H_2 cycling in Yellowstone National Park hot spring communities. M. R. Lindsay[1], K. E. Fristad[2], K. M. Fecteau[4], E. L. Shock[3,4], T. M. Hoehler[2], and E. S. Boyd[1], [1]Montana State University - Department of Microbiology and Immunology, Bozeman, MT, [2]NASA Ames Research Center, Moffett Field, CA, [3]Arizona State University – School of Earth and Space Exploration, Tempe, AZ, [4]Arizona State University – Department of Chemistry & Biochemistry, Tempe, AZ.

The interaction between crustal rocks rich in iron (e.g., basalts, peridotites) and water generates hydrogen (H_2) via several processes collectively known as serpentinization [1]. Serpentinization reactions are widespread and were likely active early during Earth's history. Comparable processes can be expected on other rocky planets that have liquid water. H_2 produced through water-rock interactions likely had an integral role in supporting early chemosynthetic life, particularly life supported by the coupling of H_2 oxidation with carbon dioxide reduction (e.g., acetogenesis, methanogenesis) as the latter was likely an available oxidant on early Earth.

There are two primary families of enzymes that activate H_2 which are termed [FeFe]- and [NiFe]-hydrogenases. [NiFe]-hydrogenases are widespread in early evolving archaeal and bacterial lineages indicating that they were likely a property of early life. [NiFe]-hydrogenases also have apparently diversified to couple with numerous oxidants (e.g. O_2, NO_3, SO_4, CO_2) as they have become available over geological time [2]. In contrast, [FeFe]-hydrogenases are present only in bacteria where they exhibit a patchy distribution, indicating that they are more recently evolved.

The relationships between the distribution of [NiFe]-hydrogenases, H_2-dependent metabolisms, the microbial communities supported by H_2, and environmental variation can be explored through the integration of geochemical, thermodynamic, molecular, and physiological data. In particular, such relationships can be explored in the accessible hot springs in Yellowstone National Park which provide a range of lithogenic H_2 sources that have been suggested to support diverse H_2 metabolizing microbial communities. High temperature putative H_2 oxidizing thermophiles have been previously studied in YNP, and it is thought that H_2 is a primary source of energy supporting production in microbial communities across YNP hot springs (>70˚C) [3].

Dissolved H_2 and CO in 27 high temperature YNP hot springs sampled from 6 geographically disctinct areas indicated that variations in dissolved H_2 concentrations within a single hot spring were often found to be multiple times greater than variations between different hot springs, indicative of a dynamic H_2 cycle. *In situ* activity assays were used to quantify rates of H_2 cycling activities in non-photosynthetic hot spring communities inhabiting Obsidian hot spring (70˚C, pH 5.5), Bison Pool (84˚C, pH 8.8), and Cinder Pool (88˚C, pH 4.0). Net H_2 oxidation was observed in communities inhabiting Obsidian hot spring and Cinder Pool, while net H_2 production was observed in a community inhabiting Bison Pool. The reduction of protons to H_2 is a strategy used by organisms to recycle reduced electron carriers when other preferrable electron acceptors (oxidants) are not available. Thus, these results suggest that gradients in oxidant availability may dictate the nature of H_2 metabolisms in geothermal communities in YNP.

To begin to probe the relationship between oxidant availability and H_2 metabolism we quantified the availability of dissolved gases (H_2, CO_2, CH_4, and CO) and aqueous geochemical parameters in the outflow channels of a paired set of hot springs – Roadside East (82˚C, pH 2.0) and Roadside West (70˚C, pH 6.4). These springs were chosen because they are likely to exhibit differences in the availability of oxidants capable of supporting H_2-dependent metabolisms. Progress on this project to date includes extraction of high quality RNA, quantification and sequencing of archaeal, bacterial, and eukaryal 16S/18S rRNA transcripts, *in situ* H_2 activity assays, and geochemical characterization. Genomes from closely related strains are currently being used to design specific PCR primers for the amplification of [NiFe]-hydrogenases which will allow for the determination of the distribution diversity of H_2 metabolisms across YNP, with implications for early forms of life that used H_2 as an energy source. The integration of these disparate datasets provide new insights into the role of environmental variation, in particular oxidant availability, in dictating the distribution and diversification of [NiFe]-hydrogenases and their potential role in supporting early H_2 dependent metabolisms.

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