

TRANSITIONING METAGENOMES INTO INTERACTOMES IN A CHEMOSYNTHETIC SULFUR-BASED HOT SPRING COMMUNITY. D. R. Colman¹, M. R. Lindsay¹, E. R. Roden², E. S. Boyd¹. ¹Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717, ²Department of Geoscience, University of Wisconsin, Madison WI, 53706

Introduction: Microbial interactions define the habitability of environments today and in the geologic past. However, little is known of the processes that influence the formation and assembly of chemosynthetic communities that likely prevailed on early Earth. The low-diversity of hot spring microbial communities make them tractable systems to understand the trophic level interactions that influence microbial community assembly processes. Moreover, they provide an opportunity to define how complexity can develop in communities that are supported by mineral sources of energy. Here, we describe a sulfur-based chemotrophic community at the level of predominant population level genomes that were reconstructed from an acidic Yellowstone National Park (YNP) hot spring ('Figure 8'; pH ~3.8, T ~62 °C) metagenome. Results are discussed in context of genome-guided hypotheses of interactions among populations and with the geochemical environment at the level of sulfur, hydrogen and carbon transformations.

Community Genomic Analyses: Multi-year 16S rRNA gene sequencing indicates that the 'Figure 8' community is relatively stable, despite minor observed fluctuations in pH over multi-year samples. Assembly and binning of metagenomic contigs from sediment samples resulted in nearly complete genomes for several community members. Phylogenetic marker-gene analyses indicated that populations included a novel euryarchaeote (Fig8-eury) distantly related to Deep-Sea Hydrothermal Vent Euryarchaeota Group 2 representatives (*Aciduliprofundum* spp.), the crenarchaeote *Caldisphaera draconis* and the bacteria *Hydrogenobaculum* sp. Y04AAS1 and *Rhodococcus qingshengii*. with $\geq 89\%$ estimated genome completeness. Further, additional populations with moderately complete genomes were recovered from the crenarchaeotes *Thermoproteus uzonensis* and *Acidilobus sulfurireducens*. Phylogenetic and metabolic reconstruction of the Fig8-eury genome suggests that it represents a novel lineage of archaeal SO_4^{2-} reducers that is only distantly related to heterotrophic, acidophilic S^0 reducers (*Aciduliprofundum* spp. and Thermoplasmatales).

Analyses of protein-coding genes within each genome bin and published cultivation information indicated that the energy metabolism of most populations is dependent on sulfur cycling: putative SO_4^{2-} reduction in Fig8-eury, S^0 oxidation in *Hydrogenobaculum*, and

S^0 reduction in *Caldisphaera*, *Thermoproteus*, and *Acidilobus* populations. *Hydrogenobaculum* encodes genes necessary for autotrophy and Fig8-eury encodes genes for a putative novel method of autotrophy, while *Caldisphaera*, *Thermoproteus* and *Acidilobus* are all heterotrophs. Comparative genomics indicates that the five dominant populations that inhabit 'Figure 8' have minimal functional overlap, with the exception of the *Acidilobus* and *Caldisphaera* populations which slightly overlap. Based on metabolic reconstructions, we propose that fixation of CO_2 in sediment communities is primarily driven by the H_2/SO_4 redox couple (Fig8-eury) through a novel pathway. Consistent with these genomic predictions, enrichments with H_2/SO_4 and CO_2 from sediment are active and transferrable.

In-Situ Microcosm Analyses: Results from H_2 oxidation assays that were deployed in 'Figure 8' were consistent with the above proposed model, whereby amendment with molybdate (Mo; an inhibitor of sulfate reduction) inhibited H_2 consumption activity in the sediment community but had little to no effect on H_2 activity in the planktonic community. However, carbon fixation assays that were deployed in 'Figure 8' suggested more complex interactions in the planktonic and sediment phases.

Conclusions: Here, we provide evidence for a lithogenically fueled chemosynthetic microbial community from a high temperature acidic hot spring in YNP. An interactive model is proposed based on genome-guided hypotheses of abiotic and biotic interactions at the level of sulfur, hydrogen and carbon transformations among both cultivated and uncultivated thermoacidophiles present in the community. Cultivation-dependent analyses provided support for hypotheses presented from the genomic data and are used to refine the geological-biological interaction model. Lastly, results will be discussed in context of connecting the interactions that occur between chemosynthetically-driven communities, their geochemical environments and among populations which ultimately lead to microbial community assemblages that are fundamentally linked to their geochemical environments.