cardinale and Martin Grube, University of Graz

The plant microbiome is contributing to both plant and human health. Raw-eaten vegetables such as arugula (*Eruca sativa* Mill.) and lettuce (*Lactuca sativa* L.) are important food plants in the health-conscious society. Here, microbiome imbalances or pathogen contamination can lead to foodborne outbreaks. To understand the microbiome of raw-eaten vegetables and to avoid both human and plant pathogen outbreaks, we studied leafy greens by a polyphasic approach. Using metagenome shotgun sequencing, sequencing of 16S rRNA genes, and complementary FISH-CLSM analyses we were able to decipher the structure, plasticity, habitat preferences, co-occurrences, and functions of the plant-associated microbiota. We found highly variable colonization patterns across different microhabitats with a major divergence for the phyllosphere compared to soil-derived habitats. An overall enrichment of *Gammaproteobacteria* including *Enterobacteriaceae*, and depletion of *Deltaproteobacteria* and *Alphaproteobacteria* on the aerial, edible plant parts and a strong rhizosphere effect were observed. Network analyses revealed *Enterobacteriaceae* as important core taxa on the aerial plant parts and as highly sensible responders to biotic stress. Spearman correlations between OTUs showed that co-occurrence prevailed over co-exclusion in the rhizosphere, and complementary FISH-CLSM analyses revealed that this pattern could result from both true interactions and habitat sharing. Predominant root taxa exhibited symbiotic interactions without participating in significant modules. In fact, we also identified a potential for organisms to heavily colonize lettuce without the need for complex interactions. The microbial network structure can explain both the plant's susceptibility to pathogens as well as to beneficial strains. These findings were supported by additional biocontrol experiments where application of a promising PGPR strain on *L. sativa* led to increased plant growth, but also highly restructured the native microbiome. Hence, robust information about the microbiome will increase both plant health and food safety and open new pathways for biological control approaches of pathogens.

12. Associations with *Azospirillum brasilense* Rhizobacteria

Abigail P. Ferrieri, Ljiljana Paša-Tolić, EMSL, Pacific Northwest National Laboratory; Charles K. Ansong and Erika M. Zink, Pacific Northwest National Laboratory; Gary Stacey, University of Missouri; Richard Ferrieri, Brookhaven National Laboratory

Plant growth-promoting rhizobacteria are considered to have a beneficial effect on host plants; however, it is controversial whether biological nitrogen fixation from associative interaction contributes to growth promoting effects. This project uses *Setaria viridis* as a model C4 grass to explore mechanisms for plant growth promotion elicited by the rhizobacteria, *Azospirillum brasilense*. Using a systems biology approach, *S. viridis* plants have been extensively studied at the physiological and biochemical levels using compositional and flux analysis with radioactive (^{11}C, ^{13}N) tracers. Here we highlight new results using a high-throughput, isobaric tags for relative and absolute quantitation (iTRAQ)-based, quantitative proteomic approach to analyze changes in protein profiles of *S. viridis* roots inoculated with an ammonium-excreting strain of *A. brasilense*. General features of the *S. viridis* root proteome profiles in response to A. brasilense inoculation are discussed in relation to known observations at the physiological and biochemical levels. These data reveal extensive changes occurring within the *S. viridis* root proteome upon bacterial inoculation, which provide valuable clues for screening key proteins during the

Azospirillum-Setaria cooperation and provide direction for future multi-scale mass spectrometry imaging studies aimed to understand nitrogen trafficking to roots and the bacterial effect on the process. Together these findings offer insight into the mechanisms by which rhizobacteria establish themselves as symbionts within their hosts during early stages of infection, and a deeper understanding of the mechanism for their promotion of plant growth through associative nitrogen fixation and other phytostimulatory actions. While nitrogen management is an important agronomic trait in any cropping system, it is of particular concern for future bioenergy crops that will be grown on sub-optimal soils lacking this essential resource for sustainable plant growth. We expect that new knowledge gained from this effort will directly translate to next-generation bioenergy cropping systems fostering more robust plants.

13. A Longitudinal Analysis of the Bacterial Microbiome of the Rocky Mountain Wood Tick (*Dermacentor andersoni*)

Cory A. Gall, Washington State University

Microbiome manipulation has proven to be an effective tool for tackling human and agriculturally important vector-borne diseases in several insect species. However, these technologies have yet to be applied to tick-borne diseases. Ticks, which are ecoparasitic arthropods, infest a diverse group of hosts across the animal kingdom and are responsible for transmitting numerous livestock and human pathogens. Recently, the microbiome of several species of ticks has been explored. However, these studies have been narrowly focused on composition and the ecological aspect of the microbiome remains unknown. There are several fundamental questions that must be addressed before considering microbiome manipulation as a feasible method to fight tick-borne diseases, such as: Does the geographic location of a population influence the bacterial microbiome? Does the microbiome fluctuate over time? Does the microbiome of labcolonized ticks represent field populations? To address these questions, we identified two populations of *Dermacentor andersoni* ticks with varying ecological conditions (Burns, OR and Lake Como, MT). The bacterial microbiome of both populations was characterized using PacBio sequencing of 16S rDNA. We analyzed the microbiome of the midgut (MG) and salivary glands (SG) in adult males over three field generations as well as three lab-colonized generations. Our results demonstrated that the microbiome is different between Lake Como and Burns tick populations. Additionally, the microbiome fluctuated differently through time based on population and tissue type, ranging from very little change to a major reorganization of the microbiome composition. Furthermore, our results indicated that lab-colonization maintained the microbiome of the original tick population, whereas the microbiome of field-collected ticks is unstable. To conclude, we have addressed important ecological factors of the bacterial microbiome that need to be considered when designing microbiome-targeted manipulation as a novel tick-borne disease biocontrol.

14. Quantitative Multi-Gene Expression Analysis in Intact Cells Using Combinatorial Barcoded FISH

Dehong Hu, Lye Meng Markillie, Will Chrisler, Siva Wu, Janet K. Jansson, Galya Orr, Pacific Northwest National Laboratory; Jian-Hua Mao, Antoine Snijders, and Manfred Auer, Lawrence Berkeley National Laboratory

Fluorescence in situ hybridization (FISH) enables the quantification of gene expression in intact cells, microbial communities or tissues while preserving the spatial information within and between cells. However, the number of distinct mRNA species that can be quantified simultaneously is limited by the number of fluorescent colors that can be clearly differentiated in an image, which is restricted by the spectral overlap between the different colors. The number of distinct mRNA species that can be resolved simultaneously in an image can increase dramatically when these colors are used in a combinatorial way to create fluorescent barcodes. This approach requires the use of super resolution fluorescence stochastic optical reconstruction microscopy (STORM), also known as photo-activated localization microscopy (PALM), for resolving the presence of multiple unique barcodes from one another in a single cell. Gene expression is then quantified by the number of times the corresponding barcode is detected in the cell. This approach, which can quantify the expression of more than 30 genes in an intact cell simultaneously, provides quantitative gene expression analysis while preserving the spatial information within microbial communities or tissues. For this approach, combinatorial barcoded FISH probes are generated from multiple sub-probes, each carries a different color and independently binds a distinct sequence along the targeted mRNA molecule. These sub-probes therefore increase the accuracy of the method by acting as a coincident detector, being counted only when they all are present. Here we demonstrate the application of combinatorial barcoded FISH to quantify gene expression in intact alveolar epithelial cells grown in culture. We also take initial steps toward establishing the technique in mouse intestine tissue sections for quantitative multi-gene expression analysis in the intestinal cells and the microbiome simultaneously.

15. Immunomagnetic Capture and Comparative Proteomic Analysis for the Characterization of *Bacillus anthracis* Sterne Spores

Janine R. Hutchison, David S. Wunschel, Brooke L. Deatherage Kaiser, Andy Lin, and Marvin G. Warner, Pacific Northwest National Laboratory

Detection and characterization of *Bacillus anthracis* spores from complex samples such as dried blood and soil can be challenging because of inhibitors present in these sample matrices that impact downstream detection. The goal of this work is to develop a method for concentration and purification of *B. anthracis* Sterne spores from dried blood and soil samples using immunomagnetic capture. This approach allows the removal of inhibitors and other contaminants, such as non-target bacterial cells and sample debris, prior to proteomic analysis with mass spectrometry. Immunomagnetic bead concentration and purification was performed using commercially available *B. anthracis* spore specific monoclonal or polyclonal antibodies. Antibodies were coupled to Tosyl-activated Dynabeads®. We found that the monoclonal antibodies failed to capture B. anthracis Sterne spores or vegetative cells at levels high enough for downstream detection (capture <10%). Polyclonal-immunomagnetic beads captured >95% of the target spores, and provided a repeatable and scalable concentration and purification method. Immunomagnetic capture was successful in solutions with 1% bovine blood, various soil extracts, and potting soil. To couple the immunomagnetic capture with proteomic characterization we compared four different treatments to dissociate spores/proteins from the antibody-bead complex. The use of 20% trichloroacetic acid (TCA) precipitation followed by enzymatic digestion and (LC-MS/MS) analysis was determined to be the most efficient and resulted in the identification of 371 proteins from spores prepared on blood agar. Our approach demonstrates that recovery and proteomic characterization of *B. anthracis* spores can be accomplished using immunomagnetic capture and LC-MS/MS.

