Integrating geochemical, microscopic and –omics analyses to understand microbial interactions in high-temperature chemotrophic communities

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Project Goals: To understand metabolic control of energy flow and nutrient cycling, and interactions among microbial community members in thermophilic chemotrophic microbial mats.

The advent of molecular tools and -omics technologies has provided opportunities for assessing the predominant and relevant indigenous organisms present in natural microbial communities, as well as their function within a connected network of different populations. High-temperature microbial communities are often considerably less diverse than mesophilic environments and are constrained by geochemical attributes such as pH, dissolved oxygen, Fe, sulfide, and or trace elements including arsenic and mercury. Consequently, one of the project goals is to understand microbial interactions among chemoautotrophic and heterotrophic members of high-temperature acidic Fe-oxidizing communities and sub-oxic elemental sulfur systems in Yellowstone National Park (YNP). Specific objectives of this work are to identify the predominant transcripts, proteins, metabolites and isotopic signatures associated with high-temperature microbial communities and to establish metabolic network models for different habitat types using consensus sequence assemblies of major phylotypes present in Fe(III)-oxide mats and sulfur sediments. Microbial community metabolic models derived from metagenome assemblies of individual populations are being used to test hypotheses regarding the role of specific phylotypes within each habitat type as well as microbial interactions occurring *in situ*.

Prior metagenome sequencing of high-temperature Fe-oxide and elemental sulfur systems from Yellowstone National Park (YNP) reveal communities dominated by thermophilic archaea and/or members of the deeply-rooted bacterial order Aquificales. Phylogenetic and functional analysis of metagenome sequence has provided an excellent foundation for hypothesizing the role of individual populations in a network of interacting community members, and for testing specific hypotheses regarding the importance of biochemical pathways responsible for material and energy cycling. For example, the predominant microbial populations present in acidic Fe-oxide microbial mats of Norris Geyser Basin (YNP) include chemoautotrophs such as *Metallosphaera yellowstonensis*, a member of the crenarchaeal order Sulfolobales. Pure-culture laboratory experiments have recently confirmed that this chemoautotroph can fix inorganic carbon (i.e. CO_2) using the 3-hydroxypropionate/4-hydroxybutyrate pathway

while obtaining energy from the oxidation of ferrous Fe. Gene expression studies under different treatment conditions showed that *M. yellowstonensis* utilizes a novel terminal oxidase complex to oxidize Fe(II) (*fox* gene complex) while fixing inorganic carbon. Consequently, *M. yellowstonensis* is an important primary producer in high-temperature acidic Fe-oxide mats, and may provide a source of organic carbon for other heterotrophs present in the community.

Metagenome sequence assemblies show that the Fe-oxide mats also contain potential heterotrophs including Desulfurococcales and Thermoproteales-like populations. Functional analyses of genes belonging to these phylotypes suggest that these organisms degrade complex carbon sources, and that specific proteins may serve as a primary carbon and energy source. Recent isolation and characterization of a representative Thermoproteales organism (strain WP30) from YNP shows that this heterotrophic population respires on elemental sulfur and/or arsenate during growth on complex carbon sources. The Mo-pterin proteins responsible for reduction of elemental sulfur and arsenate have been identified and gene expression studies are underway to confirm the role of these novel proteins in community function. A deeply-rooted archaeal population has also been identified as a major community member in high-temperature Fe mats (referred to here as 'Novel Archaea Group 1'), and *de novo* sequence assemblies suggest that this organism is heterotrophic, potentially utilizing complex carbon sources produced by *M. yellowstonensis*. Iron depositional studies have been conducted to correlate Fe-oxidation rates with O_2 flux rates measured at the Fe-mat interface (using O_2-microelectrodes) as well as 16S rRNA gene copy-numbers of *M. yellowstonensis* (measured using quantitative PCR). Results show an excellent correlation between *M. yellowstonensis* copy-number and Fe-oxide deposition rate, especially during early stages of Fe-oxide mat development.

Early results from proteomic analyses of Fe-oxide mat samples confirm the importance of *M. yellowstonensis* and Novel Archaeal Group 1 populations in high-temperature Fe-oxidizing communities. Proteins identified in Fe-mat communities are being used to refine and improve metabolic models constructed using genome sequence. Pathway specific processes are also being elucidated using isotope measurements focused on ^{13}C and ^{34}S of different chemical fractions. Additional proteomic and future transcriptomic results will be used to assess the importance of specific pathways, and will provide detailed information necessary to test hypotheses regarding the major microbial interactions that define community structure and function. Integration of genomic, proteomic, and metabolic information to understand autotroph-heterotroph interactions is tractable within high-temperature geothermal systems, in part due to the relative simplicity of the communities and the stability of key geochemical variables including pH, Fe, O_2 and dissolved sulfide/elemental sulfur.

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