WCBP 2015 ABSTRACTS

SESSION I
FRIDAY EVENING
String Me Along: Extracellular Electron Transfer in Microbial Redox Chains

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Electron Transfer is the stuff of life. The stepwise movement of electrons within and between molecules dictates all biological energy conversion strategies, including respiration and photosynthesis. With such a universal role across all domains of life, the fundamentals of ET and its precise impact on bioenergetics have received considerable attention, and the broad mechanisms allowing ET over small length scales in biomolecules are now well appreciated. Coherent tunneling is a critical mechanism that allows ET between cofactors separated by nanometer length scales, while incoherent hopping describes transport across multiple cofactors distributed within membranes.

In what has become an established pattern, however, our planet’s oldest and most versatile organisms are now challenging our current state of knowledge. With the discovery of bacterial nanowires and multicellular bacterial cables, the length scales of microbial ET observations have jumped by 7 orders of magnitude, from nanometers to centimeters, during the last decade alone! This talk will take stock of where we are and where we are heading as we come to grips with the basic mechanisms and immense implications of microbial long-distance electron transport. We will focus on the biophysical and structural basis of long-distance, fast, extracellular electron transport by metal-reducing bacteria. These remarkable organisms have evolved direct charge transfer mechanisms to solid surfaces outside the cells, allowing them to use abundant minerals as electron acceptors for respiration, instead of oxygen or other soluble oxidants that would normally diffuse inside cells. From an environmental perspective, these microbes are major players in global elemental cycles. From a technological perspective, microbial extracellular electron transport is heavily pursued for interfacing redox reactions to electrodes in multiple renewable energy technologies.

But how can an organism transfer electrons to a surface many cell lengths away? What molecules mediate this transport? And, from a physics standpoint, what are the relevant length, time, and energy scales? We will describe new experimental and computational approaches that revealed how bacteria organize heme networks on outer cell membranes, and along the quasi-one-dimensional filaments known as bacterial nanowires, to facilitate long-range charge transport. Using correlated electron cryotomography and in vivo fluorescent microscopy, we are gaining new insight into the localization patterns of multiheme cytochromes along nanowires as well as the morphology and the formation mechanism of these structures. In addition, we will examine the fundamental limits of extracellular electron transport, down to microbial energy acquisition by single cells. These findings are shedding light on one of the earliest forms of respiration on Earth while unraveling surprising biotic-abiotic interactions.
Microbial Electron Uptake in Biocorrosion and Electrosynthesis

Joerg Deutzmann, Merve Sahin, Alfred M. Spormann
Stanford University

Illicit Transport in *Escherichia coli* and Beyond: The Kasugamycin Tail

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Starting from a large-scale chemical-genomic screen in *Escherichia coli* K-12, we have discovered and characterized the bacterial import pathway for a pair of "forgotten" antibiotics. The translation inhibitors blasticidin S and kasugamycin are illicitly transported through the major peptide permeases of *Escherichia coli*. I will present these results in the larger context of illicit transport, antibiotic efficacy across species, and the recent approval of kasugamycin for agricultural use in the United States.
Biosensors for the Identification of Chemoattractants

Rita A. Luu, Richard Truong, Ceanne Brunton, Madeline Lee, Juan V. Parales, Valley Stewart, and Rebecca E. Parales

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Motile bacteria contain membrane-bound chemoreceptors that detect chemicals and upon binding and initiate the response to travel towards or away from those chemicals – a process called chemotaxis. *Pseudomonas* species have complex chemosensory systems with >20 chemoreceptors. Pseudomonads also have the ability to sense and degrade a wide range of chemicals, some of which are toxic aromatic pollutants. In order to facilitate the identification of chemoreceptors responsible for detection of specific chemicals and to catalog the range of chemicals recognized, we have developed a simple screen using hybrid 2-component sensor proteins and a colorimetric enzyme assay. To assess the feasibility of the hybrid sensors, the aromatic acid receptor PcaY from *Pseudomonas putida* F1 was selected to develop the system. The ligand-binding region (LBR) of PcaY was fused to the signaling domain of a nitrate sensor from *Escherichia coli* and introduced into a reporter strain carrying a nitrate-responsive promoter fused to *lacZ*. Four different variations of the hybrid proteins were made, each with a different junction point and HAMP domain, the structural element that mediates signal transduction in 2-component systems. Of the four constructs, the hybrid protein in which the LBR of PcaY was fused to the NarQ HAMP domain was functional in the *E. coli* reporter strain. Further analysis revealed that strong attractants, weak attractants, and non-attractants were readily distinguished and the responses can be quantitatively measured with this system. The binding sensitivity of the LBR to different aromatic attractants using the reporter system correlated well with results of in vivo chemotaxis assays. These results demonstrate that hybrid sensors may be useful for the functional characterization of chemoreceptors of unknown function.
Regulatory Analysis of the Indole-3-acetic Acid Catabolic (iac) Bacterial Gene Cluster

