Microbial Diveristy and Biogeochemical Function of the Phototrophic Microbial Mats of Epsomitic Hot Lake, WA

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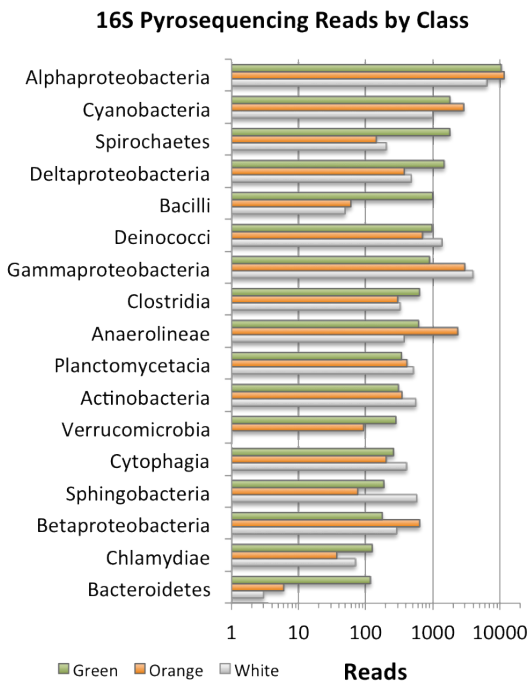
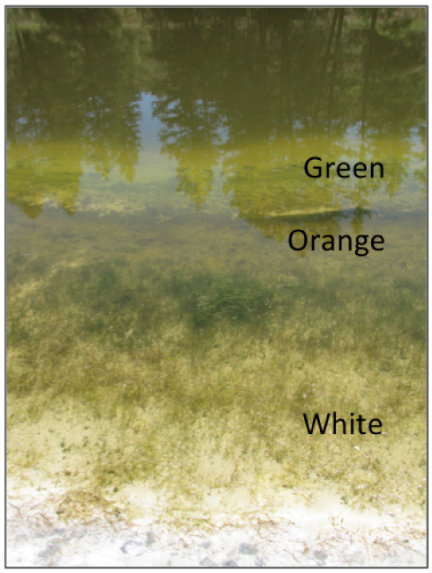
Project Goals: Determine the phylogenetic and functional diversity of members of a hypersaline microbial mat using (meta)genomics of whole mat, enrichment cultures, and sorted single cells; probe the dynamics of community function and metabolic interactions in a hypersaline microbial mat in response to changes in salinity, temperature, and photon flux using metatranscriptomics and stable isotope probing; ultimately, to generate a whole-mat model of energy and element cycling in the mat within its ecological context, especially with respect to carbon balance.

Hot Lake is a meromictic, hypersaline lake that occupies a small glacial endorrheic basin in north-central Washington and contains a benthic phototrophic microbial mat. Being highly organized, metabolically interactive, self-sustaining communities, phototrophic microbial mats are natural models for the study of energy and element cycling between phototrophs and heterotrophs and the effect these interactions have upon biogeochemical processes, especially with respect to carbon cycling. As there are no water inputs into Hot Lake besides meteoric water falling within the small basin it occupies, primary production within the lake is thought to be the major source of fixed carbon; this simplifies analysis of the carbon and energy budget of the phototrophic mat and the mat's influence on its broader geochemical context. Carbonate minerals precipitate within the mat beneath the cyanobacterial stratum by an as yet-unknown mechanism. As the metabolic activity of both sulfate reducers and oxygenic phototrophs have been independently implicated as the key driver of biogenic carbonate precipitation within microbial mats in disparate environments, study of the interactions between Hot Lake mat phototrophs and heterotrophs will shed light on the biological mechanisms of carbonate deposition common to epsomitic environments. Furthermore, Hot Lake is a dynamic system; besides variability in temperature and photon flux throughout the annum, Hot Lake also experiences a seasonal cycle in salinity, varying between observed extremes in epilimnion water of ~200 mM $MgSO_4$ after snow melt to greater than 2 M at the close of the dry season. This cycle permits analysis of the stability in composition and function of the same community under diverse chemiosmotic conditions and elucidation of the mechanisms that impart robustness. As changes in climate are likely to alter both the temperature and salinity of surface waters, the study of Hot Lake may also improve our prediction of the effects of climate change on microbial community function and biogeochemical cycling, as well as offer insight and bioprospecting for high-salt (especially epsomitic) industrial and bioenergy applications.

