Deciphering Microbial Community Interactions Using Model Complex Microbial Consortia

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Project Goals: The PNNL FSFA goal is to identify the fundamental mechanisms by which microbial interactions and spatial organization impact rates and pathways of carbon and energy flow in microbial communities. The strategy involves the study of highly interactive and tractable model autotroph-heterotroph consortia whose member genome sequences have been defined. Our project leverages unique capabilities including multi-omics measurements, advanced functional imaging, taxonomic profiling and metabolic and regulatory network modeling to elucidate underlying reaction mechanisms within complex microbial communities. Our research plan supports DOE goals to achieve a predictive understanding of microbially-mediated carbon and energy transformation.

Microbial autotroph-heterotroph interactions influence biogeochemical cycles on a global scale, but the molecular mechanisms underlying microbial community interactions and functional processes they perform are largely unknown. The diversity and complexity of natural systems and their intractability to *in situ* manipulation make it challenging to elucidate the principles governing these interactions in natural systems. We have addressed this challenge by development of tractable model microbial autotroph-heterotroph consortia as experimental platforms. We apply a genome-enabled approach together with integrated experimental and modeling to paint a cohesive picture of fundamental mechanisms of microbial community interactions, metabolic interdependencies and spatial constraints on functional processes and community properties.

The majority of our experiments have focused on the study of interactions in consortia consisting of one cyanobacterium and up to 18 associated heterotrophs. These unicyanobacterial consortia (UCC) have proven to be a unique and valuable resource for the team and are becoming recognized as the "***E. coli of complex communities***" because of the following attributes: 1) the UCC consist of a primary producer and its heterotrophic epibionts that has been stably maintained in simple defined medium, and thus must employ a complex interaction network to support all its members, 2) complete or near-complete genome sequence from nearly every member has been derived enabling the prediction of member metabolic interdependencies, testing the effect of member microdiversity, and application of transcriptomic and proteomic methodologies to construct regulatory networks, 3) axenic cultures have been developed and sequenced, filling in gaps in member sequence and allowing us to manipulate community membership, and 4) genetic systems have developed for isolates allowing us to manipulate community function. We have also developed new methods for study of communities: 1) assays to measure metabolites in high salt conditions, 2) software for quantitative analysis of cellular relationships from images, 3) chemical probes to identify metabolite- protein interactions and image substrate uptake, and 4) protein SIP to determine the fate of metabolites in communities.

Several multidisciplinary studies addressing specific hypotheses within our model communities, using novel applied imaging, metabolomic, and other spatiotemporal profiling capabilities lead to new fundamental knowledge about the realm of interspecies interactions guiding complex community functions. The main findings are briefly summarized in this FSFA overview poster as follows:

Poster by Nelson *et al*. To test the hypothesis that microbial interactions increase over community

ecological succession, we used a multi-omics approach, including a novel metabolomics methodology, to examine community composition and function over a 28-day growth of the UCCs. We found evidence of increases in efficiency of nitrogen species recycling as the community matures.

Poster by Wright *et al*. Coordination of metabolism can occur via vitamin exchange leading to a microbial division of labor. Because vitamins are synthetically expensive, vitamin sharing should conserve energy for other tasks. Importantly, we demonstrated that B_{12} utilization is widespread throughout the entire community, yet production is limited to only a few species, thus the exchange process is a necessary interaction for community success.

Poster by Lindemann *et al*. It has been observed that N affects carbon uptake and partitioning in a phototrophic dominated system, though the mechanisms driving these phenomena are not understood. We hypothesized that the cyanobacteria in the consortia served as the major primary assimilators and providers of community N, and found N availability to microbial photoautotrophs governs community C partitioning.

Poster by Renslow *et al*. Communities are typically spatially heterogeneous and the means by which microbes interact across various environmental gradients is not well understood. Here we developed a novel imaging method applied to the UCC's to decipher the spatial context of interactions occurring between members. We found the carbon based trophic relationships are dynamic and show a sharp dependence on N availability.

Poster by Moran *et al*. While photosynthetic carbon (C) fixation in vertically laminated phototrophic mat systems is generally relegated to only an upper portion of the overall community, microbial interactions must govern the availability of C to the rest of the community. We have tracked stable isotope-labeled (^{13}C) bicarbonate, acetate and glucose through the biomass of a phototrophic mat system. We identified that the community regions with highest productivity did not correlate with broadest biodiversity but that, rather, high autotrophy correlated with low biodiversity.

Poster by Song *et al*. Two complementary modeling efforts are focused on inferring microbial interactions in our model communities. We have integrated our genome and transcriptomic data into the DOE's Systems Biology Knowledgebase (KBase) platform to construct single genome and community metabolic network models as an *in silico* tool for predicting interspecies metabolite exchanges. We found coordinated and highly correlated transcriptional responses between species that could be used to infer specific interactions, particularly those that regulated synthesis and exchange of resources, such as amino acids, sugars and organic acids and organic C sources.

Poster by Bernstein *et al*. Specific mechanisms that mediate metabolic coupling and acclimation to microbial partnerships centered on C-N exchanges are not well defined. Transcriptome measurements coupled with metabolic modeling of a constructed binary phototroph-heterotroph consortium found coordinated and highly correlated transcriptional responses between species that could be used to infer specific interactions, particularly those that regulated synthesis and exchange of resources, such as amino acids, sugars and organic acids and organic C sources.