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The iac gene cluster confers the ability to mineralize the plant hormone indole-3-acetic acid (IAA) into catechol. In the soil bacterium Enterobacter soli LF7, the cluster spans 8.1 kbp and is flanked by protocatechuate- and catechol-metabolizing genes as part of the same operonic unit. Expression of the iac cluster is thought to be regulated by the product of iacR, a marR (Multiple Antibiotic Resistance Regulator) family repressor protein. The suspected promoter for the cluster, located in the iacRH intergenic region, was isolated from E. soli LF7 and fused to variants of gfp reporter genes coding for green fluorescent proteins with different susceptibility to proteolytic degradation (no tag, ASV, AAV, or LVA, respectively). When transformed into E. soli LF7, these <iacHprom-gfp> reporter constructs expressed high levels of GFP fluorescence in response to IAA. The AAV variant of gfp was selected for further experiments because of the low baseline fluorescence and optimal difference in GFP expression between induced and uninduced cells. When exposed to a range of IAA concentrations from 0 to 1mM, E. soli LF7 <iacHprom-gfp[AAV]> showed a dose-dependent response to IAA, with a lower limit of detection of 1.6 µM and maximum GFP output at 40 µM and higher. Using flow cytometry to measure induction of fluorescence expression by the <iacHprom-gfp[AAV]> promoter in E. soli LF7 cells exposed to 24 IAA- and tryptophan-derivative compounds, we found a high level of specificity for IAA (and two chlorinated IAA derivatives), with no fluorescence observed with any other compounds. To confirm the precise location of the iacH promoter sequence, deletion mutants were made by PCR to shorten the iacRH intergenic space using amplicons of 7 forward primers with the same, single reverse primer used to clone the full length bioreporter. We found that GFP is not expressed following deletion of the putative -35 binding site, or 112 bp from the predicted translational start site of iacH. This bioreporter construct can be used to investigate microbial responses to IAA in many plant-associated habitats such as the rhizosphere, phyllosphere, and soil.
Transcriptional Changes in *Vibrio fischeri* During Host Colonization

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The bioluminescent bacterium *Vibrio fischeri* is the monospecific symbiont of the Hawaiian bobtail squid, *Euprymna scolopes*. These bacteria are present as a planktonic population in the waters surrounding Hawaii, and each generation of squid isolate their symbionts after hatching by filtering *V. fischeri* from the surrounding seawater. Previous studies of adult animals have characterized strong diel rhythms in both the host tissues and symbionts’ metabolism, and shown specific transcriptional responses in the host during the earliest stages of colonization. There are numerous differences between the juvenile and adult symbiosis that have been observed to date, indicating that the metabolic rhythms observed in adult animals do not become established until about 4 weeks of age. In this study, we performed RNAseq analysis on (i) *V. fischeri* symbionts vented from recently colonized juvenile squid, (ii) viable cells incubated in sterile artificial seawater, and (iii) cells cultured in rich-media. This analysis aims to characterize the transcriptional changes undergone by *V. fischeri* during the early stages of host colonization. We found strong differential expression in over 100 genes between conditions (i) and (ii), including many that have been previously characterized as important to the squid-vibrio symbiosis. By also relating these to a rich-media grown culture, we are able to cross-compare the three conditions to further enhance our ability to predict which responses are specific to host-association. The responses we observe are consistent with expectations of genes regulated through quorum sensing, and illustrate that bacterial membranes are subjected to oxidative stress within the host. This represents the first full transcriptomic study of *V. fischeri* during juvenile host colonization.
A genome-scale metabolic model (GEM) is a structured systems-level representation of most known biochemical reactions of a target organism and their associated genes. It represents the metabolic capabilities of the organism and allows high-throughput in silico experiments to test new hypotheses. We constructed a GEM of the bioluminescent marine bacterium *Vibrio fischeri* to better understand its metabolism and, in particular, its symbiotic relationship in the light-emitting organ of the Hawaiian bobtail squid, *Euprymna scolopes*. We first set up the reaction network using the annotated genome and literature information, and then measured the biochemical composition of *V. fischeri*, growth rates and metabolite consumption and production rates to parameterize the model. To validate the model, we tested the growth phenotypes of *V. fischeri* in 181 different sole carbon-source conditions. The current *V. fischeri* GEM consists of 1725 reactions, 1019 unique metabolites, and 822 metabolic genes. It correctly predicts 86.2% of the experimentally validated growth phenotypes. It also successfully categorizes 83.4% of the 822 metabolic genes into either essential or non-essential genes in a complex-nutrient medium [1]. Finally, we performed a network analysis of the GEM based on the unpublished transcriptomics data from *V. fischeri* cells (i) grown in rich medium, (ii) incubated in seawater to mimic the planktonic state, or (iii) immediately after release from the light organ of a juvenile squid. This analysis inferred both the nutrient conditions for *V. fischeri* inside the squid’s light organ, and the changes in *V. fischeri* metabolism of oxygen, amino acids, sugars, and fatty acids that are due to its habitat transition between seawater and the symbiotic niche.

