

216. Omics and Chemical Probe Strategies for Assessing Interactions Among Microbial Community Members

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Project Goals: The PNNL FSFA is focused on discovering fundamental principles that determine microbial community structure, function, and dynamics, and applying these principles to rational design of microbial communities for desirable outcomes. Our strategy integrates field studies of microbial mat communities and laboratory studies of field-derived consortia and isolates to gain a mechanistic understanding of the environmental and biotic drivers of community higher-order properties such as resistance and resilience. Genome sequence-enabled approaches, including chemical probes directed against specific functions, are being used to elucidate underlying interaction mechanisms. These data are in turn being used to inform community metabolic, spatial and regulatory network models. Our research plan supports DOE goals to achieve a predictive understanding of microbes and microbial communities and to provide foundational knowledge enabling rational design of microbial systems.

We have used metagenome sequence to assemble complete or near-complete genome sequences from nearly all the members (~20) of two uncyanobacterial consortia (UCC) developed from benthic microbial mats associated with a saline (MgSO₄) lake in northern Washington State (see Nelson and Moran posters for details). These compositionally-stable consortia have been maintained under constant light in defined media containing salts, trace minerals, and CO₂ as the primary carbon source. This defined system has allowed us to use single organism metabolic reconstruction strategies to infer member function and metabolic requirements and to use these genomic analyses collectively to predict interactions among members and the identity of genes and processes involved. We describe two examples of how the assembled genomes provide detailed insights into microbial community interactions than would not be possible with typical incomplete metagenome sequence data. We also describe development of chemical probes intended to provide visualization of individuals contributing to carbon and nutrient interactions.

Maintaining the consortia under constant light promotes continuous O₂ production and highly oxidizing conditions. Under these conditions Fe is present as Fe³⁺ and that a strong complexing ligand (citrate) be provided to maintain its solubility. We conducted regulon and comparative genomic analysis to assess how different members acquire Fe³⁺ and maintain internal iron homeostasis. Analyses revealed that while most of the members sense iron levels directly using Fur, the seven Rhodobacteraceae and one Rhizobiales member sense it indirectly via the heme-binding Irr regulator. A review of these predicted regulons suggest that the gamma proteobacterial members have an expanded repertoire of iron uptake systems and that the Halomonas sp. are the only members with detectable siderophore biosynthetic capability. All of the members possess a ferric uptake system (FbpABC) and utilize the SUF rather than ISC iron-sulfur assembly system, likely due to its insensitivity to aerobic stress. Proteomic analysis of the UCC OSCr consortia detected ferric uptake system components from 11 members but provided no evidence for siderophore utilization. On average the number of proteins involved in Fe homeostasis detected accounted for two percent of the total for each organism which is high considering how few proteins are involved in iron metabolism and how few are detected for each organism.

It is well recognized that vitamin auxotrophy and prototrophy provide the basis for metabolic interactions between phototrophs and heterotrophs. By analyzing our UCC we are able to provide the first insights into how auxotrophy might be maintained in a self-sustaining multi-member community. As no external source of vitamin is provided to our cultures, auxotrophs must acquire this resource from other members or employ a metabolic strategy that obviates the need for vitamins that they cannot synthesize. Genomic analysis revealed that only the cyanobacteria and two *Halomonas* sp. are capable of synthesizing all required vitamins (B1, B2, B3, B5, B6, B7, B9, and B12) or coenzymes derived from them. While all members could produce FAD/FMN (B2) and pyridoxine (B6), auxotrophy for the remaining vitamins are widespread among consortia members. We also identified the vitamin-dependent metabolic processes to assess the burden that sharing these resources might have on the community. This analysis revealed an unexpected relationship between the ability to produce B12 and need for it to mediate essential processes. In general, the auxotrophs encode more enzymes that require B12 than the five producers do. Both *Halomonas* sp. are B12 opportunists, yet they utilize enzymes or pathways that obviate its requirement under all conditions except growth on ethanolamine suggesting a potential role for these organisms as producers of this commodity. We also discovered that B12 likely acts as a regulatory sensor in the phototrophic Rhodobacteriaceae and *Algoriphagus marincola*, controlling photosynthesis and carotenoid biosynthesis, respectively. Its dual role as a sensor and coenzyme suggests that it may be a key determinant controlling the community dynamics.

In order to identify proteins involved in these processes and to monitor the exchange of metabolites under different conditions we have developed activity-based protein probes including mimics of vitamins, amino acids, and sugars. These probes are designed so that they can be taken up by living cells, fixed to targets that they bind, and assayed by image analysis, flow cytometry, or mass spectrometric proteomic characterization. We have validated the specificity of the vitamin probes and successfully used them to identify transporters and enzymes in several different UCC taxa. We have initiated investigations that involve use of the probes to image the distribution of cells that assimilate them under different conditions so that the impact of changing culture and/or environmental conditions on resource allocation can be determined.

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