Characterization of Metabolites from Phototrophic Microbial Mats in Biogeochemically Unique Environments

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Project Goals: The objective of the Pacific Northwest National Laboratory Foundational Scientific Focus Area is to develop a predictive, genome-enabled understanding of how microbial interactions impart stability, robustness, and functional efficiency to microbial communities. To achieve this goal, research is being conducted on three different model systems: iron- and sulfur-oxidizing communities associated with hyperthermophilic acidophilic springs at Yellowstone National Park (YNP) and phototrophic mat communities formed in high temperature springs at YNP or in a hyper-saline lake (Hot Lake) found in northern Washington State. As part of this effort, we are analyzing intracellular and extracellular metabolites to identify those that are secreted by microbes present in these phototrophic mat systems and to monitor their exchange among different community members.

The phototrophic microbial mats in YNP hot springs have been well-studied. However, the fundamental relationships between the primary producers (autotrophs) and consumers (heterotrophs) in these mats are not fully understood. Oxygenic phototrophic cyanobacteria, such as *Synechococcus* species, produce organic molecules by fixing inorganic carbon via photosynthesis or by fermenting stored molecules at night. For example, previous research has revealed that glycolate is produced during the day, while acetate is produced from the fermentation process at night. In turn, anoxygenic phototrophic bacteria, such as *Chloroflexus* and *Roseiflexus* species, and other heterotrophic organisms utilize these primary molecules for their carbon and energy needs. In this system, the metabolic processes of different community members contribute to the accumulation or consumption of storage molecules such as polyhydroxyalkanoic acids (PHAs) and wax esters for carbon storage and cyanophycin for nitrogen, which are thought to undergo diel cycling in *Roseiflexus* and *Chloroflexus*.

PHAs are also possibly precursors for the synthesis of branched amino acids in certain bacterial species which have no enzymes to facilitate these processes. Using gas chromatography-mass spectrometry (GC-MS)-based metabolomics analyses, we have identified several key metabolites in this system. Some of these metabolites fluctuate during a diel cycle and may be exchanged between members of hot springs microbial mat communities. We will present results of metabolomics analyses of the mats, as well as the results of spent media analyses from the lab culture of *Thermosynechococcus* and *Chloroflexus* species which have been isolated from these systems.

The hyper-saline and ecologically unique Hot Lake in north-central Washington state has not been well-investigated by the scientific community. Recent studies have shown that several autotrophic and heterotrophic microorganisms comprise mats within this extreme environment, which contains almost 1 M magnesium sulfate and 0.5 M sodium chloride. Since the location of this lake is geographically isolated from any river or stream, organic carbon and nitrogen sources are not able to enter from outside sources. Therefore the majority of water input in this lake comes from precipitation, causing the lake to undergo fluctuations in salinity. The phototrophic mats in this system represent good models of energy and carbon fluxes. Importantly, the high levels of salts in this system present a challenge to the analysis of organic metabolites, which usually have similar size and polarity as those of salts. For example, high concentrations of salts may reduce extraction and derivatization efficiencies during GC-MS and generate excessive adduct ions in liquid chromatography-mass spectrometry (LC-MS) analyses. Therefore, we are developing and optimizing protocols to remove these salts to improve our measurements. We will present our on-going mass spectrometry-based metabolomics analysis of mat samples and secreted metabolites from cultures of autotrophic and heterotrophic organisms isolated from the Hot Lake community.

Results obtained from the above mentioned studies will be incorporated with data from transcriptomics and proteomics experiments and used for modeling the metabolism of the microbial mat communities.

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