Microbiota Accessible Carbohydrates as Modulators of Clostridium difficile Infection

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Several enteric pathogens, including Clostridium difficile (Cd), leverage inflammation and dysbiosis to thrive in the gut environment. We hypothesize that host diet reinforces a Cd-mediated inflammatory state that dictates the persistence of a pathogen-supportive metabolic network during Cd infection (CDI). Using murine model CDI, we show that mice fed a diet deficient in the microbiota accessible carbohydrates (MACs) found in dietary fiber exhibit persistent CDI. Conversely, mice fed a fiber-rich diet clear CDI. These states are accompanied by differences in the composition/diversity of the gut microbiota and in the secreted host stool proteome. We further demonstrate the differential ability of closely related, structurally distinct polysaccharides to clear CDI, correlating with their capacity to support the growth of Cd in vitro. These observations suggest that competition for polysaccharides between a pathogen and the microbiota is a determinant of enteric pathogen fitness during infection. Here, we describe how pathogen inaccessible, microbiota accessible carbohydrates (PIMACs) contribute to disease outcome in the context of diet driven gut metabolic ecology. Our continued efforts will reveal mechanisms underlying the transitions between healthy and dysbiotic states, which can be exploited for a better understanding of the ecology of the gut microbiota and for the mitigation of CDI via targeted dietary intervention.
A Comprehensive, CRISPR-based Approach to Functional Analysis of Essential Genes

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To take full advantage of the opportunities provided by the recent deluge of microbial genome sequencing data, we need organism-independent strategies capable of identifying phenotypes for all genes, including essential genes and synthetic lethal gene pairs. To address this issue, we established a CRISPR (clustered regularly interspaced short palindromic repeats) interference (CRISPRi) framework for comprehensive phenotypic analysis of essential genes in bacteria. CRISPRi is a powerful, yet simple, methodology for targeted repression of gene expression, wherein a synthetic guide RNA (sgRNA) directs a nuclease-deactivated variant of Streptococcus pyogenes Cas9 (dCas9) to bind target DNA. Using CRISPRi, we generated and analyzed an essential gene knockdown library in the Gram-positive model bacterium, Bacillus subtilis. First, using a chemical genomics approach, we constructed a functional network of essential genes that revealed novel inter- and intra-process connections between pathways required for cell viability. Second, we validate using the library as a platform for drug discovery and then identified Undecaphrenyl pyrophosphate synthase (UppS) as the direct target of a largely uncharacterized antibiotic with an unknown mode of action. Finally, we show that CRISPRi is multiplexable; this feature allows us to dissect gene function in highly redundant genetic pathways. Using the pathway encoding penicillin-binding proteins (PBPs) as a test set, we identified a triple genetic interaction between genes involved in peptidoglycan biosynthesis during cell division. Our work demonstrates that CRISPRi is a powerful approach for elucidating genetic networks, validating drug targets, and uncovering double and higher-order genetic interactions in complex pathways, thereby providing a framework for high-throughput analysis of gene function generally applicable to diverse bacteria.
Non-canonical Amino Acids for Selective Proteomics and Discovery of a New Slow Growth Transcriptional Regulator

