Microbial Interactions in Hot Spring Phototrophic Mats and Isolates

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Project Goals:

Microbial mats in alkaline siliceous hot springs of Yellowstone National Park, which are constructed by a variety of oxygenic and anoxygenic phototrophic microorganisms, are model systems from which to learn principles that underlie a predictive understanding of microbial community ecology. Our goal is to use multiple "omics" and cultivation approaches to understand microbial interactions that govern the capture and recycling of energy and resources in this system.

Analyses of assembled metagenomic scaffolds have identified eight major functional guilds inhabiting the photic portion of the community. These include the oxygenic phototrophic *Synechococcus* spp., five anoxygenic phototrophic guilds (*Roseiflexus* spp., *Chloroflexus* spp. and Anaerolineae-like Chloroflexi; "*Candidatus* Chloracidobacterium thermophilum" and "*Ca*. Thermochlorobacter aerophilum") and two aerobic heterotrophic populations. Detailed annotations of genes in these assemblies have permitted reconstruction of the metabolic potential within each guild and this has guided us in developing a model of how the members of different guilds might interact metabolically. For instance, consistent with previous results, *Synechococcus* spp. possess genes that should enable them to produce glycolate through photorespiration and to produce glycogen and break it down via fermentation. *Roseiflexus* spp., "*Ca*. C. thermophilum", " *Ca*. T. aerophilum" and members of heterotrophic guilds possess genes that should enable them to metabolize glycolate and/or acetate. Surprisingly, "*Ca*. C. thermophilum" and "*Ca*. T. aerophilum" are both missing the same genes involved in the synthesis of branched amino acids (though not the transaminase genes). This observation suggests that they may obtain relevant biosynthetic precursors from another mat organism(s). One hypothesis is that polyhydroxyalkanoic acids (PHAs) of *Roseiflexus/Chloroflexus* spp. which are produced from related compounds can be a source of branched amino acid precursors for above mentioned organisms. Additionally, *Roseiflexus* spp. appear to be deficient in the synthesis of riboflavin, suggesting their dependence on populations that can produce it (e.g., *Synechococcus* spp., "*Ca*. T. aerophilum"). Finally, both *Synechococcus* spp. and *Roseiflexus* spp. may have potential to fix N_2, possibly suggesting that other community members may be dependent on them for a supply of fixed nitrogen.

These metagenomic assemblies have also been useful as references for interpreting metatranscriptomics and metaproteomics databases. While transcription patterns in general confirmed previous observations, they also revealed new ways to think about the metabolisms of mat community members. For instance, it was formerly thought that *Roseiflexus* spp. were primarily photoheterotrophic during daytime, aside from a brief period of light, but anoxic conditions in early morning, when they conduct carbon fixation via the 3-hydroxypropionate pathway. It was thus surprising to find that genes in this pathway were expressed during the day.

Detailed examination of expression patterns for genes involved in central carbon metabolism and storage polymer synthesis and degradation now lead us to hypothesize that *Roseiflexus* spp. conducts a photomixotrophic metabolism throughout the day. We hypothesize that to do so they produce PHAs (and possibly wax esters) at night, which they use during the day to maintain an adequate supply of organic intermediates and reductant. If true, this could have important implications on the timing of transfer of intermediates derived from PHAs to other members of community.

These hypotheses are being tested in several ways. First, to demonstrate that *Roseiflexus/Chloroflexus* spp. incorporate CO_2 in the low-light, anoxic morning period and also in high-light oxic period, we investigated $^{13}CO_2$ incorporation into proteins of these organisms *in situ.* In a similar manner, ^{13}C-acetate has been used to assess the degree to which *Roseiflexus* spp., "*Ca*. C. thermophilum," and "*Ca*. T. aerophilum" may compete for this intermediate. Second, metabolomics analyses are being used to evaluate the hypothesis that PHAs (and possibly wax esters) undergo diel rhythms of synthesis and degradation in *Roseiflexus/Chloroflexus* spp. Third, we are developing experimental co-cultures consisting of cyanobacteria and Chloroflexi isolated from hot spring cyanobacterial mats, namely, *Thermosynechococcus sp. NAK55* (whose genome we obtained), *Roseiflexus castenholzii* and *Chloroflexus aurantiacus* J-10-fl. We found that *Thermosynechococcus* excreted a variety of metabolites such as formic, lactic, succinic, oxalic and isocaproic acids, dihydroxyacetone, sarcosine and glyceraldehyde, which have not yet been considered in our model of metabolic interactions inferred from metagenomic data. *Choloroflexus* J-10-fl was tested for its ability to utilize these metabolites and some of them were found to support growth. To investigate these potential interactions further, we are studying growth of the cyanobacterium in co-cultures with Chloroflexi isolates. Metabolites present in the growth medium and gene expression in the members of the co-cultures are being determined under light and dark conditions in two experimental settings: i) slowly mixed planktonic culture and ii) mat forming, benthic cultures growing on the surface of submerged agar. These investigations are enhancing our understanding of metabolic interactions among members of major guilds in natural hot spring cyanobacterial mats and providing new experimental approaches to evaluate the interactions.

This research is part of the PNNL Foundational Scientific Focus Area: Biological Systems Interactions.