

High-throughput sequencing of samples from the Chocolate Pots Hot Spring, Yellowstone National Park, suggests the involvement of novel taxa in dissimilatory iron reduction. B.J. Converse^{1,2}, N.W. Fortney^{1,2}, S. He^{1,2}, and E.E. Roden^{1,2}, ¹Department of Geoscience, University of Wisconsin-Madison, Madison, Wisconsin, 53706 (bconverse@wisc.edu), ²NASA Astrobiology Institute, USA.

Though microbial iron (Fe) redox cycling has been well studied in high-temperature, low-pH environments at Yellowstone National Park (YNP) [1], the microbial and geochemical processes in circumneutral pH environments remain understudied. The Chocolate Pots Hot Spring (CP) in YNP offers an ideal circumneutral pH site to further explore microbial Fe-redox cycling, and to learn more about microbial communities, genetic systems, and geochemical processes involved in dissimilatory iron reduction (DIR). In this study, we employed 16S rRNA amplicon and shotgun metagenomic Illumina sequencing to analyze DNA extracted from sediment core samples from CP. To our knowledge, this is first high-throughput sequencing study conducted from neutral pH Fe redox-active sediments.

An analysis of Illumina MiSeq 16S rRNA gene amplicon data signifies the presence of a diverse and novel group of bacteria and archaea in the upper 1 cm of sediment at increasing distances from the hot spring vent outlet. Taxa of note include potentially novel *Deltaproteobacteria*, *Chloroflexi*, *Chlorobi*, the recently described phylum *Ignavibacteriae* (of which certain species are known to reduce Fe(III) [2]), and variety of archaeal taxa related to *Crenarchaeota*. The majority of OTUs detected shared only partial homology to taxa described in the NCBI database.

Follow-up shotgun metagenomic sequencing was conducted with the Illumina HiSeq platform (University of Wisconsin-Madison Biotechnology Center), and the results corroborated those obtained with 16S rRNA gene sequencing. Sequencing of material from near the hot spring vent source (core 1) resulted in a metagenomic assembly containing contigs ranging from 86 to 513,071 bp in length, with an average contig length of 1,021 bp.

To determine the completeness of the microbial genomes obtained, CONCOCT [3] was used to bin contigs based on tetranucleotide frequency and sequencing coverage. The bins were evaluated for completeness and contamination via comparison to housekeeping genes from phylogenetically related organisms using CheckM [4].

Binning information suggested that upwards of 40 partially complete (>50%) microbial genomes were recovered with little to no contamination, though analysis of this data is ongoing. Taxonomic classification revealed that *Deltaproteobacteria* related to *Geobacter* spp. and *Thermodesulfobacteriaceae*, *Chloroflexi*, *Ignavibacteriales*, and various unclassified *Archaea* are dominant in CP sediments.

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To explore the genetic potential for DIR in core 1, the metagenome was probed for the presence of porin-cytochrome complexes (pccs), a recently described system known to be involved in the extracellular transfer of electrons to insoluble Fe(III) in several taxa [5]. Preliminary results revealed the presence of numerous homologs to this genetic arrangement with distinctive variations, suggesting that in addition to previously unidentified taxa, novel Fe(III)-reduction systems may be present in CP sediments.

Of particular note, several pcc-like systems were identified with homology to those found in the order *Ignavibacteriales*, indicating that these related organisms may be involved in DIR in this system. These findings mirror a parallel enrichment culture study with CP core sediments (Fortney et al., in prep.), where a similar pcc system was identified in an organism partially related to *Ignavibacterium* and *Melioribacter*, both members of the phylum *Ignavibacteriae*.

References:

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