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Microbial quiescence and slow growth are ubiquitous physiological states, but their study is complicated by low levels of metabolic activity. Comparative proteomics is a powerful tool for identifying the mechanisms used to survive these conditions, but in dynamic or heterogeneous systems, isolating the subset of proteins of interest can be challenging. Here we present the application of a time- and cellselective proteomics approach toward the study of Pseudomonas aeruginosa during anaerobic survival and in phenotypically heterogeneous biofilms. The BONCAT (bio-orthogonal non-canonical amino acid tagging) technique relies on pulsed metabolic labeling with an azide-bearing methionine analog, which is incorporated during cellular protein synthesis. Through controlled expression of a mutant aminoacyl tRNA synthetase, incorporation of the non-canonical amino acid can be targeted to cells of interest. Labeled proteins are chemically distinct and can be physically enriched and identified via liquid chromatography-tandem mass spectrometry. We applied this approach to identify proteins synthesized preferentially, but at extremely low rates, during anaerobic survival, and to identify proteins expressed by a biofilm subpopulation. This proteomic analysis led to the discovery and characterization of a previously unknown transcription factor, important for P. aeruginosa slow growth.

Neutral and Niche Dynamics in a Synthetic Microbial Community

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Ecologists debate about the relative importance of niche and neutral processes in structuring communities. We investigate this question in a simple laboratory experiment by transforming heritable DNA barcodes into a population of otherwise clonal E. coli and watching the population dynamics over time for a wide variety of population sizes with immigration. We find one regime which is well predicted by neutral theories and another regime which requires differences between community members to explain. We use theory and simulations to show how this transition depends on fitness, migration, and population size. Finally we show the implications for selecting the correct theory by comparing divergent predictions with experimental results of a population expansion.
**Helicobacter pylori** Utilizes the Cytoplasmic Chemoreceptor TlpD to Repel Itself from Reactive Oxygen Species

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Cytoplasmic chemoreceptors are widespread among the different prokaryotic taxons and have been implicated in many processes, but they are far less understood than transmembrane chemoreceptors. One such cytoplasmic chemoreceptor is *Helicobacter pylori* TlpD, which is required for stomach colonization and participates in energy taxis *in vitro*. Neither the signals sensed by TlpD nor its molecular mechanisms of action are known. We report here that TlpD can function independently of the other chemoreceptors. TlpD is found in both soluble and membrane-associated subcellular fractions; association with the membrane depends on the transmembrane chemoreceptors, CheA, CheW, and one of three CheV coupling proteins. When TlpD is the sole chemoreceptor, it exists in a mostly soluble form at the cell pole. TlpD recruits CheW, CheA, and at least two of the CheV proteins to this location, consistent with the idea that TlpD forms its own autonomous signaling unit. We further determined that TlpD mediates a repellent chemotaxis response to iron and hydrogen peroxide. These responses appear to be connected, as the hydrogen peroxide repellent response requires iron. Given that both hydrogen peroxide and iron generate reactive oxygen species (ROS), we propose that TlpD coordinates a signaling complex that responds to cytoplasmic ROS.
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SESSION III
SATURDAY EVENING
Methanotrophy revisited: New Insights Into How Bacteria Grow on Methane

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The aerobic bacteria that grow on methane (methanotrophs) have been studied for over 50 years. This body of knowledge has created the concept of a highly restricted metabolic network, evolved to allow growth on methane as their sole substrate. The availability of a new set of methanotroph strains that are more easily studied has provided the opportunity to revisit how bacteria grow on methane. I will discuss three major new findings regarding methanotrophy, which taken together, paint a very different picture of methanotrophy, as a flexible and robust metabolic network with a suite of branchpoints and metabolic choices.
A Matter of Life and Death: *Methylobacterium* Growth and Viability Loss on Formaldehyde

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Microorganisms in the environment face a constant onslaught of things that can kill them. Extensive work has been done in quantifying the mechanisms and dynamics of microbial survival in the face of toxic compounds such as antibiotics; however, there is a more complicated situation that may occur in nature but is much more poorly understood: when the toxin is also a food source. This is a situation faced by *Methylobacterium extorquens*, a methylotroph that is ubiquitous in the phyllosphere and serves as a model for C1 metabolism in the lab. On a regular basis, *M. extorquens* must deal with formaldehyde: it is a key metabolic intermediate produced within the cell during growth on C1 compounds. Formaldehyde can even serve as a sole carbon source to *M. extorquens* when provided externally, yet high levels of the substrate cause severe loss of viability in the population at the same time that they sustain the growth of the surviving individuals. We have recently discovered a gene (*efgA*) encoding a protein that senses intracellular formaldehyde and slows growth when levels rise, and we are only just beginning to understand the physiological basis for the fitness benefit this gene confers. For instance, mutants lacking *efgA* outcompete wild type in the presence of high levels of external formaldehyde, because they are able to continue growing. On the other hand, wild-type cells yield higher biomass when growing on methanol (which produces internal formaldehyde).