16. Altered Microbial Amino Acid Metabolism in the Gut after Roux-en-Y Gastric Bypass Surgery Might Contribute to Sustainable Post-Surgical Weight Loss

Zehra Esra Ilhan, Andrew K. Marcus, Dae-Wook Kang, Bruce E. Rittmann, and Rosa Krajmalnik-Brown, Arizona State University; Nancy Isern and David W. Hoyt, EMSL, Pacific Northwest National Laboratory; John K. DiBaise and Michael Crowell, Mayo Clinic

The long-term success of Roux-en-Y gastric bypass (RYGB) surgery to treat obesity varies among patients. RYGB patients harbor different gut microbiota than obese and lean people; however, the microbiota's role on success of RYGB surgery is unknown. We hypothesized that the microbiome plays a role in successful and sustainable weight loss after RYGB surgery. We recruited post-RYGB, normal-weight, and pre-operative morbidly obese subjects, and categorized the RYGB subjects into successful (SRYGB) and unsuccessful (URYGB) groups based on their initial weight loss and subsequent weight 7regain. We investigated gut microbiota structure and function using 16S rRNA gene sequencing and its metabolome using H-NMR. Phylotypes from *Gammaproteobacteria*, *Bacilli*, and *Clostridia* were more abundant in SRYGB compared to URYGB fecal samples. Differences in microbiota composition were reflected in fecal metabolites, especially in amino acids and their fermentation products. Based on Receiver Characteristics Operator analysis, we identified specific amino acids (tryptophan and serine), amino acid fermentation products (isobutyrate and isovalerate), and amino acid degradation products (p-cresol and phenyl acetate) as biomarkers of successful surgery. Among these metabolites, serine is the precursor of tryptophan, and tryptophan is known to reduce food intake by stimulating increased serotonin secretion. Amino acid fermentation products (isovalerate and isobutyrate) were significantly more abundant in the SRYGB fecal samples, possibly due to the prevalence of *Clostridia* and *Bacilli* species, which can ferment leucine and valine, amino acids that have been associated with weight regulation. The prevalence of amino acid producing and fermenting *Gammaproteobacteria*, *Clostridia*, and *Bacilli* along with amino acids and their fermentation products in SRYGB samples suggests a potential weight-loss mechanism that involves the gut-brain axis. In conclusion, enhanced microbial amino acid biosynthesis and fermentation in SRYGB patients might be contributors to enhanced weight loss post-RYGB surgery.

17. A Multi-Omic View of Host-Pathogen-Commensal Interplay in Salmonella-Mediated Intestinal Infection

Brooke L. Deatherage Kaiser, Jie Li, James A. Sanford, Young-Mo Kim, Scott R. Kronewitter, Samuel O. Purvine, Thomas O. Metz, Richard D. Smith, and Joshua N. Adkins, Pacific Northwest National Laboratory; Fred Heffron, Oregon Health and Sciences University; Marcus B. Jones, J. Craig Venter Institute

The potential for commensal microorganisms indigenous to a host (the 'microbiome' or 'microbiota') to alter infection outcome by influencing host-pathogen interplay is largely unknown. We used a multi-omics "systems" approach, incorporating proteomics, metabolomics, glycomics, and metagenomics, to explore the molecular interplay between the

murine host, the pathogen *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), and commensal gut microorganisms during intestinal infection with *S*. Typhimurium. We find proteomic evidence that *S*. Typhimurium thrives within the infected 129/SvJ mouse gut without antibiotic pre-treatment, inducing inflammation and disrupting the intestinal microbiome. Alteration of the host microbiome population structure was highly correlated with gut environmental changes, including the accumulation of metabolites normally consumed by commensal microbiota. Finally, both proteomic and glycomic evidence suggests *S*. Typhimurium may take advantage of increased fucose moieties to metabolize fucose while growing in the gut. The application of multiple omics measurements to *Salmonella*-induced intestinal inflammation provides insights into complex molecular strategies employed during pathogenesis between host, pathogen, and the microbiome.

18. Linking the Microbiome to Plant Metabotype and High-value Phytochemicals

Martina Köberl, Ruth Schmidt, and Gabriele Berg, Graz University of Technology; Richard A. White III and Janet K. Jansson, Pacific Northwest National Laboratory; Tarek F. El-Arabi, Ain Shams University; Rudolf Bauer, University of Graz; Christer Jansson, EMSL, Pacific Northwest National Laboratory

Plants form close interactions with microorganisms that are essential for their performance and survival. Thus, plant-microbe interactions are key for understanding and improving plant health and productivity and for sustainable agricultural management practices. It is well known that plants use metabolites to direct organization and growth of their associated microbial communities. However, vice versa, the plant-associated microbiome influences the metabolic activity of the plant leading to different metabotypes. A significant number of plant metabolites are produced by associated microbes, or through interaction with their plant host (prominent, e.g., paclitaxel). To analyze microbiome-metabolome interactions, we used the grass model *Brachypodium distachyon* as well as different species of medicinal plants with particularly high levels of complex constituents, including *Matricaria chamomilla* and *Calendula officinalis*. These two medicinal plants are cultivated all over the world, however with different chemical profiles. We observed a plant-specific selection of rhizospheric microbes associated with medicinal plants grown on an organically managed Egyptian desert farm. The soil microbiome comprised a high abundance of spore-forming *Firmicutes* (esp. *Bacillus* and *Paenibacillus*) and *Actinobacteria* (*Streptomyces*), which were linked to pathogen suppression under arid soil conditions. The desert agro-ecosystem exhibited a higher microbial diversity and better ecosystem function for plant health in comparison to the native desert soil. Promising antagonistic counterparts to soil-borne phytopathogens were selected by a hierarchical screening for field evaluation. The priming of chamomile seedlings had a stabilizing effect on plant performance, and indigenous *Bacillus* and *Paenibacillus* strains were also able to elevate the plants' flavonoid production. These findings suggest that a targeted bacterial treatment can influence the metabolic activity of the plant. We aim to reveal the underlying mode of actions at the genomic and transcriptional levels and to develop an effective biocontrol strategy on the basis of these promising antagonists.

19. Learning Sparse Generalized Aitchison Embeddings for Microbial Compositions

Zachary Kurtz, New York University School of Medicine; Christian L. Müller and Richard A. Bonneau, Simons Foundation

Genomic survey by 16S amplicon sequencing has enabled new appreciation for the role of complex microbial communities in diverse ecosystems. However, due to the unique properties of community composition data, standard analysis methods are likely to produce statistical artifacts. First, the abundances of microbial operational taxonomic units (OTUs) from 16S datasets are compositional. That is, counts are normalized to the total number of counts in the sample. Thus, microbial abundance measurements are not independent, and traditional statistical tools (correlation, differential abundances, etc.) can lead to spurious results. Second, microbial sequencing-based studies typically measure hundreds to thousands of OTUs on only tens to hundreds of samples; thus, inference of taxa associations is severely underpowered. Third, high taxonomic richness in the limit of available sequencing power results in the inflation of observed 'zero counts' - a regime of missing data.

In previous work, Generalized Aitchison Embeddings were proposed as an extension of John Aitchison's log-ratio based framework to map image histograms from the Simplex to a suitable Euclidean space. We propose a novel algorithm to learn a sparse Mahalanobis metric from microbial compositions given some meta-data (i.e., sample class), using the Split Bregman method for L1-regularized optimization problems. We learn an embedding together with pseudo-counts, which regularizes taxa importance from zero-inflated data, to find an embedding under sample similarity constraints.

We demonstrate the relationship with our previously published tool for estimating microbial ecological networks (SPIEC-EASI), as well as geometrically pleasing results on data sampled from manifolds. Further, we benchmark our method against other metric learning and classification tools on synthetic data. Finally, we apply it to an environmental microbiome dataset and train a new classifier for ecological perturbation.

