**487b Proteomics-Based Systems Biology Study of the Phosphorus Starvation Response in the Cyanobacterium *Synechocystis* Sp. Strain Pcc6803**

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Cyanobacteria have recently come to the attention of researchers for their ability to produce hydrogen and a myriad of other potentially industrially important secondary metabolites. *Synechocystis* sp. strain PCC6803 is frequently used as a model cyanobacterium in biochemical and genetic studies. As is the case for most microorganisms, inorganic phosphate is the preferred source of phosphorus for *Synechocystis* sp. strain PCC6803, and within laboratory processing this organism is cultured in the presence of excess quantities of this nutrient (mM concentrations). However, increasingly it is becoming apparent that phosphorus limitation occurs for sustained periods in many geographically dispersed freshwater lakes and marine sites (pM levels). To date, however, no attempts have been made to understand the global response of this and other cyanobacteria to this form of nutrient stress. An understanding of these processes will be of use not only in the basic understanding of these microbes and the niches they occupy (and potential role in process culture), but could be applied to develop biomarkers to follow stages in the P biogeochemical cycle within aquatic environments. In addition, the identification of novel proteins associated with Pi uptake could be exploited in the removal of Pi for water quality improvement, and enzymes involved in the catabolism of organophosphorus molecules are of interest in the pharmaceutical and agribiotech industrial sectors.

The goal of this research is to understand the global response of *Synechocystis* sp. strain PCC6803 to phosphorous starvation using proteomics in conjunction with assays of phosphatase activity and quantitative measurements of gene expression. Cyanobacteria were grown in media containing 0.12 - 40 mg/L potassium phosphate and samples collected for protein and mRNA analysis. Differences in protein expression were assessed using gel-free ("shotgun") proteomics as well as 2-D gel electrophoresis. In the former case, quantitative measurements were obtained using an isobaric peptide tagging technique (iTRAQ, Applied Biosystems). Over 300 proteins were identified per phenotype. In addition, the levels of several mRNAs, including those from hydrogenase-related genes, were measured separately using real time RT-PCR. Key proteins whose expression is affected by Pi limitation were identified and included several phosphatases. Phosphatase activity assays were performed and the activity levels found to correspond with the trends in the proteomic data. Protein level alterations were related to existing genomic knowledge to provide a systems-level understanding of the P-stress response in this organism. This will allow identification of uptake mechanisms and catalytic proteins used by these organisms for Pi scavenging in the environment. To our knowledge, no previous proteomic studies on Pi stress within this and other cyanobacteria have been made. Furthermore, the quantitative, reproducible proteomic analysis described here illustrates the development of a method that could be integrated into other systems biology studies.