To understand ecologically relevant processes, it often helps to study extremes. We are therefore examining how *M. extorquens* lives and dies in response to formaldehyde generally. Here, we present preliminary results on growth rates and death rates across a range of formaldehyde concentrations, morphological changes resulting from formaldehyde exposure, and phenotypic diversity in recovery from formaldehyde shock. We combine these data in a rudimentary model to describe the effect of formaldehyde on *M. extorquens* populations, and propose hypotheses for the role of *efgA*. To explain our observations quantitatively, the dual nature of formaldehyde as substrate and toxin forces us to consider a model that includes both growth and death, in which the “maximum inhibitory concentration” is not constant but depends on a suite of environmental factors, such as initial population density, the presence of alternative substrates, and the metabolic states of the cells.
Lanthanide-dependent Methanol Growth in *Methylobacterium extorquens* AM1

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Lanthanides have played an important role in modern industry. They are present in everyday use items such as cell phones, hybrid-car batteries, computer hard-drive magnets and are essential components of our military weaponry. Currently, the United States depends on lanthanide imports from China, resulting in decreased lanthanide reserves and increased cost. The high demand for lanthanides necessitates the need to develop new cost-effective strategies to recycle lanthanides from discarded products. Despite having industrial relevance, lanthanides were thought to have no biological significance due to their low solubility in the environment. However, it has recently been shown that some methylotrophic bacteria are capable of acquiring and using lanthanides as enzyme cofactors to support growth. *Methylobacterium extorquens* AM1 serves as a model organism for the understanding of methylotrophic metabolism and physiology. This bacterium possesses at least three types of pyrroloquinoline-quinone (PQQ)-dependent methanol dehydrogenases (MeDH) that catalyze the oxidation of methanol to formaldehyde. The first type of MeDH is encoded by the *mxaFI* genes and uses calcium as a cofactor. The second type of MeDH is encoded by the *xoxF* genes and requires lanthanides such as lanthanum and cerium. Using growth curve analyses, we show that *M. extorquens* AM1 has a third lanthanide-dependent methanol oxidation system capable of supporting methanol growth and have identified this enzyme as ExaF. Our studies expand these findings to show that growth is supported by a variety of lanthanides including lanthanum, cerium, praseodymium, neodymium and to a smaller extent, samarium. Transcriptional reporter fusion assays suggest that the expression of the *mxa* and *xox1* genes is differentially controlled by the presence or absence of these same exogenous lanthanides in methanol medium. When lanthanides are absent, expression from the *mxa* promoter is induced and expression from the *xox1* promoter is repressed. However, the opposite expression pattern is observed when lanthanides are present. Using transcriptional reporter fusions, we show that expression from the *xox1* promoter is very sensitive to lanthanum, showing induction with concentrations as low as 2.5 nM, suggesting that this reporter fusion could serve as a very sensitive biosensor of lanthanides. To determine if lanthanides can be acquired from electronics waste, computer hard-drive magnets were crushed and added to *M. extorquens* methanol grown cultures and fluorescence from the *xox1* transcriptional reporter fusion was assessed. Fluorescence/OD increased over time in the presence of 0.1 gm of magnet but not in the absence of magnet suggesting that cells are able to acquire and transport lanthanides from lanthanide-containing commercial products. These studies serve as a first step in developing *M. extorquens* as a biorecycling strategy.
A LysR Type Transcriptional Regulator Is Required for the Expression of a Newly Discovered Lanthanide-dependent Methanol Dehydrogenase in *Methylobacterium extorquens* AM1

