Geochemical and genomic evidence for an *in situ* lithoautotrophic Fe-oxidizing microbial community at Chocolate Pots hot springs, Yellowstone National Park, USA. Nathaniel W. Fortney[1], Shaomei He[1], Eric S. Boyd[2], and Eric E. Roden[1], [1]Department of Geoscience, NASA Astrobiology Institute, University of Wisconsin-Madison, Madison, WI, 53706, USA, (nfortney@wisc.edu), [2]Department of Microbiology and Immunology, NASA Astrobiology Institute, Montana State University, Bozeman, MT, 59717, USA

Chocolate Pots hot springs (CP) is a circumneutral pH, Fe-rich geothermal feature located in Yellowstone National Park. Fe-based metabolic processes are deeply rooted in the tree of life and studying environments like CP are important for us to study to gain insight into ancient Earth ecosystems. Recently identified features on Mars are indicative of near-surface hydrothermal environments and studies of modern Earth systems like CP allow us a glimpse into how life may have arisen on other rocky worlds. The evidence for both a carbon cycle and Fe-redox cycle to be operating *in situ* at CP is important because it suggests the potential for a self-contained ecosystem, similar to that at CP, to have existed on Mars.

Previous studies, utilizing both enrichment cultures and *in vitro* incubations, have demonstrated the ability of the endogenous microbial community present at CP to reduce native Fe-Si oxides. Analyses of 16S rRNA genes extracted from enrichment cultures identified close relatives of known dissimilatory Fe-reducing bacteria (DIRB), including *Geobacter metallireducens* and *Melioribacter roseus*. Metagenomic analyses identified homologues to the well-characterized extracellular electron transfer (EET) system, *pcc* [1, 2].

The question of the composition and activity of the *in situ* Fe-cycling microbial community at CP has more recently been addressed using ^{13}C stable isotope probing using. Fe-Si oxide sediments were collected from near the CP vent and incubated under *in situ* conditions amended with ^{13}C-acetate or -bicarbonate to target DIRB and Fe-oxidizing bacteria, respectively. Relatives of known DIRB were once again identified in 16S rRNA gene amplicon libraries. However sequences related to the known lithoautotrophic Fe-oxidizer, *Sideroxydans* [3], were absent, despite the presence of related sequences identified in previously collected core samples. Metagenomic analyses of DNA extracted from Fe-reducing and -oxidizing incubations targeting both putative EET systems as well as known CO_2-fixation pathways revealed a handful of metagenomic bins containing both systems, potentially indicating members of an *in situ* lithoautotrophic Fe-oxidizing community [Fortney et al., *in prep.*].

In vitro Fe-reducing incubations showed a high amount of DIR potential in materials collected from the CP vent, which diminishes quickly, within just a couple meters of the vent pool. This is in agreement with Fe geochemistry of Fe-Si oxide sediment core samples collected along the CP flow path which shows a higher concentration of Fe(II) in the core material collected from the Vent than anywhere farther down stream. 16S libraries collected from the Vent material do not immediately suggest an abundance of DIRB as would be implicated by the Fe geochemistry, and investigation for genomic evidence of putative DIRB is underway.

Presently, metagenomic libraries have been co-assembled from DNA extracted from Fe-Si oxide sediment from the bottom of the CP vent pool, and two additional coring sample sites one and two meters further down stream. Over 250 metagenomic bins have been identified in the combined assembly. Twenty-five bins contain homologues to the *pcc* porin, and several bins contain multiple copies. Additional homologues to putative EET systems from Fe-reducing, and -oxidizing microorganism alike, including *Shewanella*-like *mtrAB*, *Acidithiobacillus*-like *Cyc2*, and *Sideroxydans*-like *mtoAB* have been identified in the coassembly as well. Ultimately, bins containing both putative EET systems as well as CO_2-fixation pathways will be targeted. So far, only RuBisCO, the marker gene for the Calvin-Benson-Bassham cycle has been searched for, and several candidate bins containing RuBisCo also contain a putative EET system.

Shotgun metagenomic sequencing of filtered biomass from the CP pool water column is currently underway. Homologues to genes in well-characterized EET systems and CO_2-fixation pathways will again be targeted to gain insight into which microbes might possess both systems *in situ*. Assembled libraries from samples collected directly from the environment have the benefit of avoiding potential biases in microbial community composition introduced in the incubation studies conducted previously.

Additional water and sediment samples are being analyzed for lipid biomarker analyses and DOC measurements in order to identify the potential carbon source for the heterotrophic (e.g. Fe-reducing) microbial community within the CP environment.

References:

[1] Fortney, N. W., et al. (2016) *Geobiology*, *14*, 255-275. [2] Shi, L., et al. (2014) *Front Microbiol,* 5, 657. [3] Weiss, J.V., et al. (2007) *Geomicrobiol J,* 24, 559-570.