Metagenomic Characterization of Serpentinization-Influenced Groundwater collected at the Coast Range Ophiolite Microbial Observatory A. J. Hyer[1] and W. J. Brazelton[2], [1]University of Utah, Department of Biology, Alex.Hyer@utah.edu, [2]University of Utah, Department of Biology

Introduction: Serpentinization is an array of geochemical reactions that occur when the mineral olivine interacts with water. Serpentinzation and associated processes produce heat, hydrogen gas, methane gas, and small organic molecules [1]. This chemical cocktail provides a reducing environment readily utilized by many microorganisms for energy and carbon. As serpentinization reactions are largely subterranean and energetically rich, they may be the primary energy source for some Bacteria and Archaea in dark subsurface environments. Phylogenetic analyses of actively serpentinizing hydrothermal vents [2] and continental springs [3] have identified known chemolithoautotrophs. Serpentinization was likely present during the Hadean Earth and on Mars [1], thus serpentinization-associated microbes may serve as models of early and extraterrestrial life.

In most serpentinizing environments, serpentinization-associated subsurface fluids mix with ambient ocean water, stream water, or other surface runoff. This nearly unavoidable source of contamination makes it difficult to determine if organisms in an environmental sample came from subsurface fluids, ambient water, or are specialized at surviving in the gradient between the two. The Coast Range Ophiolite Microbial Observatory (CROMO) is located at a research field station in California and consists of two boreholes and six monitoring wells drilled directly into serpentinite rocks [4]. By directly accessing serpentinites and associated groundwater, the CROMO wells enable unprecedented opportunities to investigate the issue of environmental fluid contamination. Thus, a robust knowledge of microbial communities at CROMO is essential to understanding microbial composition and activity in more complex serpentinite environments.

Here, we propose using both metagenomic and metatranscriptomic data from several temporally and geographically distributed samples from CROMO to identify evolutionary and metabolic patterns of serpentinization-associated microbes. Specifically, we utilize all sequence data from the wells to create a "master" metagenomic assembly that individual samples can be compared against to identify microbial and metabolic shifts in time and space

Methods: All DNA samples from CROMO monitoring well QV1.1 were concatenated and quality filtered for inaccurate bases and replicate reads. High-quality reads were assembled to form a single 23.7 Mbp "master" assembly. The master assembly was then annotated for various RNAs and CDSs. Finally, the master assembly was binned, generating several high-completion, low-contamination bins. The taxonomy and metabolic pathways of each bin were identified. All of these data were either literally or effectively encoded into a database for downstream analysis.

Both metagenomic (DNA) and metatranscriptomic (cDNA) data from six time samples (four metagenomes, two metatranscriptomes) were mapped against the master assembly. Coverages were then normalized and compared visually using the software suite anvi'o [5].

Results: Binning produced six mid-to-high completion, low contamination bins. Four of these bins had similar taxonomic assignments (*Clostridia*, *Firmicutes*, *Dethiobacter alkaliphilus*, *Dethiobacter alkaliphilus*) and encoded genes associated with the acetogenesis pathway, multiple forms of denitrification, ammonia assimilation, and the reverse acetyl-CoA pathway. The other two bins (*Xanthomonadaceae*, *Comamonadaceae*) contained genes associated with pathways for pyruvate fermentation to ethanol and nitrate reduction respectively. Interestingly, the *Clostridia* bin dropped in metagenomic coverage nearly 10-fold when the *Xanthomonadaceae* bin's coverage increased roughly 10-fold. Otherwise, each bins' metagenomic and metatranscriptomic coverages were remarkably similar: indicating a stable environment.

Future Directions: We are currently implementing this methodology with a more comprehensive dataset from CROMO that includes 28 metagenomic and metatranscriptomic samples. Additionally, we will incorporate robust statistical analyses to compare samples in a meaningful and efficient manner. For the current study, samples from the monitoring wells surrounding QV1.1 will be analyzed to determine if the identified organisms are vertically stratified within the borehole. This additional analysis will indicate if the taxonomically similar bins are specialized to different microenvironments within CROMO well QV1.1. We will also analyze the sequences of key metabolic genes vs. taxonomic markers to identify possible HGT.

References: [1] Schrenk M. O. et al. (2013) *Reviews in Mineralogy, 75*, 575-606. [2] Brazelton W. J. et al. (2006) *AEM, 72*, 6257-6270. [3] Brazelton W. J. et al. (2013) *AEM, 79*, 3906-3916. [4] Cardace D. et al. (2013) *Scientific Drilling, 16*, 45-55. [5] Eren A. M. et al. (2015) *PeerJ Preprints, 3*, e1319.