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*Methylobacterium extorquens* AM1 is a facultative methylotroph and a model organism for methylotrophic studies. It is a platform for the production of value added chemicals such as biofuels and bioplastics from methanol but also has the potential to function as a biosensor of lanthanides and to recover lanthanides from discarded electronics. Lanthanides are essential components of our computers, cell phones and military weaponry. Currently, the United States is dependent on China for lanthanide supply necessitating new strategies for lanthanide recycling and recovery. It was recently discovered that *M. extorquens* AM1 can acquire these insoluble metals and use them as cofactors for several methanol dehydrogenase (MeDH) enzymes, XoxF1 and XoxF2. We have discovered the existence of a third lanthanide-dependent MeDH encoded by the *exaF* gene. To facilitate strain engineering we need a better understanding of the lanthanide-dependent MeDH enzymes and how they are regulated. Transposon mutagenesis was carried out to specifically identify genes required for the MeDH step during lanthanide-dependent methanol growth. In addition to genes involved in metal transport, protein processing and PQQ synthesis, mutations were isolated in the *xoxF1*-MeDH gene and a putative LysR-type transcriptional regulator. Transcriptional reporter fusion studies suggest that LysR is an activator of *exaF* and may function as a repressor of the calcium-dependent MeDH genes, *mxaF1*. However, these studies do not show whether the requirement for LysR-dependent regulation of MeDH gene expression is direct or indirect. Future studies will involve microscale thermophoresis and electrophoresis mobility shift assays to determine which transcriptional regulators bind to which promoter regions and if binding is facilitated or inhibited by lanthanides. These studies will facilitate engineering efforts to create strains of *M. extorquens* AM1 that can efficiently acquire and retain lanthanides for recovery.
PQQ-dependent Ethanol Dehydrogenase Supports Methylo trophic Growth in the Presence of Lanthanides

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Lanthanides have recently been reported to stimulate expression of XoxF-type bacterial methanol dehydrogenases (MeDH). Lanthanum and cerium have been found in the active sites of some XoxF’s and demonstrated to be important for activity. In the model methylotroph Methylobacterium extorquens AM1, activity of XoxF1 was found to be lanthanide-dependent, and its expression supports methylotrophic growth in a strain lacking the calcium-dependent methanol dehydrogenase, MxaF. Surprisingly, a mutant strain lacking MxaF, XoxF1 and XoxF2 is still able to grow on methanol, albeit at a slower rate, when lanthanides are added to the medium suggesting there is another unknown enzyme capable of oxidizing methanol. Methanol oxidation was detected in cell-free extracts prepared from the triple mutant strain using a dye-linked assay. Gene expression profiles of the wild-type strain grown in minimal media with methanol containing calcium or calcium plus lanthanum were analyzed using RNAseq. Under these conditions a PQQ-dependent ethanol dehydrogenase (exa) was up-regulated ~4-fold in the presence of lanthanum.

Exa was expressed in M. extorquens grown on methanol in the presence of lanthanum and purified to homogeneity by Immobilized Metal Affinity Chromatography. Purified Exa has the characteristic absorbance signature of PQQ as a cofactor and lanthanum was detected by ICP-MS, demonstrating that it is loaded into the active site. Enzyme kinetics showed that Exa is a relatively poor methanol dehydrogenase as expected from the low growth rate on methanol. Interestingly, Exa is 100-times more catalytically efficient with formaldehyde as the substrate suggesting that it may be oxidizing methanol directly to formate. This was confirmed by measuring the end product of the reaction using colorimetric assays. The same trend was observed when formaldehyde and formate were measured in cell-free extracts of the mxaFxoxF1xoxF2 mutant and wild-type strains. This increased oxidation capacity is likely to affect carbon distribution through the methylotrophic pathways, impacting assimilation and dissimilation.

These results confirm Exa can function as a methanol dehydrogenase in the presence of lanthanides, and as such the methylotrophic bacterial community may be underestimated. This is also the first ever report of lanthanides being utilized by an ethanol dehydrogenase, expanding the importance of lanthanides in bacterial physiology.
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SESSION IV
SUNDAY MORNING
Sterol Biosynthesis in the Bacterial Domain

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Sterols are ubiquitous and essential components of eukaryotic membranes that have long held the interest of scientists from various fields. Over the last 50 years, biochemists have characterized the unique biochemical reactions required for the biosynthesis of these polycyclic molecules while cell biologists have revealed vital roles for sterols in intra- and intercellular signaling, stress tolerance, maintaining cell membrane integrity, and human disease. In addition, organic geochemists have been able to identify sterol biosignatures preserved in sedimentary rocks that are millions and even billions of years old. These sterane signatures are utilized as biomarkers, or “molecular fossils”, for ancient unicellular eukaryotes and have allowed geochemists to physically link the existence of eukaryotes to the rock record and inform our understanding of life on the early Earth. Despite decades of study on eukaryotic sterol biology, one aspect that has not been fully explored is the bacterial production of sterols. Although sterol biosynthesis is often viewed as a uniquely eukaryotic feature, sterol production has been demonstrated in a few bacterial taxa from three distinct groups: the methanotrophic bacteria, the myxobacteria, and the planctomycetes. Yet, very little is known about the evolutionary and physiological significance of this eukaryotic pathway in the bacterial domain. Here, we utilize comparative genomics, genetic and biochemical approaches to better assess the distribution of sterol synthesis in bacteria and to better understand how bacteria synthesize sterols. Our results show that bacterial sterol synthesis extends beyond the three taxa mentioned above and that the bacterial sterol biosynthetic pathway is distinct from what is observed in eukaryotes.
A Novel protein Distinguishes Tetrahymanol Lipid Synthesis in Bacteria and Eukaryotes