20. Nanospray Desorption Electrospray Ionization (nano-DESI) Imaging of Biological Systems

Julia Laskin, Pacific Northwest National Laboratory

Mass spectrometry imaging (MSI) is ideally suited for the label-free spatially resolved analysis of molecules in complex biological samples with high sensitivity, speed and unprecedented chemical specificity. Ambient pressure (AP) surface ionization MS is particularly attractive for. We have developed nanospray desorption electrospray ionization (nano-DESI) for ambient pressure imaging of biological samples without special sample pre-treatment. Furthermore, we have developed approaches for quantitative nano-DESI imaging of lipids and metabolites. In these experiments, quantification is performed by doping the nano-DESI solvent with appropriate standards and normalizing the ion image of the endogenous lipids and metabolites to the ion image of the standard. This approach enables online quantification of biomolecules extracted from the biological sample without introducing any complexity into the experimental protocol. Several examples will be presented highlighting the power of nano-DESI MSI for quantitative imaging of chemical gradients resulting from metabolite exchange between living microbial and yeast communities. These studies open unique opportunities for molecular-level understanding of interactions on both cellular and community level.

21. Features of Plant Associated Bacteria

Asaf Levy, Scott Clingenpeel, Susannah Tringe, Bryan Rangel Alvarez, Benjamin Cole, Tijana Glavina del Rio, and Tanja Woyke, DOE Joint Genome Institute; Scott Yourstone, Sur Herrera Paredes, Sarah Lebeis, and Jeffery L. Dangl, University of North Carolina, Chapel Hill; Derek Lundberg, University of Tennessee, Knoxville

Plants tightly associate with an array of phylogenetically and functionally diverse bacteria, with which they share complex and fascinating interactions. Greatly influencing host fitness, some plant-associated (PA) bacteria supply essential nutrients to plants thereby promoting plant growth, other PA bacteria serve as pathogens, and some PA bacteria protect the plants from those pathogens. To fulfill their mutualistic, parasitic, or commensal functions the PA bacteria have evolved a large set of genes that enable them to adapt to the plant environment. Here, we employed a large-scale comparative genomics study to characterize the core genome of PA bacteria. This was done by comparing the genomes of 3149 bacterial species from 15 taxa (from phyla *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*) that were classified as being PA bacteria or non-plant associated (NPA) bacteria based on their isolation site. This set includes a list of 112 newly sequenced bacterial genomes that were isolated from the root endophytic compartment of *Arabidopsis*. Multiple taxa present a significant genome expansion as part of adaptation to plant environment. Notably, genes related to carbohydrate metabolism and transport were expanded in diverse PA bacteria. Many of the significant PA genes are ordered in operons that are involved in nitrogen fixation, antibiotics and plant hormones production, and effector protein secretion. Interestingly, this analysis also revealed a large set of uncharacterized genes that are enriched in PA bacteria. These genes are conserved within and across taxa, clustered in operons, and are expressed in the plant environment, underscoring their roles in plant interactions. Understanding the functions of these genes has an enormous potential to improve plant growth efficiency and carbon sequestration by plants via microbiome manipulation.

22. Nitrogen Dynamics Control Carbon Partitioning in Mat-Derived Phototrophic Consortia

Stephen R. Lindemann, Christopher R. Anderton, Jessica K. Cole, Jennifer M. Mobberley, Amy A. Boaro, Beau R. Morton, William C. Nelson, Margaret F. Romine, and James K. Fredrickson, Pacific Northwest National Laboratory

Predicting the effects of changing environmental conditions on microbial community function or engineering synthetic communities requires elucidation of the principles driving the dynamics and properties of these communities. Phototrophic microbial associations are ubiquitous planet-wide; therefore, interactions within them exert globally significant impacts upon biogeochemical cycling. Initial species-resolved metagenome reconstruction of unicyanobacterial consortia from a saline phototrophic microbial mat revealed that most member species within the consortia are unable to directly assimilate nitrate as a nitrogen source. These consortia were isolated and are routinely cultivated with nitrate as the sole nitrogen source, meaning that these organisms are dependent upon other members for nitrogen acquisition. We hypothesized that cyanobacteria served as major primary assimilators and providers of community nitrogen and examined member niches over 28 days of biofilm succession using an integrated transcriptomic and proteomic approach. Interestingly, variable expression of terminal cytochrome oxidases suggested that subpopulations of member species were spread across multiple O_2 tensions, though the bulk fluid remained O_2-supersaturated throughout. *Rhodobacteraceae* spp. capable of reducing NO_3^- to NO_2^-, but not further, exhibited significant expression of the typically O_2-repressed narGHIJ respiratory NO3- reductase. Coordinate

expression of nrfAH, which reduces NO_2^- to NH_4^+, by a *Bacteroidetes* sp. incapable of reducing NO_3^- to NO_2^- suggested multi-species coordination to acquire nitrogen. NH_4^+ producers transitioned from early expression of NO_3^- —acquisition genes to genes for recycling reduced nitrogen (e.g., urease) over succession. Amendment of consortia with reduced N (NH_4^+) at inoculation drastically altered community dynamics. Spatially resolved analysis of ^{13}C and ^{15}N flow via nanoSIMS suggested a probable cause – that cyanobacteria share carbon with their heterotrophic partners much more rapidly when NO_3^- was the sole nitrogen source than they do with NH_4^+ amendment. These data suggest that consortial nitrogen dynamics feed back to control the partitioning of carbon, which in turn governs community composition.

23. Temporal RNA-Seq Analysis of Enterobacter lignolyticus SCF1 During Growth in Lignin-amended Medium

Hugh Mitchell, Lye Meng Markillie, Ron Taylor, Galya Orr, Pacific Northwest National Laboratory; Roberto Orellana and Kristin DeAngelis, University of Massachusetts Amherst

The production of lignocellulosic-derived biofuels is a highly promising source of alternative energy, but one that has been constrained by the lack of a way to efficiently deconstruct lignin from cellulose. A species that naturally grows in lignin-rich and fast decomposing environments, *Enterobacter lignolyticus* SCF1 is a facultative anaerobic Gammaproteobacteria isolated from tropical soil of El Yunque forest (Puerto Rico). SCF1 is capable of anaerobic growth on lignin and is tolerant of growth in the presence of 1-ethyl-3-methylimidazolium chloride, a toxic compound utilized as a pretreatment in the biofuel production. While a previous study of lignin degradation by SCF1 illuminated a potential mechanism for lignin degradation, the expression profile was obscured because the cultures were sampled at stationary phase, when lignin degradation was completed. In order to improve this approach, we characterized the temporal dynamics of lignin degradation, using a whole transcriptome analysis of samples taken at 7 h (early log phase), 22 h (late log phase), and 31 h (early stationary phase). A total of 380 genes were found to be differentially expressed, which represents ~8% of the 4,445 predicted protein-encoding ORFs. The most differentially expressed genes were found after 7 h (216), at which point >50% of the lignin available was degraded. At this time, genes encoding two extracellular laccases were up-regulated. Also, genes involved in the metabolism of sugars (xylose) and central metabolism (formate hydrogenlyase complex) were upregulated. Partitioning of genes that were differentially expressed in a single time point from those were differentially expressed in more than one time point suggested that the process of lignin degradation is dynamic. These results suggest an increased metabolism of sugar associated with lignin degradation, and a mechanism for optimizing growth during lignin metabolism involving formate hydrogenlyase and the hydrogen cycle.

24. Depth-resolved Metabolic Capabilities of Microbes Within an Epsomitic Microbial Mat of Hot Lake, Washington

Jennifer Mobberley, Stephen Lindemann, and William Nelson, Pacific Northwest National Laboratory; Jerome Babauta and Haluk Beyenal, Washington State University

Microbial communities form preferentially at environmental gradients. A model system to study this relationship is the phototrophic microbial mats from the epsomitic Hot Lake (Oroville, WA) that develop at the sediment-water interface. Initial microbial diversity studies suggested that the Hot Lake mat hosts hundreds to thousands of taxa, however little is known about the spatial distribution and metabolic diversity relative to in situ environmental gradients. In this study, we linked functional gene distribution from depth-resolved metagenomic data with confocal microscopy and microsensor biogeochemical profiles. In order to examine key genes involved in biogeochemical cycling pathways we generated functional gene libraries from a *de novo* metagenomic coassembly of five horizontally- sectioned microbial mat layers. The microsensor profiles suggest that light is a major driver of biogeochemical cycling during the day through energy and carbon acquisition from oxygenic photosynthesis. Confocal microscopy indicated that filamentous cyanobacteria are primarily found in the upper sections of the mat, though *rpoB* gene abundance suggested that there are three dominant cyanobacteria that are differentially distributed within this depth profile. While cyanobacterial photosynthesis is the main conduit for energy and carbon acquisition in the upper photic layers of the mat, the diversity of rhodopsin and bacteriochlorophyll a biosynthesis genes indicate a complex phototrophic and mixotrophic community. The metabolic diversity of energy and carbon, nitrogen, and sulfur cycles in the deeper portions of the mat, corresponding to decreased oxygen concentration and light penetration, indicates that the organisms exploit the available environmental gradients. This study provided insights into the distribution and diversity of metabolic capabilities within the Hot Lake hypersaline microbial mat system, and provides the basis for understanding the mechanisms of functional gene distribution within microbial communities.

