PROJECT REPORT

This project elucidated the cellular mechanisms involved in phosphate acquisition and *in situ* metabolism of novel phosphorus species by thermophilic cyanobacteria found in the hot spring microbial mats of Mushroom and Octopus Spring, located at the lower Geyser Basin in Yellowstone National Park (**Fig. 1**). Although significant advances have been made in characterizing the alkaline hot spring microbial mat ecosystems of Yellowstone National Park, relatively little is known about the physiological adaptations of microbes in the mats to nutrients. My research focused on characterizing the cellular mechanisms involved in phosphate acquisition and metabolism in hot spring microbial mats, specifically Mushroom and Octopus Spring, which have been studied for over 30 years. I also examined the ways in which cyanobacterial ecotypes of the genus *Synechococcus* acclimate to low phosphorus (P) conditions. Ecotypes are physiologically specialized populations that may, for instance, have unique nutrient requirements and preferences. *Synechococcus* are one of major primary producers in the hot spring microbial mats. This integrative study was also informed by full-genome sequences of two *Synechococcus* ecotypes, OS-A and OS-B', that are adapted to different temperature niches within the mat.

In this research, I performed a series of investigations to determine: 1) the P stress response genes present in the *Synechococcus* ecotype genomes, 2) the abundance of P metabolic transcripts in the mat environment, and 3) the *in situ* expression of these gene products linked to environmental variations in nutrients and temperature.

In silico analysis involved mining both *Synechococcus* ecotype genomes and the environmental genomic data set acquired from hotspring mat communities to investigate the potential P uptake and utilization genes within the microbial mat. This analysis revealed that the *Synechococcus* ecotypes have an extensive suite of novel genes that are likely responsive to environmental P levels, perhaps the largest set of such genes characterized in any organism to date. Interestingly, the genome of the *Synechococcus* OS-B' cyanobacterial ecotype, but not the *Synechococcus* OS-A ecotype, harbors a cluster of genes encoding proteins that would enable the organism to transport and utilize phosphonate compounds. Phosphonates, characterized by a C-P linkage, are thought to be relics from a time prior to oxygen evolution on Earth. These compounds occur naturally, but are also anthropogenic and widely used as herbicides, flame retardants, plasticizers, corrosion inhibitors, and drugs.

These genomic-level analyses informed the collection and field studies conducted at Mushroom and Octopus springs. I investigated the *in situ* differential regulation of the transcriptionally relevant P-stress response genes and how fluctuations in P bioavailability, oxygen availability, and temperature may regulate the P stress response of the *Synechococcus* ecotypes. These studies focused on utilization of the *phn* cluster present in *Synechococcus* OS-B' and how it may provide a competitive advantage for this ecotype in comparison to *Synechococcus* OS-A. Phosphonate compounds are not found in all environments and thus the OS-B' ecotype may have unique physiological adaptations to deal with phosphonate metabolism.

Mat samples were collected from Mushroom and Octopus hot springs over the diel (light/dark) cycle, to begin assessing when the genes may be differentially expressed in the two ecotypes, lending to investigations of what environmental factors affect and/or regulate uptake and metabolism of P species in the mat. Quantiative PCR (qPCR) analysis was performed on RNA extracts from mat samples collected over a 24 hour cycle at 2 hour intervals coupled to NMR analysis to quantify phosphonate concentrations and other potential P sources. Mat samples

were collected in September of 2007 at temperature sites where the *Synechococcus* ecotypes are predominantly distributed in Mushroom and Octopus Spring. Mat cores were frozen on liquid nitrogen immediately after extraction from the mat with a 6mm diameter core borer.

For molecular analyses, RNA was then extracted and gene expression on mat samples collected from the selected sites was quantified with primers designed from the OS-B' genome. Genes of interest include those of the putative *phn* operon. RT-PCR of RNA extracted from mat samples collected at Mushroom Spring in July revealed that the full *phnC-phnM* operon is present and expressed in the mat environment. *Phn* gene transcripts were quantified directly in Mushroom and Octopus hot spring mat samples over a diel cycle and found that they increase during the day and are reduced at night. Furthermore, these results indicate that the *phnC*, *phnI*, and *pstS* transcripts were up regulated at 7 am. This is a dark/light transition point when the mat is still anoxic. Phosphonates are known to accumulate in anoxic environments and perhaps they can be scavenged, though the mechanisms underlying this are unknown. These results were further corroborated via NMR indicating a decrease in phosphonate concentrations after expression of the *phn* genes. In summary, *in situ* investigation of *Synechococcus* gene expression and the cellular phenotype linked to P metabolism in the hot spring microbial mat over the diel cycle revealed that Phn degradation and utilization by OS-B' is tightly regulated by environmental cues, specifically light/dark metabolism and substrate availability. These results provide us a glimpse into the strong metabolic switching in the cyanobacteria over a diel cycle.

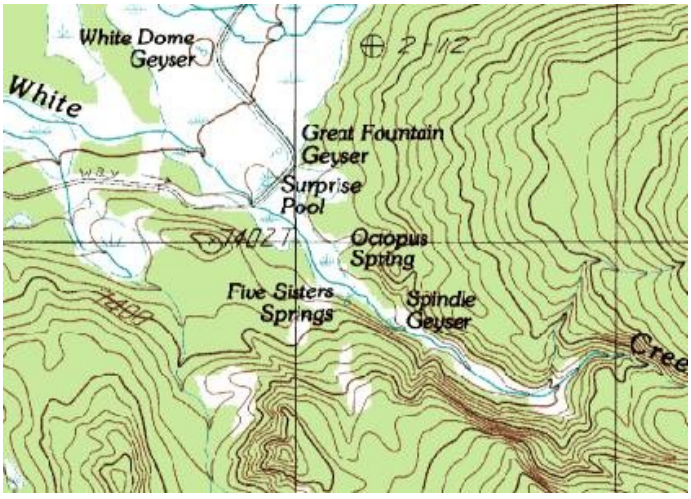

Figure 1: Study site of Octopus Spring (Yellowstone National Park) located at GPS coordinates N44.53382 and W-110.79743. Topography map courtesy of United States Geographical Survey.

Figure 2: Collection of hot spring microbial mat samples for biological and chemical analyses from Octopus Spring, Yellowstone National Park in August, 2007. Field research funded by the Lewis and Clark Fund for Exploration and Field Research in Astrobiology.