Proteomic Characterization of Microbes and Microbial Communities

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Project Goals: The overarching goal of the PNNL Foundational Scientific Focus Area (FSFA) is to investigate fundamental scientific issues in microbial interactions – those that occur between different microbes as well as between microbes and their extracellular environment – to understand how microbial communities work collectively to carry out complex biogeochemical processes. This research component will develop and apply proteomic approaches to define these interactions, to track the flow of carbon from the autotrophic population to the heterotrophic population and to elucidate the metabolic control of energy flow and nutrient cycling in thermophilic microbial mats.

The challenges associated with the proteomic characterization of natural microbial mat communities growing in extreme environments are as varied as the types of environments. These challenges range from extremely small sample size and the presence of contaminating inorganic and organic materials in the mats to the difficulties in delineating which proteins and which organisms are responsible for the function that is present. To address these challenges, we are developing and applying methods for the extraction and analysis of proteins from the mats that include in situ stable isotope probing, advanced protein extraction methods, and subcellular fractionation methods in mixed populations.

Microbial autotroph- heterotroph associations are metabolically interactive, self-sustaining biological systems that are widespread in terrestrial and aquatic environments. Of specific interest to the FSFA program are phototrophic microbial mats found in alkaline siliceous hot springs in Yellowstone National Park (YNP), and chemoautotrophic iron and sulfur-oxidizing mats found in acidophilic, hyperthermophilic springs in YNP.

While the characterization of the microbial mats at Mushroom springs at YNP is limited by small samples sizes, the well annotated metagenome and relative ease of sample processing allow advanced analysis using *in situ* Protein Stable Isotope Probing (Pro-SIP) labeling strategies. Specifically, Pro-SIP has been applied to samples taken directly from the field with the immediate short-term exposure to $^{13}C\text{-}CO_2$. Advanced analytics and

informatics developed at PNNL have allowed for identification of taxa and associated proteins that are expressed and are actively taking up CO_2 during the incubation at the tested time point. A preliminary analysis of the first 10% of the data yielded 24 proteins validated by two or more unique labeled peptides. Since 14 were from *Roseiflexus RS-1*, 9 were from *Synechococcus* and only 1 was from *Candidatus Thermochlorobacter* aerophilum, it was determined that *Roseiflexus RS-1* is active in reducing CO_2 in the morning period of sunrise through the beginning of high light, and *Synechococcus* is active to a lesser extent in this time frame. During this time frame, these organisms are mainly synthesizing chaperones and transcription associated proteins.

In contrast to the phototrophic mats, the chemoautotrophic mats from YNP present different challenges for proteomic characterization. The relative high level of archaeal components and the high concentration of iron and sulfur in the mats confound cell lysis and protein extraction. To overcome these challenges, we have developed novel protein extraction techniques and have successfully detected a suite of iron, sulfur and arsenic oxidation proteins from the organisms present in the these communities. The meta-genome analysis suggests that the organism *Thermoproteales* (strain WP30) is present and further that this heterotrophic population respires on elemental sulfur and/or arsenate during growth on complex carbon sources. This genomic hypothesis is supported by the identification of the Mo-pterin proteins responsible for reduction of elemental sulfur and arsenate in these communities.

We will present the new and developing technologies for protein extraction, protein labeling and subcellular fractionation for organisms inhabiting natural microbial mats in extreme environments along with information on the interactions among these organisms and their relationship to that environment.

This research was supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Foundational Scientific Focus Area.