25. A Multi-omics Approach Coupled to Stable Isotope Probing to Reveal Microbial Interactions and Preferred Carbon Sources in a Photosynthetic Microbial Mat

James Moran, Alexandra Cory, Amy Boaro, Krystin Riha, Hans Bernstein, Jennifer Mobberley, Megan Nims, Eric Huang, Young-Mo Kim, William Nelson, Thomas Metz, and Mary Lipton, Pacific Northwest National Laboratory

Hot Lake is a hypersaline (espomitic) lake in north central Washington State. The lake's extreme salinity limits aquatic vegetation and in so doing helps enable seasonal establishment of benthic microbial mats. These robust phototrophic-driven mats are cyanobacteria dominated and contain a number of associated heterotrophic members. We performed a series of near in situ incubations using freshly sampled microbial mat to probe the fundamental relationships between the photo- and heterotrophic community members and shifts in these relationships across a diel cycle. We amended the lake water used for the incubations with stable isotope labeled (^{13}C) bicarbonate, glucose, and acetate to track the substrate incorporation and accumulation into different biomass constituents. A series of stable isotope, proteomic, and metabolomic analyses provided insights into fundamental changes in the mat's C-cycling over the incubation period. Specifically, we integrated the multi-omics dataset to determine substrate bioaccumulation into the mat at spatial (10s µm, laser ablation isotope ratio mass spectrometry), phylogenetic (proteomic), and chemical (metabolomics, spectroscopic, and compound-specific isotope analysis) scales. Spatial analysis both highlighted specific regions of substrate accumulation and provided timing constraints on the transport of photosynthetically derived biomass below the mat's maximum photic depth. Proteomic analysis revealed the primary consumers of each substrate and also the key heterotrophs benefiting from metabolic interaction with the mat's cyanobacteria. Examination of specific chemical compounds (lipids, amino acids, etc.) indicate that the mat can quickly shift to an exogenous C source (e.g., acetate or glucose) during daylight but that bicarbonate-derived C remained the main anabolic building block at night. Taken together, this experiment demonstrated that resource availability can direct microbial activity and biosynthetic patterns but that substrate addition alone does not completely overprint established diel cycles within the mat.

26. A Vitamin B12 Probe Reveals Unexpected Pathway Interactions and a Novel Light Sensitive Transcriptional Regulator in a Unicyanobacterial Consortial Isolate

Premchendar Nandhikonda, Yukari Maezato, Young-Mo Kim, Thomas Metz, Lindsey Anderson,Margaret F. Romine, and Aaron T. Wright, Pacific Northwest National Laboratory; Dmitry Rodionov and Irina Rodionova, Sanford-Burnham Medical Institute

We have established that extensive vitamin interdependencies exist among members of two stable 20 member unicyanobacterial consortia (Ana and Oscar) and now seek to understand whether this interdependency is important for establishing community stability. We developed an affinity-based probe (ABP) that mimics vitamin B12, which is produced by only four members of the community. The probe was tested for its ability to capture B12-binding proteins expressed by Oscar consortium isolate *Halomonas* sp. HL-48, which is capable of both synthesizing and salvaging B12. A total of 45 proteins, identified by proteome analysis, were captured by the probe. Components of all three expected B12-dependent enzymes (methionine synthase, ribonucleotide reductase, and ethanolamine ammonia-lyase) were among those detected. A review of the remaining proteins, suggest that B12 may act as an allosteric regulator of enzyme activity, a finding that has previously not been observed in any organism.

A single regulator that we named PhrR was also captured by B12-ABP yet lacked an easily recognizable B12-binding motif. Since B12 is susceptible to photolysis it can potentially control DNA binding of PhrR in the presence of light. We purified PhrR and validated that it is able to bind B12 as well as several DNA fragments that encode a palindromic binding motif that we predicted to be the target binding sites for PhrR. This motif was found at 15 sites in the genome and predicted to control 27 different genes encoding functions associated with photodamage repair, folate biosynthesis, and SAM-dependent processes. This unexpected connection between B12 and folate metabolism suggests that this vitamin maybe mediate a light-dependent of coordination of community member metabolism.

27. A Modified Folch Extraction Multi-omic Technique for Sample Preparation Yields the Highest Number of Unique Peptides in Complex Soil Samples

Carrie D. Nicora, Richard Allen White III, Eric M. Bottos, Taniya Roy Chowdhury, Kristin Burnum-Johnson, Lee Ann McCue, Erin Baker, and Janet K. Jansson, Pacific Northwest National Laboratory

The extraction of proteins, lipids, and metabolites from soil samples is time and sample consuming. Significant challenges occur in soil preparation for each molecule type since there is great microbial complexity in the soil ecosystem and high concentration organics (humic acids) and minerals exist. Kansas native prairie (KNP) soil presents the greatest complexity in soil microbial communities due to its high microbial diversity (e.g., >1,000 OTUs g^{-1}) and high microbial cell density (>107 g^{-1}). The complex nature of soil makes sample preparation the most critical step in obtaining clearly representative and meaningful data from metaproteomics. We compared protein yields from a modified Folch extraction multi-omic technique, which isolates proteins, lipid and metabolites concurrently, to five methods of proteomic-only sample preparations: 1) SDS direct lysis, 2) boiling SDS direct lysis, 3) indirect cellular separation, 4) Novipure® MoBio commercial kit soil protein extraction, and 5) soil binding prior to direct extraction. Among the six methods, indirect differential centrifugation yielded the lowest amount of unique peptides and the Novipure® MoBio commercial kit had polymer contaminants, making spectra unreliable. The modified Folch extraction preparation had the highest yield of unique peptides overall. COG pathways represented in the metaproteomic data from KNP prepared using the modified Folch extraction method suggest diverse metabolism in digesting recalcitrant carbon sources (e.g., lignin and xylan). The modified Folch extraction was further used with permafrost and wetland soils also yielding higher protein yield and unique peptide identifications. This modified Folch extraction method is robust for isolating proteins, lipids and metabolites simultaneously saving time and sample consumption.

28. Unraveling the Transcriptomic Organization and Regulatory Landscape of Cyanobacteria and a Two-Member Community Using Co-expression Network Analysis

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Background: Although governed by principles common to all living organisms, cyanobacterial gene expression must contend with a genome organization that lacks apparent functional context, as the majority of cellular processes and metabolic pathways are encoded by genes found at disparate locations across the genome. This organization makes functional grouping of genes by genomic context difficult. The fact that coordinated regulation of cyanobacterial cellular machinery takes place with significantly fewer transcription factors, compared to other *Eubacteria*, suggests the involvement of post-transcriptional mechanisms and regulatory adaptations that are not fully understood.

Results: Global transcript abundance from the model cyanobacterium *Synechococcus* sp. PCC 7002 grown under 42 different growth conditions was analyzed using context-likelihood of relatedness. The resulting 903-gene network, which was organized into 11 modules of highly co-expressed genes, not only allowed for the classification of cyanobacterial responses to specific environmental variables but also provided insight into the transcriptional network topology and led to the expansion of predicted regulons. When used in conjunction with genome sequence data, the global transcript abundance information identified novel potential targets of both DNA binding and post-transcriptional antisense small RNA regulators.

Conclusions: The results offer new perspectives into the multi-scale regulation that governs cellular adaptations of fast-growing physiologically robust cyanobacterium *Synechococcus* sp. PCC 7002 to changing environmental variables. It also extends a methodological knowledge-based framework for studying multi-scale regulatory mechanisms that operate in cyanobacteria. Finally, it provides valuable context for integrating systems-level data to enhance evidence-based genomic annotation, especially in organisms where traditional context-based methods cannot be implemented due to lack of operon-based functional organization.

29. Exploring Clonal Populations of Verrucomicrobia from Grassland Soil Metagenomes Using Moleculo

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We investigated soil bacterial community structure in a switchgrass stand at high spatial resolution to determine whether biogeographic trends occurred at the centimeter scale (O'Brien at al. 2015; in review). Bacterial communities were highly heterogeneous, with abrupt changes in relative abundance of bacterial phyla from samples collected only centimeters apart. Such heterogeneity did not obscure larger-scale trends; however, at the ecosystem scale bacterial community composition and structure were subtly, but significantly, altered by fertilization, with higher alpha diversity in fertilized plots. We also identified a single Verrucomicrobia-derived OTU that dominated many of our small-volume soil cores (<2 g each). Here, we use shotgun sequencing to determine whether this OTU is taxonomically coherent or is comprised of multiple species/strains. We hypothesize that if it is many species/strains, they may have conserved elements (core genome) and variable regions that suggest variations in ecology or function, potentially defining a pan-genome for the Verrucomicrobia in grasslands (Fierer et al. 2012, 2013; Bergmann et al .2011). To test our hypothesis, we selected 4 samples rich in Verrucomicrobia for Moleculo long-read sequencing and shotgun sequencing, and generated shotgun metagenomic sequences (Illumina Hiseq2000) for an additional set of 16 soil samples. Samples from fertilized and unfertilized soils were sequenced to determine whether this field treatment impacts the gene content of the species/strains of Verrucomicrobia observed. Long-read Moleculo data will allow us to better understand the highly diverse soil microbial communities by providing access to long (5-10 kb) contigs representing individual strains. In addition, a comparison of raw shotgun reads with assemblies of shotgun reads and synthetic long reads will provide an assessment of the accuracy of current methods and will shed light on any potential biases in using high-throughput and long-read sequencing to understand the biochemistry of microbes in soil and elsewhere.

