

METHANE CYCLING BY NOVEL ARCHAEA IN YELLOWSTONE NATIONAL PARK HOT SPRINGS.

L. J. McKay^{1,2}, R. Hatzepichler^{1,3}, M. W. Fields^{1,4}, and W. P. Inskeep², ¹Center for Biofilm Engineering, Montana State University (luke.mckay@montana.edu), ²Department of Land Resources and Environmental Sciences, Montana State University, ³Department of Chemistry and Biochemistry, Montana State University, ⁴Department of Microbiology and Immunology, Montana State University.

Introduction: The discovery of near-complete methanogenesis pathways in the genomes of members of recently described phyla Bathyarchaeota [1] and Verstraetearchaeota [2] has drawn into question whether the ability to generate or oxidize methane and other hydrocarbon gases is limited to a single archaeal phylum, the *Euryarchaeota*. This phylum-level taxonomic expansion supports the hypothesis that methanogenesis could be one of the first metabolic modes of life [3], because an early metabolism would likely leave genetic remnants across broad, deeply-rooted lineages.

Sample site description and justification: Washburn Hot Springs (WS) in Yellowstone National Park (YNP) is an anoxic, highly sulfidic geothermal system where source gases are comprised almost entirely of carbon dioxide (~92%), hydrogen (~2%), and methane (~6%)—one of the highest known methane fluxes in YNP. In subsurface sediments at WS, porewater methane concentrations are between 50 – 100 ppm. Stable isotopic signatures of methane gas from WS have suggested that the majority of methane flux is geogenic, but these high levels of methane could also reflect a contribution from methanogens and/or support anaerobic methanotrophy even if the major flux is not biotic.

Results and Conclusions: We employed a combined approach of targeted amplicon and random metagenomic sequencing at WS to investigate the likelihood that novel methane cycling archaea are present and/or active in this extreme environment. Our data indicate a predominance of *Bathyarchaeota* in WS and a wide diversity of *mcrA* genes related to the *Euryarchaeota*, *Verstraetearchaeota*, and *Bathyarchaeota*. Sequences detected from RNA demonstrate that *mcrA* genes are actively transcribed by all three phyla. Further, reconstructed genomes yielded information regarding the ubiquity and phylogeny of single carbon processing genes across the archaeal domain.

We interpret these results as an indication that previously unidentified archaea are likely major contributors to global fluxes of methane, and that the WS system is replete with the ingredients for a chemolithotrophic origin of life: abundant carbon dioxide and hydrogen, intense thermal activity, and a

cesspool of deeply-rooted archaea involved in single carbon metabolism.

References:

- [1] Evans P. N. et al. (2015) *Science*, 350: 6259, 434–438. [2] Vanwonterghem I. et al. (2016) *Nature Microbiology*, doi:10.1038/NMICROBIOL.2016.170. [3] Weiss M. C. (2016) *Nature Microbiology*, doi:10.1038/nmicrobiol.2016.116.