

## 18. FSFA Poster #2: Metabolic Interactions Structuring Microbial Communities

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The overarching goal of the PNNL Foundational SFA is to identify the principles that govern assembly, stability, and resilience of self-organized microbial communities to enable prediction of response to perturbation and control for maintenance of system function. Interactions between component members of communities are major forces maintaining community function in the face of perturbations. Complementary sequence-based functional predictions and experimental validation studies are being applied in iterative fashion to identify the spectrum of interactions that occur in natural communities, derived consortia, or synthetic co-cultures. Hypotheses-driven experimental approaches involve a suite of physiological, biochemical, and 'omics measurements performed in conjunction with controlled cultivation to systematically perturb organisms and measure response to determine network structure and identify potential interactions between members.

Through the application of next-generation sequencing technology in conjunction with controlled cultivation and metabolomic profiling, we carried out analysis of differentially regulated transcripts in a synthetic cyanobacterium-heterotroph consortium grown on either organic or inorganic C source. The obtained information led to the development of a conceptual model of interactions between phototautotrophs and heterotrophs as a function of carbon sources and flux directions (Fig. 1). Although the overall response was broad and complex, the global transcription patterns indicated competition for available carbon resources and maximization of metabolic capacity. This was manifested through the induction of transport and catabolic pathways involved in the assimilation of both monomeric and complex polymeric substrates, such as exopolysaccharides, peptides, and nucleic acids. This was particularly evident in the organism that was incapable of utilizing the primary carbon source (inorganic carbon vs. lactate). Substantial evidence supported metabolic coupling via the exchange of amino acids and co-factors. Co-cultivation with the heterotroph stimulated expression of facile and

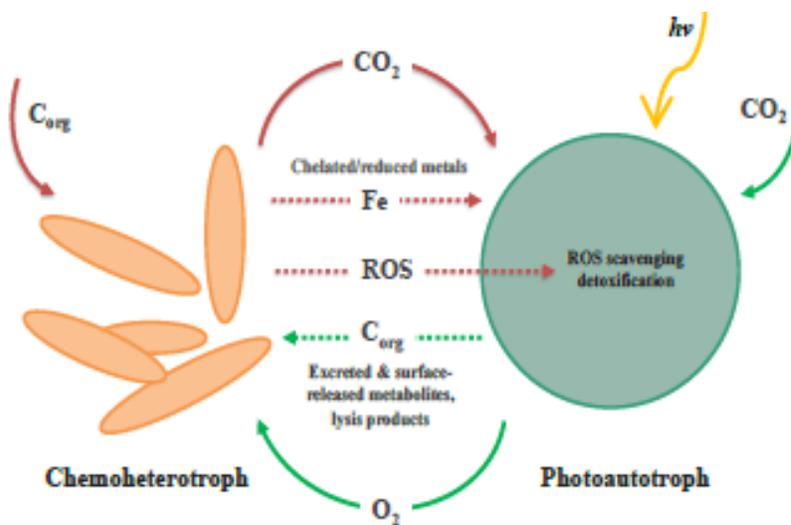


Fig. 1. Proposed mechanisms of interactions between of photoautotrophic and heterotrophic cells growing in co-culture. Arrows represent fluxes driven by photoautotrophic (green) and heterotrophic (red) metabolism.

energy-efficient pathways for Fe acquisition by the autotroph; Fe is required for the extensive electron transport pathways of photosynthesis. In contrast to other studies where heterotrophs were hypothesized to reduce phototroph oxidative stress via catalase-dependent ROS scavenging, the transcript levels of genes involved in oxidative stress response and scavenging of ROS radicals were down-regulated under co-culture conditions. This suggests that some phototrophs may be the provider of protection against oxidative stress in phototroph-heterotroph consortia. Isolates and consortia from hot spring and hypersaline mats are now allowing investigation of whether observed behaviors in synthetic consortia hold for functionally similar consortia derived from natural systems.

Complementary comparative analyses of genome sequences (derived from mat isolates and metagenome subassemblies) have developed predictions of resource sharing that are being tested experimentally using gene knock-outs/knock-ins, biochemical assay, or targeted synthetic chemical probes. B-type vitamin biosynthesis and salvage are broadly recognized as key interactions driving autotroph-heterotroph associations, but knowledge gaps in genes responsible for salvage and transcriptional control of co-factors prevent accurate prediction, probing, and modeling of exchange in microbial communities. Chemical probes have been developed for vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>7</sub>, B<sub>9</sub>, and B<sub>12</sub>, and coenzymes derived from them, and have been shown to selectively target specific vitamins *in vitro*. Probes are currently being used on whole cultures (axenic or mixed) to assay uptake and identify proteins associated with uptake. Using comparative genomics we have identified the first archaeal transcriptional regulators of riboflavin, cobalamin, and thiamin biosynthesis; in bacteria control is mediated by riboswitches rather than via protein binding near promoters. An archaeal NAD-specific transcriptional regulator was also identified in Thermoproteales and Metallosphaera. Regulon and genome context analysis enabled discovery of three candidate salvage systems (transporters) for riboflavin and thiamin and one for niacin.

In both the chemotrophic and phototrophic communities it appears that vitamin auxotrophy is limited primarily to heterotrophs suggesting that autotrophic organisms are an important source of essential co-factors early in community assembly. Analysis of thermophilic iron mat metagenome subassemblies suggest that *Hydrogenobaculum*, an early colonizing chemoautotrophic bacterium, is the only source of biotin. Similarly, in uncyanobacterial consortia derived from a saline mat, cobalamin is predicted to be synthesized by only two of 17 taxa (Phormidium and Rhizobiales) present in co-cultures grown in the absence of external vitamin supplementation.

Using our uncyanobacterial consortia (UCC) we are also examining the potential role of extracellular polysaccharides (EPS) in metabolic coupling of autotrophs and heterotrophs. Metabolite analysis of loosely associated EPS extracted from an 18 member UCC identified tartaric acid, glutamate, D-xylose, fucose, glucose, and mannose as primary carbohydrate constituents. Analysis of the metagenome subassemblies from the 18 taxa present in this UCC suggest that the potential to metabolize each of these carbohydrates are present in community members. Mannose metabolic potential was found in all but four of the taxa, which was consistent with this also being the most abundant metabolite detected in EPS. A mosaic distribution of catabolic pathways for the other carbohydrates was observed and hypothesized to minimize competition for EPS constituents as carbon sources.