30. Assessment of Microbial Community Structure Inhabiting Long Term Oil Contaminated Soil: Tracking Taxonomic Profiling and Functional Characterization using Next Generation Sequencing Technology

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Oil spills have been pivotal in delineating microbial diversity at the affected site in contrast to the pristine soil. To understand the impact of long-term oil contamination on microbial community structure soil samples were taken from oil fields located in different industrial regions across Kadi, near Ahmedabad, India. Soil collected was hence used for metagenomic DNA extraction to study the capabilities of intrinsic microbial community in tolerating the oil perturbation. The extracted metagenomic DNA was preceded for sequencing on Ion Torrent platform using 318 sequencing chip. Taxonomic profiling was carried out by two different complementary approaches i.e. 16S rDNA and lowest common ancestor. The community profiling revealed the enrichment of phylum *Proteobacteria* and genus *Chromobacterium*, respectively, for polluted soil sample. Our results indicated that soil microbial diversity (Shannon diversity index) decreased significantly with contamination. Further, assignment of obtained metagenome reads to Clusters of Orthologous Groups (COG) of protein and Kyoto Encyclopedia of Genes and Genomes (KEGG) hits revealed metabolic potential of indigenous microbial community. Enzymes were mapped on fatty acid biosynthesis pathway to elucidate their roles in possible catalytic reactions. To the best of our knowledge this is the first study for influence of edible oil on soil microbial communities via shotgun sequencing. The results indicated that long-term oil contamination significantly affects soil microbial community structure by acting as an environmental filter to decrease the regional differences distinguishing soil microbial communities.

31. Metagenomic and Metatranscriptomic Insights into the Role of Iron-dependent Carbon Dioxide Reduction Metabolism During Microbial Corrosion of Steel

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Microbial corrosion of steel can negatively affect the operations of the oil and gas industry. The unpredictability of microbial growth and subsequent localized corrosion can be highly expensive in terms of maintenance and eventual replacement of the affected materials. Despite a long tradition of academic research and industrial experience, microbial corrosion is neither fully understood, nor completely mitigated. We anaerobically enriched a natural microbial community from beach soil with acetate and sulfate as electron donor and acceptor, respectively, and in the presence of carbon steel. Within two months, thick corrosion biofilms developed in association with sulfate reduction as well as acetogenesis. Microbial community characterization via 16S rDNA sequencing revealed *Desulfovibrio* spp. being highly enriched concomitantly with higher acetate production and independently from sulfate reduction or consumption of organic carbon. To better elucidate this

phenomenon, we further enriched such corrosive community without sulfate and organic carbon and were still able to observe acetogenesis and corrosion. Initial results from metatranscriptomic analyses suggest a relevant role for iron-dependent metabolisms of hydrogenotrophic carbon dioxide reducers. In particular, along with transcripts from methanogens, mRNA from *Desulfovibrio* spp., rather than from "typical" acetogens, was enriched. These results may extend the role of sulfate reducing bacteria in corrosion beyond their classic metabolism and further explain the affinity of these microbes for steel-containing anaerobic environment.

32. Integrative Genomics and Flux Chamber Approach to Understanding Microbial Influences on the Oxidative Capacity of the Atmosphere

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Nitrous acid (HONO) is a photochemical source of hydroxyl radical and nitric oxide in the atmosphere that stems from abiotic and biogenic processes, including the activity of ammonia-oxidizing soil microbes. HONO fluxes were measured from agricultural and urban soil in mesocosm studies aimed at characterizing biogenic sources and linking them to indigenous microbial consortia. Fluxes of HONO from agricultural and urban soil were suppressed by addition of a nitrification inhibitor and enhanced by amendment with ammonium (NH_4^+), with peaks at 19 and 8 ng m^{-2} s^{-1}, respectively. In addition, both agricultural and urban soils were observed to convert $^{15}NH_4^+$ to $HO^{15}NO$. Genomic surveys of soil samples revealed that 1.5 to 6% of total expressed 16S rRNA sequences detected belonged to known ammonia oxidizing bacteria and archaea. Peak fluxes of HONO were directly related to the abundance of ammonia-oxidizer sequences, which in turn depended on soil pH. Peak HONO fluxes under fertilized conditions are comparable in magnitude to fluxes reported during field campaigns. The results suggest that biogenic HONO emissions will be important in soil environments that exhibit high nitrification rates (e.g., agricultural soil) although the widespread occurrence of ammonia oxidizers implies that biogenic HONO emissions are also possible in the urban and remote environment. In contrast to agricultural soil where ammonia oxidizing bacteria were the major source of reactive nitrogen, Thaumarchaeota are the dominant ammonia oxidizers in subniveal environment studied during the Uintah Basin Winter Ozone Study (UBWOS-2014), and their activity may explain the anomalously low $^{15}N/^{14}N$ isotope ratios derived from nitrate present in snow during this field campaign.

33. Affinity-based Probe Enabled Enrichment and Characterization of RNA Bound to Vitamin B12

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Cobalamin (B12) is an important enzyme cofactor for the synthesis of methionine and is required by many organisms for DNA synthesis. Many microbes are unable to produce vitamin B12, and must procure it from a producing organism; this auxotrophy generates obligate interspecies interactions in microbial communities. Expression of several vitamin bio-synthesis or salvage pathways are repressed by the binding of the vitamin to a regulatory motif in the messenger RNA transcript (aka riboswitch). This causes termination of transcription or reduction in translation (see below). To determine whether affinity-based probes (ABPs) can be used to target and enrich for RNA that binds B12, we used a B12-mimicking ABP to validate whether it repressed a translational reporter controlled by a known B12 riboswitch from E. coli or predicted B12 riboswitches from a novel organism, *Halomonas* sp. HL-48.

34. Microbial Community Composition and Biogeochemistry of an Arctic Permafrost and Freshwater Wetland Sediments

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Climate extremes will significantly impact regional and global hydrology. Changing precipitation patterns and warmer temperatures are altering the magnitude and dynamics of hydrologic flow which significantly affect aquatic-terrestrial transition zones like permafrost and wetlands. As the Arctic becomes warmer, permafrost zones are vulnerable to deepening of the active layer and thawing of the near-surface permafrost. Coastal wetlands are susceptible to fluctuating conditions due to storm events, salt-water intrusion and increasing water levels. Such perturbations expose organic matter protected in the soils to microbial degradation leading to increased carbon dioxide (CO_2) and methane production. Permafrost drainage and rapidly fluctuating wetland hydrology can activate and de-activate microbial populations that in turn quickly alter the nature

and rate of biogeochemical transformations. As changing soil redox conditions alter electron acceptor availability and recovery strengths, a disproportionate release of soil carbon as CO_2 can stem from alternative anaerobic degradation processes like sulfate and iron reduction in addition to methanogenesis. The sensitivity of the soil microbiome to altered hydrologic regime and effects on ecosystem functions has not been elucidated. Our objective, therefore, is to understand responses of the soil microbiome to hydrologic regime shifts using a multi-omic approach.

We identified two model systems, boreal discontinuous permafrost zone in interior Alaska and wetlands along a coastal gradient in the lower Columbia River, Oregon to investigate the impacts of hydrologic perturbation on soil microbiomes. The aim of the study presented here is to obtain knowledge about the microbial community composition and geochemical signatures. Specific objectives are to (i) investigate site-specific differences in the microbial community composition and (ii) to elucidate the relationship between community composition and geochemical parameters. We will present results for soil physicochemical properties, microbial community composition obtained by 16S rRNA gene sequencing and process rates for respiration and methanogenesis.