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Cyclic triterpenoid lipids such as sterols, hopanoids and tetrahymanol are important both biologically, because they profoundly affect cellular membrane structure and integrity, as well as geologically, because their stability allows them to serve as biomarkers for ancient microbial life preserved in sedimentary rocks. In eukaryotes, such as the ciliate Tetrahymena, tetrahymanol is synthesized directly from squalene by Squalene-tetrahymanol cyclase (Stc) when dietary sources of sterols are scarce. Because a bacterial Stc homolog has never been identified, trace amounts of tetrahymanol found in several bacteria was thought to be a side product of hopanoid synthesis by the enzyme Squalene-hopene cyclase (Shc). Here, we demonstrate that the obligate aerobic methanotrophic bacterium Methylomicrobium alcaliphilum synthesizes significant amounts of tetrahymanol and production varies with growth conditions. We use comparative genomics to identify a gene encoding a hypothetical protein of unknown function that is necessary for synthesis of tetrahymanol in bacteria. We designate this novel protein Tetrahymanol synthase (Ths) and identify homologs in alphaproteobacterial Rhizobiaceae, gammaproteobacterial Methylococcaceae and sulfate-reducing deltaproteobacterial Desulfovibrionaceae. Using engineered Escherichia coli strains and a cell-free in vitro system, we establish that enzymatic synthesis of tetrahymanol in bacteria proceeds via a hopanoid intermediate by a novel protein, Ths, in contrast to the direct route from squalene by Stc in eukaryotes. We must now assess the physiological role of tetrahymanol in bacteria and reconsider the interpretations of tetrahymanol-derived biosignatures in the rock record.
Correlation Between Rippling, Aggregation and Motility in *Myxococcus xanthus*

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*Myxococcus xanthus* is a bacterium that grows as a biofilm called a swarm and exhibits multicellular behavior in response to environmental stress. As part of its response to starvation, an *M. xanthus* swarm will sometimes create a series of density waves called ripples. *M. xanthus* ripples are one of the few observed instances of non-reaction/diffusion waves in a biological system; cells align into alternating regions of higher and lower density, which coordinate their reversals to create a standing wave pattern that appears as two sets of opposing traveling waves. Ripples are produced in part through a contact dependent extracellular C-signal cascade that coordinates the temporal and spatial movement of cells across a swarm. Under starvation conditions, this signaling cascade controls a developmental process that ultimately transforms an *M. xanthus* swarm into multicellular aggregates, also known as fruiting bodies. Any connection between these rippling formations and the ability to form fruiting bodies has never been established, in part because ripple formation is not very reliable and reproducible. Therefore, we developed assay conditions that will produce reliable, reproducible ripples in wild-type *M. xanthus*. This “rippling” assay has allowed us to characterize rippling behavior both wild-type and mutant strains. Based on the result of our assay we have determined that there is a correlation between rippling and fruiting body formation, and that both rippling and fruiting body formation correlate strongly with cell motility.

To accomplish this, we identified 60 mutant strains (including csgA\(^-\)), 30 of which have no known fruiting defects, and 30 of which had significant fruiting defects. These 60 strains also have varying motility. We used all 60 strains strains to study the correlation between motility and rippling, motility and fruiting, and rippling and fruiting. In general, mutant strains that form fruiting bodies like wild-type cells also ripple like wild type cells and have wild-type motility. However, we identified four outlier strains that led to our conclude that rippling and fruiting body formation may not have a direct functional connection. Image analysis of both wild-type and mutant ripples revealed that the speed, amplitude and wavelength are all under genetic control. We are now studying the movement of individual cells within these mutant ripples, to see if there is a connection between the behavior of ripples (speed, amplitude, wavelength) and the behavior of individual cells (speed, reversal frequency).
Phenotypic Variation among Developing Populations of *Myxococcus xanthus* DK1622

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Laboratory evolution is inevitable for a model microbial system like *Myxococcus xanthus*. As long as a group of *M. xanthus* cells swarm on solid media, double every ~5 hours in liquid media, and aggregate on starvation media, subtle genomic changes with a less than catastrophic impact on these behaviors may go unnoticed. Over time, accumulation of these changes might cause the phenotypes of wild-type strains in different laboratories to deviate.