The first aim of this project is to understand the dynamics of community structure and function in response to the annual cycle of the major physicochemical parameters of Hot Lake, especially salinity, temperature, and photon flux. Initial analysis of community diversity by 16S rRNA pyrosequencing of mat obtained during near the seasonal maximum in photon flux revealed taxonomic units consistent with oxygenic and anoxygenic photosynthesis, sulfur oxidation and reduction, and halotolerance. Nearest-neighbor phylogeny inferred two main filamentous cyanobacteria, one of order *Oscillatoriales* and one of order *Nostocales* present within the mat. Halophilic purple sulfur bacteria of genus *Halochromatium* and *Thiohalocapsa* as well as purple non-sulfur bacteria such as *Roseobacter* and *Rhodovulum* compose the anoxygenic phototrophic guild. 16S sequences consistent with the presence of bacteria similar to the nitrate-reducing, obligately chemolithotrophic sulfur oxidizer *Thialkalivibrio nitratireducens* as well as the halophilic deltaproteobacterium *Desulfosalina* suggests active sulfur cycling within the mat. Samples were collected from three points along the depth/salinity gradient of the lake; while sequences from the major members of the expected major functional guilds were observed in all three samples, elevations in *Desulfosalina*, *Spirochaeta*, and *Bacteroidetes* in the region of the halocline suggest an increased role for anaerobic degradation of mat biomass in the transition from the oxic epilimnion to the anoxic hypolimnion. Seventeen unique strains have been isolated from the mat to date, including a previously uncultivated *Rhodobacteriaceae*, *Marinobacterium*, and *Salinibacterium* species. Both of





the primary oxygenic phototrophs have been grown in unicyanobacterial consortia with mat heterotrophs with bicarbonate as the sole carbon source. These cultures are currently being analyzed by HR-NMR and LC-MS to determine the nature of the organic compounds that are being exchanged between the cyanobacterial phototrophs and associated heterotrophs. Furthermore, under a JGI Community Sequencing Program project, metagenomes of these co-cultures and whole mat will be generated and single cells from interesting taxa derived from whole mat will be isolated and sequenced. Paired samples will be utilized for metatranscriptomic analysis of *in situ* mat collected around the diel cycle. The resultant sequences, in concert with empirical data from naturally-derived simplified communities, will be used for predictive modeling of community membership and function, with an emphasis upon exchanged metabolites, in response to changes in physicochemical parameters.

The second major goal of this project is to generate a whole-mat model of energy and element cycling in the mat within its ecological context, especially with respect to carbon balance. Initial efforts towards this goal are focused on broad chemical classes; including dissolved organic carbon, sediment carbon, mat carbon, and surrounding landscape carbon. We are inventorying these pools to quantify where carbon is stored in the system and employing NMR and mass spectroscopy to identify key chemical species within each of these larger inventory pools. Stable isotope analysis is being used to link different pools and help identify carbon sources metabolized within the mat. Such surveys of total carbon and inventories of specific carbon compounds allow detection of connections between mat metabolic activity and the surrounding geochemical landscape. We are exploring metabolites produced in representative cultures and enrichments from Hot Lake to identify important potential targets for use in future SIP experimentation. This groundwork will provide a foundation for *in situ* stable isotope probing experiments to occur in the coming field season. A solid understanding of the carbon landscape within and surrounding the mats coupled with the SIP experiments should illuminate potential key carbon currencies in the mat and provide clues to carbon exchange between species within the mat community.

We are also working to understand how the system geochemistry influences the mat community. We are using a simple geochemical model to predict supersaturated species in the lake over very large seasonal fluctuations in water temperatures, salinity, and redox conditions. Initial measurements of microgradients within *ex vivo* mats have been measured with dissolved oxygen, pH and redox microelectrodes over a variety of light regimes, which is being coupled to the predictive model to refine our understanding of what geochemically-important processes are occurring within microenvironments inside the mat.

The combined genomic, enrichment incubation, and geochemical data collected to date will be compiled to guide sample collection during the summer of 2012, culminating in generation of a comprehensive dataset permitting multifaceted analysis of the activity of the mat and interactions of its members around the diel cycle near the seasonal photic maximum.

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