35. Coupled Spatiotemporal Dynamics of Microbiomes, Metabolites, and Hydrologic Mixing

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The hyporheic zone (HZ) is a critical ecosystem component that links terrestrial, surface water, and groundwater ecosystems. A dominant feature of the HZ is groundwater-surface water mixing and the input of terrestrially—as well as aquatically—derived organic carbon. In many systems the HZ has a relatively small spatial extent, but in larger riverine systems groundwater-surface water mixing can occur 100s of meters beyond the surface water shoreline; we consider these more distal locations to be within the 'subsurface interaction zone' (SIZ) as they are beyond the traditional HZ. Microbial communities in the HZ and SIZ drive biogeochemical processes in these system components, yet relatively little is known about the ecological processes that drive HZ and SIZ microbial communities. Here, we applied ecological theory, metabolomics, aqueous biogeochemistry, and DNA sequencing to field samples collected through space (400 m spatial extent) and time (7 month temporal extent) within the Hanford Site 300 Area. These data streams were integrated to evaluate how the influence of groundwater-surface water mixing on microbial communities changes when moving from the HZ to the broader SIZ. Our results indicate that groundwater-surface water mixing (i) consistently stimulated heterotrophic respiration, but only above a threshold of surface water intrusion, (ii) did not stimulate denitrification, (iii) caused deterministic shifts in HZ microbial communities associated with changes in the metabolite profile, and (iv) did not cause shifts in SIZ microbial communities. These results suggest that microbial communities and the biogeochemical processes they drive are impacted by groundwater-surface water mixing primarily in the HZ and to a lesser extent in the SIZ.

36. Coupling High-resolution Soil Carbon Metabolomics with Microbial Profiling Techniques in a Permafrost Transition

James C. Stegen, Malak M. Tfaily, and Carolyn G. Anderson, Pacific Northwest National Laboratory

Permafrost thaw has complex effects on the carbon cycle in northern high latitudes. Because permafrost soils harbor twice the amount of C as contained within Earth's atmosphere, thaw may cause net loss of C to the atmosphere in sufficient quantities to result in a positive feedback, whereby warming drives the loss of C, which drives further warming. The response of C cycling in permafrost zones to ongoing warming will therefore have a major influence over future climate. There is limited understanding, however, of the interplay among soil-atmosphere C fluxes, microbial communities, and soil organic matter (SOM) chemical composition. To address this knowledge gap, we leveraged the distinct spatial transitions in permafrost-affected soils at the Caribou Poker Creek Research Watershed, a 104 km^2 boreal watershed ~50 km north of Fairbanks, AK. We couple field C flux and vegetation structure measurements with soil analyses, including metabolomics (ultrahigh resolution SOM chemical composition from Fourier-transform ion cyclotron resonance mass spectrometry, FT-ICR) and microbial community composition (16S rDNA sequencing). Structural equation modeling suggests linkages among abiotic and biotic variables that control microbial community richness, including novel contributions of high-resolution SOM data. Specifically, we find that while some compounds in SOM directly govern microbial diversity levels (e.g., CHO and CHOSP compounds), others indirectly control diversity levels through cell density (e.g., amino sugars). Additionally, the model suggests that total soil C levels strongly mediate cell density, which in turn controls microbial diversity levels. Coupling high-resolution SOM metabolomics and microbial profiling techniques, in tandem with soil physicochemical characterization and ecosystem structure and process measurements, enables a novel analysis of the

connections between complex microbial communities, the soil environment, and C cycling in a permafrost system. This work will ultimately provide key insights needed to improve the representation of interactions among SOM, microbial communities, and C cycling across permafrost transition zones, thereby helping to reduce uncertainty in global climate predictions.

37. Hydrology Defines Microbial Communities and Functions Across Arctic Polygonal Grounds

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Permafrost soils store almost as much organic carbon as is found in the rest of the world's soils. With increasing global temperatures, permafrost soils are becoming a potential source of greenhouse gas emissions (GHGs), but the vulnerability of permafrost and the potential response of soil microorganisms to availability of new carbon sources remains unknown. In this project, we aim to define microbial processes leading to GHG emissions at the NGEE-Arctic Barrow site where formation of ice wedges and permafrost thaw create polygonal ground. We collected seasonally thawed active layer soil samples and several deep cores—up to 3 m—along two polygon transects containing high (HCP), flat (FCP), and low (LCP) centered polygons for three consecutive years. The microbial community composition and genomic potential were determined via metagenomics. The sequence data were correlated to in-situ GHGs and soil geochemistry. The polygon type was the primary driver of microbial community composition and metabolic potential. Not all the key metabolic pathways leading to GHGs were found across the landscape. For example CH4 production potential was only found in LCP whereas CH_4 oxidation was prevalent in the HCP and FCP. The metagenome sequence data suggested that nitrate was utilized as a nitrogen source, but not lost through denitrification. Deep permafrost layers were dominated by several different species of Actinobacteria, which are known to have a diverse metabolic capability and to adapt to stress. Permafrost layers with high salt content—and potential liquid water—contained anaerobic CH_4 producing and oxidizing Archaea and chemolithoautotrophic ammonia-oxidizers. We hypothesize that nutrients and available carbon in these layers are tightly regulated and recycled. In this ongoing project, the long-term goal is to use omics datasets to aid in understanding of the biogeochemical cycles and explore methods for their integration into models.

38. Comparison of Metagenomes and De Novo-Assembled Metatranscriptomes as Mapping References: A Possible Bootstrap Approach to RNA-Seq Analysis of Complex Communities

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Metatranscriptomic analysis can provide valuable insights to microbial community activity and response patterns. However, a corresponding assembled metagenome is not always available for short read mapping of metatranscriptomic reads. To address this challenge, here we explored the use of a *de novo* assembled metatranscriptome, built from RNA-Seq data, to replace the metagenome as a reference for mapping the same RNA-Seq data to obtain quantitative gene expression analysis. To test this bootstrap approach, we used genomic and RNA-Seq data from a simplified Hot Lake bacterial community consisting of 20 species with 61,186 identified genes. We built a metatranscriptome from the RNA-Seq data using Trinity *de novo* assembler software, and compared its composition with gene sequences identified in a corresponding high-quality metagenome, built from random shotgun sequencing performed at the JGI. We identified differences in gene prediction by BLASTing the open reading frames predicted on *de novo* assembled RNA-Seq data against open reading frames predicted in the metagenome and the NCBI RefSeq database. We then identified common and unique genes based on set difference. We found that 21,309 genes in the metagenome were missing from the metatranscriptome. Conversely, 224 genes found in the transcriptome were found to be present and annotated in RefSeq but were not found in the metagenome. Using the MetaPathways software platform, we are now comparing the mapping of the RNA-Seq data using the metatranscriptome vs. the metagenome as the reference assembly. By performing mapping runs on RNA-Seq data taken at multiple time points against both assemblies, it will be possible to evaluate the efficiency and accuracy of this bootstrap approach to achieve quantitative gene expression data using de novo assembled metatranscriptome as the reference. We note that de novo assembled metatranscriptomes can also potentially be used as a check for the completeness of metagenomes.

39. Metabolomics and Inferred Metagenomics of the Human Lung Microbiome During HIV Infection

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As for any single organism, the metabolic potential for communities of bacteria in the human lung can be studied at multiple levels, including the metagenome, metatranscriptome, and (meta-)metabolome. Here we compare the (meta-)metabolome with the inferred metagenome in the same individuals by looking for enriched metabolic pathways and differential abundance of potential pathways during HIV infection. We collected broncheoalveolar lavage (BAL) fluid from 43 individuals, 23 of whom were HIV-infected. All individuals were without acute respiratory infections or symptoms, and had not received antibiotics in the previous 6 months. BAL samples were subjected to both liquid chromatography-high-resolution mass spectrometry and 16S rRNA gene sequencing.

Based on 134 (out of 5,868) C18 chromatography input masses that were over-expressed in the HIV-infected individuals, we used mumminchog to identify enriched metabolic pathways. We identified three such pathways: parathio degradation ($p=0.0043$), glycosphingolipid metabolism ($p=0.0143$), and omega-3 fatty-acid metabolism ($p=0.0228$).

We inferred the metabolic potential of the lung bacteria community from 16S rRNA genes sequenced from each BAL using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). Based on the assigned phylogeny, PICRUSt assigns individual operational taxonomic units (OTUs) to Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO). We identified two out of 273 KOs that were more abundant in the HIV-infected individuals: drug metabolism – cytochrome P40 ($p=0.0479$) and flavonoid biosynthesis ($p=0.0337$). Neither of these KOs is traditionally associated with bacteria, but these are small pathways (23 and 19 genes, respectively) and the over-abundance may be driven by a smaller number of genes.

While the metabolic pathways identified are not associated with either over-abundant KO, they may be driven by the same bacteria. Others may be coming from the human host or external influences. Metatranscriptomics may be useful to resolve the dissimilar results of the metabolome and the predicted metagenome.