We have carefully measured the phenotypic profiles of wild-type DK1622 obtained from nine different labs and report considerable differences in aggregate number, aggregate size, and/or recovered spore counts across all samples. Whole genome sequencing of all nine strains revealed few genomic differences between them, supporting the hypothesis that a few mutations may have a significant impact on phenotype\(^1\). By reconstructing the mutations, we have converted one lab strain with a mean developmental phenotype to resemble another lab strain displaying a more distinct developmental phenotype.

Evidence that O-GlcNAc Protein Modification is Essential for Hormogonium Development in *Nostoc punctiforme*

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Filamentous cyanobacteria commonly exhibit gliding motility which facilitates a number of biological processes, including phototaxis, the formation of colonial aggregates, and the establishment of plant-cyanobacterial symbioses. In a subset of these organisms, gliding is restricted to hormogonia, differentiated motile filaments. Currently, the gene regulatory network that governs hormogonium development is not well defined. To identify components of this network we employed a forward genetic screen to isolate non-motile mutants of the filamentous cyanobacterium *Nostoc punctiforme*. This screen identified *ogtA*, which encodes a putative O-linked b-N-acetyl glucosamine transferase (OGT), as essential for normal hormogonium development and motility. In many eukaryotes, OGTs modulate protein activity via transient O-GlcNAcylation of target proteins, resulting in subsequent effects on a number of cellular and developmental processes. In *Nostoc punctiforme*, transcription of *ogtA* is upregulated early in hormogonium differentiation and deletion of *ogtA* completely abolished motility. The *ogtA*-deletion strain could be induced to differentiate filaments with hormogonium-like morphologies, but unlike wild-type hormogonia, these filaments fail to accumulate PilA or secrete hormogonium polysaccharide. Preliminary immunological assays indicate an increase in the abundance of O-GlcNAc-proteins during the progression of hormogonium development, and detection of O-GlcNAc-proteins was drastically reduced in the *ogtA*-deletion strain. These results implicate O-GlcNAc protein modification as an essential early event in hormogonium development.
The Stringent Response Regulates Adaptation to Darkness in a Cyanobacterium

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The cyanobacterium *Synechococcus elongatus* is an obligate phototroph. Darkness thus represents a starvation-like state for *Synechococcus*, which dramatically decreases macromolecular synthesis in the dark via unknown mechanisms. Here, we show that the stringent response, a stress response whose enzymes are conserved across bacteria and in plant plastids, contributes to dark adaptation. Cells lacking the stringent response display pronounced growth defects in light/dark cycles. Levels of the stringent response alarmone ppGpp rise after a shift to darkness and regulate expression of a number of genes in *Synechococcus*, including ribosome hibernation promoting factor (*hpf*). HPF, in turn, controls translation in response to light status. Although the metabolism of *Synechococcus* differentiates it from typically studied bacterial model systems, the logic of the stringent response remains remarkably conserved, while at the same time having adapted to the stresses of the photosynthetic lifestyle.

Diversity of Lactic Acid Bacteria in Ethiopian Teff Injera

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Lactic acid bacteria (LAB) and yeasts are essential for the production of teff (*Eragrostis tef*) injera, an Ethiopian sourdough crepe made by fermenting teff flour. Although teff injera is a staple in the Ethiopian diet, the biodiversity of microorganisms in teff injera remains largely unknown. In this study, LAB were isolated and identified from individual brown and white teff injera cultures produced in Ethiopia. LAB *Lactobacillus plantarum, Lactobacillus sanfranciscensis, Lactobacillus rossiae, Lactobacillus spicheri* dominated both brown and white teff cultures according to the 16S rRNA gene sequences of individual injera isolates. *Lactobacillus xiangfangensis* and *Lactobacillus casei* were also isolated but from the brown and white teff injera, respectively. *L. xiangfangensis*, a newly identified species of Lactobacillus, was isolated from a grain culture for the first time. Notably, except for *L. plantarum*, the LAB species identified here have not been previously detected in teff fermentations. The *L. plantarum* isolates identified here were distinguished by their different capacities to consume glucose, sucrose, and fructose as sole carbon sources for growth. Each of the three isolates tested exhibit different growth rates and cell yields on these mono- and di-saccharides. These investigations can be used for the development of commercial teff injera starter cultures as well as provide novel insight into the bacterial diversity and functional traits of grain-based food fermentations.