40. Visual Data Integration of Multi-Omics Data

Allison Thompson, Lisa Bramer, and Lee Ann McCue, Pacific Northwest National Laboratory

Exploratory data analysis (EDA), data integration, and visualization are critical steps in analyzing high-throughput biological data. In addition to challenges presented by data volume from high-throughput experiments, the disparate data types often require specific EDA and visualizations designed for each type of data. Here we focus on visualization approaches for EDA and integration of statistical modeling results for high-throughput metagenomics sequencing data and Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry data using tools implemented in the Tessera open source environment (tessera.io). We use boxplots to observe the quantitative differences in metagenomic features between samples, presence/absence heatmaps to qualitatively assess the data, and a hierarchical view to narrow the scope of the features of interest. We have also linked the analysis of metagenomic features to the FTICR carbon chemistry data, to enable simultaneous visual exploration of these disparate data types and a more profound view of the underlying biological mechanisms. We will demonstrate these Tessera tools that allow researchers to perform exploratory data analysis and pinpoint features of interest in a user interface requiring no programming expertise.

41. Microbial Community Characterization of Fecal Samples from Healthy Premature Infants Compared with Those That Develop Late Onset Sepsis

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OBJECTIVE: Extreme low birth weight (ELBW) infants are at very high risk for developing necrotizing enterocolitis (NEC), a common cause for morbidity and mortality among these infants. Premature birth and early feeding are both important risk factors. A majority of infants with NEC go on to develop severe late onset sepsis (LOS) requiring prolonged broad spectrum antibiotics and debilitating surgical intervention. We aim to characterize gut microbial communities from otherwise healthy premature infants in relation with previous antibiotic use and feeding patterns, and to compare them to those from ELBW

infants who develop LOS (with or without NEC). This will help devise strategies to preserve a healthier microbiome and improve outcomes in ELBW infants.

METHODS: Fecal samples have been collected from 37 ELBW infants, including 24 controls, 5 with NEC, and 7 septic non-NEC. Metagenomic DNA was extracted, and barcoded 16s rRNA (V3-V4 region) sequencing libraries were prepared with the NexteraXT primers followed by Illumina sequencing. Fecal samples were also analyzed for metabolites using GC-MS. Prinseq was used for quality control of the sequencing data and further analysis was performed using Qiime and the R packages Vegan and randomForest. Clinical characteristics of these subjects were combined with sequencing and metabolomics data to identify trends between the sick and healthy premature infants.

RESULTS: As expected the microbial composition of the infant guts varies greatly from patient to patient. Because of the widespread use of antibiotics on premature infants, the gut microbial composition often varies greatly at different timepoints in a single infant. Analysis is ongoing and the addition of metabolomics data could reveal important trends between the bacterial composition and metabolites present in healthy and sick premature infants.

SIGNIFICANCE: A large number of ELBW infants develop NEC and LOS. Understanding the characteristics of normal gut microbial communities in ELBW infants will enable strategies to preserve or reconstitute healthy microbial community assembly after modification by diverse therapeutic interventions.

42. Proteomic and Cytometric Analysis of Microbial Community Function Using Activity-Based Probes

Christopher Whidbey, Natalie Sadler, Brian Thrall, and Aaron Wright, Pacific Northwest National Laboratory

Communities of microbes perform diverse functions involved in processes ranging from biogeochemical cycling to alteration of host metabolism. However, the molecular basis for many of these functions remains unknown. Identification of the proteins and organisms responsible for various functions in situ would provide key information for modulation and design of microbial communities. To this end, our group is developing an approach to use activity-based probes to label proteins and cells in microbial communities. These probes consist of a binding group mimicking the natural substrate of interest, a reactive group to covalently attach the probe to the protein target, and a click chemistry handle. Once labeled, the cells can be lysed for proteomic analysis or sorted using fluorescence-activated cell sorting (FACS) to enrich for cells with the target function for genomic analysis. To date, we have applied this approach to mouse gut, soil, and model hypersaline lake microbial communities. Preliminary results of sequencing and proteomic analysis from these communities will be presented.

43. Chemical Imaging of Live Biofilms and Extracellular Polymeric Substance by Time-of-Flight Secondary Ion Mass Spectrometry

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A vacuum compatible microfluidic reactor, SALVI (System for Analysis at the Liquid Vacuum Interface) was employed for in situ chemical imaging of live biofilms using time-of-flight secondary ion mass spectrometry (ToF-SIMS). Depth profiling by sputtering materials in sequential layers resulted in live biofilm spatial chemical mapping. Two-dimensional (2D) images were reconstructed to report the first three-dimensional (3D) images of hydrated biofilm elucidating spatial and chemical heterogeneity. 2D image principal component analysis (PCA) was conducted among biofilms at different locations in the microchannel. Our approach directly visualized spatial and chemical heterogeneity within the living biofilm by dynamic liquid ToF-SIMS. In addition, dry extracellular polymeric substance (EPS) extracted from *Shewanella* biofilms was investigated using ToF-SIMS. Spectral PCA was conducted to understand the chemical differences among loose, bound and total EPS. Preliminary results will be presented and showcase the direction of ToF-SIMS applications in studying biofilms and EPS with high spatial resolution.

44. Characterizing an Autotrophic Biocathode Microbial Community Using Electrochemistry, Spectroscopy, and Meta-omics

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Pure cultures of the iron-reducing bacteria *Geobacter* and *Shewanella*, as well as acetogenic, methanogenic, and iron-oxidizing bacteria have all been shown to generate current at the biocathode through extracellular electron transfer (EET). In the environment microorganisms exist as microbial consortia where their microbial diversity confers metabolic flexibility to respond and adjust quickly to conditions encountered outside of a controlled reactor. In fact, the highest current densities at a biocathode and highest yields from microbial electrosynthesis have been recorded when a mixed microbial community was used as the inoculum. Molecular redox mediators for cathode EET remain largely unknown in both pure culture and mixed communities, inhibiting our ability to direct or engineer electron flow for biocathode applications. Here we present a comprehensive analysis using microbial electrochemistry, meta-omics (genomics, proteomics, and transcriptomics), microscopy and spectroscopy to identify the biocathode constituents that play key roles in EET and carbon fixation in an autotrophic, O_2-respiring marine biofilm. A previously uncharacterized member of the family *Chromatiaceae* mediates EET and carbon fixation and cannot be cultivated outside of the electrode environment. Proteins identified using metaproteomics indicate a potential pathway for EET similar to that proposed for some iron-oxidizing bacteria and confocal raman microscopy showed that a rubredoxin-like protein was abundant within heterogeneous cell clusters at the electrode surface. Heterotrophic members of the biocathode consortia interact with the electrode when cultured independently, however, differential expression of redox proteins and carbon flux is primarily associated with the *Chromatiaceae* when the electrode potential is changed. Genetic tools aimed at modifying carbon storage pathways of heterotrophic members of the community have been developed in order to manipulate cross-feeding between syntrophic partners with the goal of developing an engineered biocathode community for production of biocommodities. This work also highlights the importance of using cultivation independent methods to study electroautotrophs.

45. Hemicellulose Powered Microbial Methanogenesis Revealed by Metaproteomics, Metabolomics, and Metabolic Pathway Reconstruction

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Odontotaenius disjunctus (e.g., Jerusalem beetle) consumes >4.5 times its body weight in wood everyday with a colony consuming >7 kg of wood per year (twice as much as termite colony). The gut microbiome of *O. disjunctus* is categorized into four distinct compartmentalized regions: foregut (FG), midgut (MG), anterior hindgut (AHG), and posterior hindgut (PHG). Acetoclastic and hydrogenotrophic methanogens were found to be localized to the AHG. Moreover, whether any functional methanogenesis or other functional potential can be attributed to these groups is unknown in the *O. disjunctus* gut. We conducted a high-throughput metaproteomic study that included 829,099 raw spectra, 75,754 unique peptides, and 31,749 unique proteins from four inbred homogeneous male *O. disjunctus*. Metaproteomic analysis revealed enrichment of hemicellulosic (e.g., xylan degradation) pathways primarily over lignocellulosic pathways. A complete xylan degradation pathway was reconstructed based on expressed protein levels that was partitioned over various genomic bins related to *Clostridiales*, *Bacteroides*, and *Bacillus*-like Firmicutes. CAZy glycoside hydrolase GH43 (EC 3.2.1.37 or Xylan 1,4-betaxylosidase), the enzyme responsible for breaking hemicellulose to xylose, was compartmentalized to the MG-AHG regions to *Clostridiales* (e.g., Firmicutes) genomic bins. Cellulases (e.g., Beta-glucosidases) from CAZy families GH1/GH3 were found throughout the gut. Lignin degradation (e.g., lignin peroxidases, EC 1.11.1.15) proteins were detected amongst *Clostridiales* (e.g., Firmicutes) genomic bins in the MG gut region. *Methanobacteriaceae* proteins were highly enriched for methanogenesis based on formate oxidation to CO_2. Methyl-coenyzme M reductase (EC 2.8.4.1), F420-dependent dehydrogenase and methanol-corrinoid co-methyltransferase proteins were elusively compartmentalized to AHG by *Methanobacteriaceae* genomic bins. Methanol-corrinoid Co-methyltransferase protein allows for the transformation of methanol to methane. *Methanobacteriaceae* within the AHG are represented at <5% of the total population but are the sole contributors of methanogenesis in the *O. disjunctus* gut. Our findings suggest functional evidence for hemicellulose (e.g., xylan degradation) powered microbial methanogenesis.

46. Moleculo Hybrid Synthetic Long Reads Reconstruct Full Genomes from the Rare Biosphere with Functional Potential Elucidated By High Resolution Mass Spectrometry

Richard A. White III, Eric M. Bottos, Taniya Roy Chowdhury, Carrie D. Nicora, Kristin Burnum-Johnson, Lee Ann McCu1, Erin Baker, and Janet K. Jansson, Pacific Northwest National Laboratory

Hybrid synthetic long reads from the high-throughput sequencing Illumina platform (e.g., Moleculo) provide >8 kb read lengths with 99.99% accuracy. Moleculo enables resolution of complex repeats in eukaryotic genomes including *C. elegans* and *Botryllus schlosseri*. Kansas native prairie soil presents the greatest challenge for de novo assembly from metagenomic shotgun reads due to its high microbial diversity (e.g., >1,000 OTUs g^{-1}) and high microbial cell density (>107 g^{-1}). Approximately 300 million reads from the HiSeq 2500 rapid mode (e.g., 250 bp paired reads) using hybrid synthetic long reads Moleculo yielded 16.9 k reads >9 kbp and 6.2 k reads >10 kbp. By comparison, assembly attempts from this same ecosystem with >600 Gbs of 80 bp reads provided only 9 contigs >9 kbp. Moleculo synthetic long reads data resulted in >100 genomic bins, 20 partial genomes, and 3 complete genomes from the rare biosphere (e.g., TM7, Zixi, OD1). Using this approach, a novel candidate phyla was detected using Moleculo in the Kansas native prairie soil by binning with tetranucleotide, GC content, and codon bias using self-organizing maps (ESOM). Amongst the completed genomes in this analysis, the *Candidatus Zixibacterium* strain JKJ-1 in Kansas soil has the potential for hemicellulose degradation (e.g., xylan metabolism) and has two copies of arylsulfatase that are linked to soil desiccation and environmental warming. Greater than 100,000 high resolution mass spectra (e.g., Q-Exactive) mapped to moleculo synthetic long reads, resulting in 2,000 peptides with >100 proteins relating to members of the rare biosphere (e.g., TM7, Zixi, OD1). Proteomic analysis suggests many of these proteins have the potential for digesting recalcitrant carbon such as ligin and Xylan. This work demonstrates the synergistic potential of integrating Moleculo hybrid synthetic long reads in systems biology approaches, whereby a functional linkage between metagenomics and metaproteomics may be established.

TERRAGENOME YOUNG SCIENTIST TRAVEL AWARD WINNERS

Methylotrophic Methanogenesis in Deep Shales, Something New from Something Old

Daniel Marcus, Rebecca A. Daly, Richard Wolfe, Michael J. Wilkins, Paula J. Mouser, and Kelly C. Wrighton, The Ohio State University; David W. Hoyt, Pacific Northwest National Laboratory

Over the last decade shale gas obtained from hydraulic fracturing of deep shale formations has become a sizeable component of the US energy portfolio. Through both sequencing based community assessments and isotopic analyses, there is a growing body of evidence indicating that methanogenic archaea are both present and active in hydraulically fractured shales. However, the mechanisms employed by these microorganisms to cope with injected chemical additives, along with brine-level salinities, high pressures, and temperatures, have yet to be systematically understood. Here we leveraged natural gas extraction activities in the Appalachian region to gain access to fluid samples collected over approximately a year period from hydraulically fractured shales at ~2 km depth. Using assembly-based metagenomics, we reconstructed two nearly complete (90% & 99%) methanogen genomes. 16S rRNA phylogenetic analysis revealed that the genome of the methanogen present in the terminal sample (328 days post fracturing) belongs to the genus *Methanohalophilus*. Based on metabolic reconstruction, we infer this organism utilizes methylamines, not hydrogen or acetate as substrates for methanogenesis.¬ Consistent with this finding, amendment of production fluids with mono-methylamine resulted in biogenic methane production. To adapt to the high salinities of the shale environment, the genome encodes multiple pathways for the synthesis and transport of compatible organic solutes (e.g., betaine, N-Acetyl-β-Lysine). ^{1}H and ^{13}C NMR-based metabolomics corroborated these metagenomic inferences by measuring a depletion of methylated methanogenic substrates and an increase of betaine from fluid samples collected over the course of energy extraction. In addition to adaptation to the physical environment, the genome encodes a CRISPR-Cas adaptive immunity system. A spacer sequence match linked the *Methanohalophilus* host to a complete circularized viral genome, suggesting active viral predation in the subsurface. Collectively, this work provides the first genomic insight into physiological strategies enabling proliferation and methane production in deep shale environments.

Detecting CRISPRs in Solar Saltern Metagenomes: Analysis of Viral-Host Interactions in a Model Ecosystem

Abraham Moller and Chun Liang, Miami University (Ohio)

Solar salterns are large, shallow, salt-crystallizing ponds with salt concentrations ranging from 10 to 37%, well above the seawater salt concentration of 3.5%. Because of their low microbial diversity, relative environmental stability, lack of protistan bacterivory, and extremely high viral concentration, salterns are an excellent model system for exploring viral interactions with bacterial and archaeal hosts. The microbial metagenomes studied represent a geographically diverse set of salterns, located in Santa Pola and Isla Cristina (Spain), Chula Vista (California), and Cahuil (Chile). Current research projects have included taxonomically profiling these saltern communities, assembling metagenomic reads into contigs, detecting new CRISPRs directly from reads, and searching assembled contigs for halobacterial Cas homologs. Taxonomic profiling with MetaPhlAn and MetaPhyler suggested diverse halobacterial populations primarily composed each saltern community. After taxonomic profiling, the metagenomes were assembled with the programs Newbler and Velvet. Across all metagenomes, Newbler assembled fewer and longer contigs than Velvet. *De novo* CRISPR detection with Crass identified multiple CRISPR arrays in the metagenomes, including one that likely corresponds to a CRISPR array in *Haloferax volcanii*. Crass failed to identify expected *Haloquadratum* and *Halorubrum* CRISPRs, however, necessitating a novel search strategy for these arrays. Multiple contigs assembled from each metagenome matched halobacterial Cas homologs, thus indicating functional Cas genes were present in the metagenomes. Future

studies will include taxonomy-guided CRISPR detection, mapping virus-host interactions using detected CRISPR spacers, and comparing virus and host sequence characteristics to further confirm the determined virus-host interactions. These projects together will further elucidate the relationships between haloviruses and the halobacterial communities that inhabit these extreme environments. These projects will also examine the roles of CRISPR-Cas prokaryotic immune systems in regulating virus production and proliferation in solar salterns.

Integrative Multi-Meta-Omic Analysis of Microbiome Taxa and Metabolites Reveals Community Metabolic Trends and Putative Mechanistic Links

Cecilia Noecker and Elhanan Borenstein, University of Washington

"Multi-meta-omic" technologies have enabled comprehensive profiling of the composition and activity of the human microbiome. Typical analyses of these datasets are correlation-based, despite extensive prior knowledge of links between them. We developed a framework to systematically interpret and compare variation in metabolomics data in terms of associated taxonomic variation across microbial community samples. To do so, we adapted the Predicted Relative Metabolomic Turnover method (Larsen et al., 2011) to calculate an approximate metric of community metabolic potential based on gene or species composition. Our framework compares this metric with measured metabolomic variation to evaluate the mechanistic concordance between species and metabolites, and identifies the major taxa and reactions contributing to predicted metabolite variation. We applied this pipeline to a collection of datasets pairing 16S rDNA community profiling and global metabolomics. In two datasets of samples from the vaginal microbiota of women with and without bacterial vaginosis (BV) (Srinivasan et al., 2015), we observe that variation in 37% of detected and identified metabolites is significantly predicted by estimated metabolic shifts based on species composition. Notably, the number of significantly predicted metabolites is higher than observed with scores based on randomized metabolic networks, and includes 55% of disease-associated metabolites. Metabolite variation predicted well by this method tends to result from secondary metabolism present only in sample subsets, including the synthesis of novel products and the depletion of accumulated metabolites in the more diverse BV state. The disease-enriched taxa *Megasphaera* and *Eggerthella* are enriched contributors to these significant metabolite predictions. Additionally, negative correlations between predicted and observed metabolite variation suggest hypotheses about environmental impacts on community metabolism, including a known role for glycerol availability in *Lactobacillus* iners metabolism and growth. This framework is a first step towards the use of mechanistic multi-omic analyses to identify microbiome community features involved in health-relevant metabolite shifts.

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Thank you to our internal partners:
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